2023 AACC Annual Scientific Meeting & Clinical Lab Expo

Poster Session Schedule

Posters of accepted abstracts were viewed in the Poster Hall of the of the Anaheim Convention Center on Tuesday, July 25 and Wednesday, July 26.

Below are the topics and their scheduled times.

TUESDAY, JULY 25, POSTER SESSIONS

9:30 am – 5:00 pm

WEDNESDAY, JULY 26, POSTER SESSIONS

9:30 am – 5:00 pm

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

Tuesday, July 25, 2023

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

A-002

The Prevalence of the High Sensitivity Cardiac Troponin I Outlier and False Positivity Rates on Beckman Coulter Access DxI

X. Li[1], J. Bryksin[2]. *[1]Emory University, Atlanta, GA, [2]Emory Unversity, Atlanta, GA*

Background: The false positive elevation in high sensitivity cardiac troponin I (hsTnI) can be triggered by many factors, for example, fibrin clots, heterophile antibodies, or other sources of troponin interferences. These interferences lead to inaccurate hsTnI results. These inaccurate results described in the literature as the "outliers". Published studies use statistical tools to identify the outliers based on the discrepancies between the two repeat values. Depending on the instrument platform and the study design, the outlier rate for the conventional troponin I assay was reported from 0.44% to 1.01% (1,2). In our laboratory, the false positivity rate for the conventional troponin assay on Beckman Coulter DxI platform was determined as 1% (unpublished data). This study aim was to determine both the statistically and clinically significant outliers for hsTnI assay on the Beckman Coulter Access DxI instrument in the academic medical center clinical laboratory setting.

Methods: A unique test repeat protocol was created. Two parallel hsTnI measurements are conducted in lithium heparin tubes (BD Vacutainer) on Beckman Coulter DXI 800 systems. When the difference between the two results is greater than 30%, a third repeat is automatically ordered and run after additional centrifugation at a higher relative centrifugal force. The outlier determination is based on assessing the difference between the first two values using previously described statistical analysis tools (1). The clinically significant outlier was determined by counting positive results in the first two runs which are not unconfirmed by the third run, i.e. false positive results. Gender specific cutoffs, 14 ng/L for female and 19 ng/L for male, were applied to identify the positive results. Overall, 2935 patients' results were evaluated during the study.

Results: Based on the analysis of the first two repeat runs, out of 2935 samples 16 samples, or 0.54%, were identified as the statistical outliers. 2 out of these 16 outliers, or 12.5%, were negative results. Taking into consideration the third repeat data, 13 out of 2935 samples, or 0.44%, were identified as false positive results. Only 9 out of 13 false positive results, or 69.2%, were identified using the statistical approach above.

Conclusion: The false positivity rate of hsTnI testing has dropped significantly from 1% to 0.44% in a setting of our laboratory. Our refined repeat protocol approach significantly increases the clinician's confidences in the hsTnI results by capturing potential false positive results. This approach addresses the outliers at an additional expense like reagents, instrument wear and tear, and labor. Therefore, a more robust methodology is still desired by the industry.

References: 1. Pretorius, Carel J et al. "Outliers as a cause of false cardiac troponin results: investigating the robustness of 4 contemporary assays." Clinical chemistry vol. 57,5 (2011): 710-8. doi:10.1373/clinchem.2010.159830 2. Li, Yirong et al. "Rapid identification of falsely elevated serum cardiac troponin I values in a stat laboratory." Laboratory medicine vol. 45,1 (2014): 82-5. doi:10.1309/lmo7hli8eodnazsg

A-003

Point of Care BNP Values Obtained by the Abbott i-STAT are not Interchangeable With Laboratory Determined Values Using Beckman DXI 800 Analyzer

K. Zinn, M. Boyd, A. Dasgupta. *University of Kansas Health System, Kansas City, KS*

Background: B-type natriuretic peptide (BNP) is used as a biomarker of heart failure. In our hospital, point of care (POCT) BNP test is performed on whole blood using the i-STAT (Abbott, Abbott Park, IL) while in clinical laboratory, the DXI 800 analyzer is used (Beckman, Brea, CA) for determination of BNP in EDTA plasma. Sometimes clinicians interchangeably use POCT BNP with laboratory BNP test for monitoring heart failure patients, but values are sometimes significantly different causing diagnostic confusion. We compared POCT BNP values in 88 patients with laboratory-based BNP values analyzed between less than one hour to less than 12 h. In addition, 12 specimens were simultaneously analyzed for POCT BNP and lab-based BNP. No additional blood was drawn from patients and medical record was not accessed. The study had exempt status: by the IRB of University of Kansas Medical Center.

Methods: POCT BNP values were obtained using the i-STAT analyzer following manufacturer's protocol. It is a two-site enzyme-linked immunosorbent assay (ELISA) with an analytical measurement range of 15-5000 pg/mL. In the laboratory BNP concentrations were obtained using the DXI 800 analyzer. It is also a sandwich immunoassay with the analytical measurement range of 5-5000 pg/mL.

Results: Overall comparison using lab-based BNP as x-axis and POCT BNP as y-axis produced the following regression equation; y=1.48 x -23.45 (n = 88, r = 0.96) indicating that although POCT BNP values correlated well with lab-based BNP values, POCT values showed an average 48% positive bias. When specimens analyzed within 4 h were compared, the regression equation was: y = 1.40 x + 74.12 (n = 20, r = 91) and when specimens analyzed within 1 h were compared, the regression equation was: y = 1.56 + 59.0 (n = 23, r = 0.97). In contrast, when 12 specimens were analyzed simultaneously using POCT and lab-based BNP, using x-axis as the lab-based BNP and y-axis as the POCT BNP, the following regression equation was observed: y = 1.83 x - 102.8 (n = 12, r = 0.99).

Conclusion: Higher positive bias in specimens analyzed simultaneously indicates true positive bias with POCT BNP compared to lab-based BNP because lab-based BNP in real patients were ordered later after POCT BNP. To our knowledge i-STAT BNP values were never compared with DXI 800 BNP values. We communicated with our clinicians not to use lab-based BNP test and POCT BNP in monitoring patients being treated for heart failure interchangeably. POCT BNP can be used for initial screening to identify potential patients with heart failure.

A-004

Analytical Assessment And Expanded Stability Studies Of The Abbott Alinity i STAT High Sensitivity Troponin-I Assay

D. C. Kirchhoff, F. K. Arowolo, G. M. Pinero, D. R. Mendu. *The Mount Sinai Hospital, New York, NY*

Background: The Mount Sinai Health System has recently integrated the Abbott Alinity i STAT High Sensitivity Troponin-I Assay (HS-Trop) into its 'Suspected Acute Coronary Syndrome Algorithm'. Prior to doing so, a comprehensive evaluation of the assay was performed. In particular, the manufacturer's specifications state that HS-Trop samples (plasma) are stable for 8 hours at room temperature (RT), and that plasma and red cells must be separated by centrifugation within 30 minutes of draw. This centrifugation time window and RT storage requirements are not practical for most large hospital laboratories. Sample delivery, accessioning, and centrifugation are often greater than 30 minutes, and in most laboratory automation systems, sample storage modules are refrigerated. RT storage would require the removal of the HS-Trop sample from the automation line, and storing the sample elsewhere. This adds additional work to laboratory staff, and makes add-on testing challenging. As such, refrigerated storage and a longer centrifugation time window would be ideal. In this study, we sought to assess the analytical performance of the HS-Trop assay, and validate expanded centrifugation and stability requirements.

Methods: Inter and Intraday precision was assessed through replicate measurements (n=10) of low, mid, and high Technopath Quality Controls. Linearity was determined through triplicate analysis of Maine Standards linearity standards. Accuracy was assessed through split sample (n=40) analysis on two distinct reagent lots. Stability was assessed in centrifuged samples at RT and 4°C through analysis at 0, 4, and 8 hour time points, with an additional 16 hour time point assessed at 4°C. Unspun sample stability was assessed at RT at 0, 2, 4, and 8 hour time points. LoQ was assessed through a sensitivity profile, by performing a serial dilution spanning 1:2 - 1:128, measuring each dilution in triplicate on 10 different days. Reference range verification was performed through the assessment of 20 sex-stratified normal donor samples.

Results: Intraday precision afforded CVs of 5.1%, 5.6%, and 2.9% for the low, mid, and high controls, respectively, while Interday precision at low, mid, and high controls yielded CVs of 4.3%, 6.3%, and 3.3%. Accuracy assessment resulted in a slope of 0.928, y-intercept of 6.613, and R^2 = 0.9943. Linearity was confirmed between 12.3 and 4216.5 ng/mL with a slope of 0.997, y-intercept of 0.261. Spun and Unspun sample stability was confirmed at RT and 4°C for a maximum of 8 hours, affording percent difference values with respect to the initial time point of <20%. Stability studies failed at the 16 hour time point. The LoQ was determined to be 4.4 ng/L at 20% CV. The reference range was verified at: Males, <34, and Females, <18 ng/L.

Conclusions: Our study has shown the Abbott HS-Trop assay performs well analytically, with acceptable precision, accuracy, linearity, and analytical sensitivity for the assessment of HS-Trop. The manufacturer-specified reference range was confirmed.

Our study has validated and confirmed an expanded stability and centrifugation window, which will allow for the easy integration of the assay into large hospital networks, and for its use in many laboratory automation systems.

A-006

Evaluation of the ADVIA Centaur NT-proBNPII Assay Versus Standard-of-care Natriuretic Peptide Testing in the Emergency Department

J. Snyder[1], S. Bermel[1], C. Brown[2], C. Cooper[2]. *[1]Siemens Healthineers, Newark, DE, [2]Siemens Healthineers, Tarrytown, NY*

Background: The Siemens Healthineers ADVIA Centaur® NT-proBNPII (PBNPII) assay* was developed for in vitro diagnostic use in the quantitative determination of N-terminal pro-brain natriuretic peptide (NT-proBNP) in human serum and plasma (EDTA and lithium heparin) using the ADVIA Centaur XP Immunoassay System. Siemens Healthineers performed a clinical study to evaluate the ADVIA Centaur XP PBNPII assay in an Emergency Department (ED) patient population. The study also involved collection of standard-of-care (SOC) natriuretic peptide (NP) results, either NT-proBNP or BNP. **Objective:** To compare results from the ADVIA Centaur PBNPII assay in ED populations with SOC NP results and adjudicated diagnosis of heart failure. **Methods:** 3128 subjects were enrolled from 30 ED sites. Blood was collected at time of enrollment for subsequent ADVIA Centaur PBNPII measurement; results from SOC NP testing were obtained from chart review. Available SOC testing was performed within 24 hours of enrollment draw for all except four patients. Adjudication of acute HF (de novo or worsening) or non-acute HF was determined by an independent central adjudication panel of expert clinicians. **Results:** Of the 3128 patients in the ED study, 1646 (52.6%) had an SOC BNP test performed, 1020 (32.6%) had an SOC NT-proBNP test performed, and 462 (14.8%) had neither. Agreement between the SOC NP results and PBNPII results was assessed based on concordance using cutoffs described in heart failure guidelines that include an indeterminate zone.[1] For those tested with SOC NT-proBNP, there was 94.7% concordance, 5% partial concordance (indeterminate by one assay and positive or negative by the other), and 0.3% discordance. For those tested with SOC BNP, there was 73.8% concordance, 25.5% partial concordance, and 0.7% discordance. Agreement with adjudication was virtually identical for both the PBNPII assay and SOC PBNP at 63% concordance, 17% partial concordance, and 20% discordance. For those tested with SOC BNP, there was 63% concordance, 29.5% partial concordance, and 7.5% discordance, while in this same population the PBNPII assay had 67.8% concordance, 15.7% partial concordance, and 16.5% discordance. **Conclusion:** Upon comparison of ADVIA Centaur PBNPII assay results to SOC NP testing in an ED population, Siemens Healthineers concludes that the ADVIA Centaur PBNPII assay performs similarly to commercial natriuretic peptide assays in routine use. These results agree with publications comparing diagnostic testing of NT-proBNP and BNP for suspected heart failure.[2] **References**1.Mueller C, McDonald K, de Boer RA, Maisel A, Cleland JGF, Kozhuharov N, Coats AJS, Metra M, Mebazaa A, Ruschitzka F, Lainscak M, Filippatos G, Seferovic PM, Meijers WC, Bayes-Genis A, Mueller T, Richards M, Januzzi JL Jr; Heart Failure Association of the European Society of Cardiology. Heart Failure Association of the European Society of Cardiology practical guidance on the use of natriuretic peptide concentrations. Eur J Heart Fail. 2019 Jun;21(6):715-31.2.Farnsworth CW, Bailey AL, Jaffe AS, Scott MG. Diagnostic concordance between NT-proBNP and BNP for suspected heart failure. Clin Biochem. 2018 Sep;59:50-55.*Disclaimer: The products/features mentioned herein are not commercially available in all countries. Their future availability cannot be guaranteed.

A-007

Cardiomyocyte-monocyte cross-talk exploits cellular stress pathways leading to myocyte apoptosis in dilated cardiomyopathy of idiopathic origin

K. Sikder[1], D. Talukdar[1], A. Haldar[1], R. Dastidar[2], S. Kumar[3]. *[1]Ramakrishna Mission Vivekananda Educational and Research Institute, Kolkata, India, [2]Ramakrishna Mission Seva Pratishthan hospital and Nirnayan health care pvt. ltd., Kolkata, India, [3]Vivekananda Institute of Medical Sciences, Kolkata, India*

Background: Dilated cardiomyopathy (DCM) is the most prevalent form of cardiovascular diseases (CVDs) results in dilation and disruption of pumping mechanism of heart. Most strikingly, about 50% DCM cases do not have any identified aetiology, hence known as idiopathic DCM (IDCM). The aim of this study is to investigate major inflammatory markers and cellular stress pathways in the circulating leukocytes and cardiomyocytes to find out the cross-talk between these cell types in vitro condition by mimicking the in vivo inflammatory milieu.

Methods: The study is divided into two parts, first part comprising patient study and the second part is the in vitro cell culture based mechanistic study. 50 IDCM patients and 30 age and sex matched control subjects were recruited from our in-house Ramakrishna Mission Seva Pratishthan (RKMSP) hospital based on their NYHA discomfort criteria followed by intra thoracic echocardiography. Exclusively the IDCM subjects were selected for this study. Blood was collected from the patients for biochemical and inflammatory parameters and to isolate PBMCs. mRNA and protein expressions were studied by real time PCR and immunoblotting respectively. H9C2 cardiomyocyte cells alone or with LPS-activated THP-1 monocyte/macrophage line were co-cultured in presence of the major inflammatory cytokines in the same in vivo concentration as found in the IDCM patients in presence or absence of ER stress agonist or antagonists. Immunofluorescence, RT-PCR and immunoblotting were done for markers specific to myocyte stress, inflammation and apoptosis. Results: Inflammatory and cardiac blood biomarkers NLR, hs-CRP, IL-6, pro-BNP and troponin-I were found to be significantly high ($P<0.05$) in IDCM. Subsequently, the mRNA expressions of pro-inflammatory genes TNF-α (2.6 fold), IFN-γ (10.2 fold), IL-1β (8.5 fold) and NF-κβ (2.427 fold) were all significantly increased ($P<0.05$). Looking into the cellular stress pathways, we further found upregulations of antioxidant genes SOD (2.59 fold) and catalase (2.17 fold) and ER stress markers Grp78 (2.794 fold), CHOP (3.407 fold), IRE1-α (2.104 fold) and ATF-6 (40.618 fold) mRNA expression in the PBMCs. When we stimulated the H9C2 cardiomyocytes with IL-1B and TNF- α at comparable conc. as found in the IDCM patients, the ER stress and inflammation were observed. Further, when we co-cultured these H9C2 with LPS stimulated THP-1 macrophages an exaggerated inflammation and myocyte stress was observed that led to apoptosis of the cells. Interestingly using ER stress inhibitor TUDCA, remarkably blocked the apoptosis and promotes cell survival. Conclusion: Our results showed that a systemic inflammation caused by stressed PBMCs leads to cardiac deterioration and dilation in the unknown DCM cases. So far we have established that an exaggerated inflammation induce the circulating PBMCs to migrate and infiltrate the cardiac musculature where they induce oxidative stress and ER stress to the cardiomyocytes leading to apoptosis of the later. In long run these unknown forms of dilated cardiomyopathy patients suffer from a dilated heart due to a decline in the myocyte population. Our findings may warrant for a treatment strategy that alleviate the intracellular stress of the circulating leukocytes and cardiomyocytes to minimize the inflammatory changes of the heart.

A-008

Comparison of Roche 5th generation Troponin T analytical outlier rates between the e411 analyzer using a biotin-sensitive reagent and the e601 analyzer using reformulated (biotin-insensitive) reagent

B. R. Kelley, A. M. Wockenfus, B. M. Katzman, G. M. Spears, B. S. Karon, A. S. Jaffe, L. J. Donato. *Mayo Clinic, Rochester, MN*

Background Analytical outliers (fliers) occur with most troponin methods and can adversely affect patient management. We compared the analytical outlier rates for the Roche 5th generation Troponin T STAT (cTnT) reformulated (biotin-insensitive) reagent using a Cobas e601 analyzer (Indianapolis, IN) to rates observed in a previous study using the biotin-sensitive reagent on a Cobas e411 analyzer. **Methods** Plasma separator tubes (PST) were collected from hospital patients with orders for clinical cTnT testing. PST samples were centrifuged for 3 minutes at 4000 x g and analyzed using the biotin-insensitive cTnT Gen 5 assay on a Roche Cobas e601 immunoassay system. If samples displayed measurable cTnT Gen 5 (≥6 ng/L), samples were stored at 2-8°C within 2 hours of initial measurement (N=2555). Within 24 hours of initial analysis, samples were warmed to room temperature, aliquoted, re-centrifuged at 4000 x g for 3 minutes, and cTNT was re-measured on the same analyzer. Outlier rates were compared to a previous study (N=1997) using nearly identical study design but using biotin-sensitive Roche 5th Gen cTnT reagent on an e411 analyzer. We defined analytical outliers by three methods:•Repeat value (after aliquoting and re-centrifugation) that differed from the initial value for all samples with measurable cTnT by a critical difference (CD): where $CD = z \times \sqrt{2} \times SD_{analytical}$, with a z-value of 3.719 and using SD from best fit line of precision vs cTnT concentration. Precision experiments were performed using four plasma pools in 5x5 precision experiment on the e411 analyzer.•Using the same CD but considering only initial cTnT values < 100 ng/L, those are most likely to be clinically significant or lead to misinterpretation.•Using CD for initial values < 100 ng/L, and a fixed 10% difference between replicates for initial values ≥ 100 ng/L, accounting for our practice of reporting the cTnT "delta" in percent (rather than absolute) for values ≥ 100 ng/L. •Comparisons of outlier rates between the biotin-sensitive and biotin-insensitive reagents were performed using

Pearson's chi-squared test. **Results** Using the CD the total number of outliers that were observed for the biotin-insensitive reagent on the e601 was 208/2555 (8.1%), compared to 68/1997 (3.4%) on the biotin-sensitive reagent measured on the e411 ($p<0.0001$). Outliers were observed in 138 (6.4%) of samples with initial values < 100 ng/L using the biotin-insensitive reagent on the e601 compared to 45 (2.6%) of such samples on the e411 ($p<0.0001$). Using the Z score method to define outliers for initial values <100 ng/L and a fixed 10% to define outliers for initial values ≥ 100 ng/L, the outlier rate was 151 (5.9%) for the biotin-insensitive reagent on the e601 compared to 53 (2.7%) on the e411 ($p< 0.0001$). **Conclusions** Analytical outliers occurred more frequently using the reformulated (biotin-insensitive) reagent on the Roche Cobas e601 analyzer, compared to outlier rates observed for biotin-sensitive reagent on the Cobas e411.

A-009

Analytical Concordance of High-Sensitivity Cardiac Troponin I and T Assays in Patients From the CONTRAST Trial Presenting to an Emergency Department

I. L. Gunsolus[1], K. M. Schulz[2], Y. Sandoval[3], S. W. Smith[4], F. S. Apple[4]. *[1]HealthPartners, Bloomington, MN, [2]Hennepin Healthcare Research Institute, Minneapolis, MN, [3]Minneapolis Heart Institute and Abbott Northwestern Hospital, Minneapolis, MN, [4]Hennepin Healthcare/Hennepin County Medical Center & University of Minnesota, Minneapolis, MN*

Background: There are limited data on the analytical concordance of high-sensitivity cardiac troponin (hs-cTn) T and hs-cTnI assays. The goal of this study was to evaluate the concordance of one hs-cTnT and one hs-cTnI assay relative to each assay's limit of quantitation (LoQ) and 99th percentile upper reference limit (URL).

Methods: The Comparison of Troponin I and T Assays Trial (CONTRAST) NCT03214029 is an observational study that evaluated the concordance of two FDA-cleared hs-cTn assay concentrations measured at presentation (0h) and 2h by the Abbott ARCHITETCT i2000 hs-cTnI (clinically used assay) and Roche cobas 601 Gen 5 hs-cTnT (measured for investigational purposes) from consecutively enrolled patients presenting to an urban US medical center with symptoms suggestive of ischemia. Concordance at presentation (0h) was assessed relative to each assay's FDA-cleared reporting concentration (LoQ), and FDA-cleared sex-specific 99th percentile URLs. The incidence of myocardial injury, defined as at least one concentration above the 99th percentile URL over serial sampling, was also evaluated.

Results: At presentation hs-cTn concentrations <LoQs (Abbott 3.5 ng/L, Roche 6 ng/L) were 834/2270 (37%) and 530/2270 (23%), respectively. The concordance of results <LoQ was 81%. The Abbott assay showed a higher percent of concentrations below both female and male URLs than did the Roche assay. For females, concentrations below the sex-specific 99th percentile URLs were 707/945 (75%) for Abbott hs-cTnI (16 ng/L) and 545/945 (58%) for Roche hs-cTnT (14 ng/L, $p < 0.03$). For males, concentrations below the sex-specific 99th percentile URLs were 1100/1325 (83%) for Abbott hs-cTnI (34 ng/L) and 900/1325 (68%) for Roche hs-cTnT (22 ng/L). The concordance of results below the sex-specific 99th percentile URLs for both assays was 80%. Among males with presentation concentrations <URL, the incidence of myocardial injury (hs-cTn >URL at 2h) was approximately two-fold greater for the Roche hs-cTnT (59/900, 6.2%) than Abbott hs-cTnI (31/1110, 2.8%). Females with presentation concentrations <URL showed similar rates of myocardial injury at 2h for the Roche (24/545 [4.4%]) and Abbott assays (36/707 [5.1%]).

Conclusion: We report a 20% discordance between Abbott hs-cTnI and Roche hs-cTnT assays in detecting myocardial injury at respective 99th percentile URLs and at each assays' FDA allowed LoQ reporting limit.

A-010

Systemwide implementation of high sensitivity Troponin T in a multi-center hospital set up with instrument variability: the Baystate Health experience

M. Pandiri[1], D. Kotorobay[2], S. Ibrahim[1], J. Rogers[2], A. Paz[2], R. Moccio[3], G. Ouimet[3], D. Fleischmann[4], J. Paadam[5], R. Baevsky[6], A. Lotfi[7], R. Quarles[8], S. Gemme[6], V. Johari[1], M. A. Barbhuiya[9]. *[1]Department of Pathology, UMass Chan Medical School-Baystate, Springfield, MA, [2]Department of Pathology, Baystate Medical Center, Springfield, MA, [3]Division of Laboratory Informatics, Baystate Health, Springfield, MA, [4]Division of Clinical Informatics, Baystate Health, Springfield, MA, [5]Division of Clinical Informatics, Baystate Health; Department of Medicine, UMass Chan Medical School-Baystate, Springfield, MA, [6]Department of Emergency Medicine, UMass Chan Medical School-Baystate, Springfield, MA, [7]Division of Cardiology, Department of Medicine, UMass Chan Medical School-Baystate, Springfield, MA, [8]Division of Internal Medicine, Department of Medicine, UMass Chan Medical School-Baystate, Springfield, MA, [9]Department of Pathology; Healthcare Delivery and Population Sciences, UMass Chan Medical School-Baystate, Springfield, MA*

Background: High-sensitivity cardiac troponin (hs-cTn) testing helps in rapid risk stratification in patients with acute coronary syndromes resulting in early identification of low-risk patients eligible for early discharge. A high sensitivity troponin test can measure the 99th percentile upper reference limit with an analytical imprecision ≤ 10 % and it can measure cTn above the limit of detection in ≥ 50% of healthy subjects. We carried out method validation of the Elecsys Gen 5 hsTnT assay with variable instrument capacities like Roche cobas e801, e601, and e411 in our health system. The analytical variability of these instruments was studied and standardized to implement the Gen 5 hsTnT assay systemwide for clinical use in four hospitals.

Methods: The cTnT control samples with low, medium, and high concentrations were used for precision studies (N=20). The calibration materials at five levels ranging from 8 ng/L to 10000 ng/L were used for evaluating linearity, accuracy, and analytical measurement range. The observed and assigned values were compared using linear regression analysis. Heparinized plasma samples were tested using the Gen 5 hsTnT assay. Method comparisons were carried out on different Roche cobas analyzers in each hospital splitting remnant specimens. Analyzer e801 performance was compared with a reference laboratory. Simultaneously, the e601 and e411 analyzers were compared with the e801. Statistical analysis was carried out using EP Evaluator and GraphPad Prism.

Results: The Roche e801 demonstrated imprecision of 0.5%- 2.2% (N=20), the e601 had imprecision of 0.4%- 1.4% (N=20) and the e411 had 0.8%- 4.8% (N=20) across the three levels of control samples tested. These were well below the expected imprecision criteria of ≤10 % for troponins. Deming regression analysis of the 5th GEN assays showed linear relationship across platforms: y (e801 5th GEN)= 1.007x (Reference Lab 5th GEN)+50.65, R=0.9899 with positive bias of 8% (N=45); y (e601 5th GEN) = 0.929x(e801 5th GEN)+ (-1.426), R=0.9995 with negative bias of 7% (N=48); y (e411 5th GEN) = 0.942x(e801 5th GEN)+ (-7.044), R= 1.000 with negative bias of 7% (N=49). The accuracy, reportable range, and linearity of Gen 5 hsTnT were tested on e801, e611 and e411 analyzers over a measured range of 8 ng/L to 10000 ng/L. Allowable systematic error (SEa) was 1.5 ng/L (conc) or 5.0% (e801); 1.5 ng/L (conc) or 7.5% (e611 and e411). The maximum deviation for a mean recovery from 100% was 2.4% (e801), 2.7% (e601) and 6.2% (e411). The 5 of 5 mean recoveries were accurate within the SEa for all the three analyzers. The 15 out of 15 results were accurate within the allowable total error (TEa) of 3 ng/L (conc) or 10.0% (e801), 3 ng/L (conc) or 15.0% (e601 and e411). The three analyzers passed reportable range and accuracy tests, and the results were linear.

Conclusion: The TEa was determined from instrument specific imprecision, systemwide medical decision limits and parameters of rule out criteria. The standardization of analytical performance characteristics is important in a large health system with instrument variability and provision of transfer of care across health systems.

A-011

High sensitive troponin assays for differentiating the patients with unstable angina from stable angina

Y. Kim[1], H. Cho[2], S. Kim[2], H. Lee[3], H. Kwon[3], J. Lee[4]. *[1]The Catholic University of Korea, St Vincent's Hospital, Suwon, Korea, Republic of, [2]The Catholic University of Korea, St. Vincent's Hospital, Suwon, Korea, Republic of, [3]The Catholic University of Korea, Seoul, Korea, Republic of, [4]The Catholic University of Korea, Eunpyeong St. Mary's Hospital, Seoul, Korea, Republic of*

Background: There have been no cardiac biomarkers used in the diagnosis of unstable angina. In this study, we examined high sensitive troponin T and I as a marker to differentiate the patients with unstable angina from the patients with stable angina. **Methods:** A total of 816 patients (59 AMI, 94 unstable angina, 99 stable angina, 623 healthy individuals) were included. All patients had glomerular filtration rate of 60 ≥ml/min/1.73m^2. Leftover serum samples were obtained from the patients who visited The Catholic University of Korea, St Vincent's Hospital from April to November, 2019. Cardiac troponin was measured using Beckman Coulter Access high sensitivity cTnI assay, Abbott Architect high sensitive TnI assay, and Roche Elecsys Troponin T-high sensitive assay. **Results:** The 99th percentile URLs (Upper Reference Limits) of Beckman Coulter cTnI, Abbott cTnI, and Roche cTnT are 9.8 ng/L, 17.9 ng/L, and 16.4 ng/L, respectively. In the 99 patients with stable angina, 9.1% (9/99), 5.1% (5/99), and 6.1% (6/99) of the patients showed higher values than each the 99th percentile URL with Beckman Coulter cTnI, Abbott cTnI, and Roche cTnT, respectively. In the 94 patients with unstable angina, 24.5% (23/94), 24.5% (23/94), and 25.5% (24/94) showed higher values than each the 99th percentile URL with Beckman Coulter cTnI, Abbott cTnI, and Roche cTnT, respectively. There are significant differences between the frequencies of the patients with stable angina and unstable angina showing higher values of cardiac troponins than each the 99th percentile URL. **Conclusion:** High sensitive troponins could be a useful marker for the differentiation of the patients with unstable angina from stable angina.

A-012

Novel method for determining the concentration of total N-terminal pro-hormone BNP (NT-proBNP) in a sample with mass spectrometric detection

M. Jaquemar, T. Knüttel, L. Thi Tran, J. Weikum, V. Schmelck, T. Nilsen, A. David. *Gentian AS, Moss, Norway*

Background:

NT-proBNP, the N-terminal fragment of the hormone B-type natriuretic peptide (BNP), is a useful clinical biomarker for diagnostic and prognostic evaluation of heart failure (HF). Recent studies show that commercially available NT-proBNP assays underestimate concentrations of NT-proBNP due to the usage of antibodies targeting the glycosylated central region of the NT-proBNP molecule. With this, a clinical need arises to establish a robust and reliable assay method which can measure the total amount of NT-proBNP, independent of the glycosylation status of the molecule.

Methods:

To determine the concentration of NT-proBNP in patient samples, a known quantity of labelled NT-pro-BNP was added to each sample as standard. The capture of the analyte (native and standard) was performed by Dynabeads™ M-270 Epoxy beads coated with polyclonal goat antibodies, targeting non-glycosylated sites of the NT-proBNP molecule. Magnetic beads were added to the sample and incubated overnight. The isolated analytes were trypsinated directly on the beads after treatment for denaturation by unfolding and oxidation/alkylation. The reaction was stopped by addition of formic acid and the tryptic peptides were cleaned on C18 SPE columns, dried by SpeedVac and resuspended in acetonitrile for separation and analysis on LC-MS/MS (Agilent 1200 Series LC/MS system coupled to an Agilent G6490 triple quadrupole mass spectrometer).

Results:

The Gentian LC-MS/MS validation was conducted including five studies such as Linearity, LoQ, Trueness, Precision and Carry-Over. All studies passed our defined acceptance criteria. To elucidate how glycosylation influences measurements of NT-proBNP, 81 plasma and serum samples covering a concentration range from 100 ng/L to 25000 ng/L were measured by the Gentian LC-MS/MS method and the Roche Elecsys® proBNP I assay. Within the clinically relevant range of 100 - 2000 ng/L, the results between both methods exhibit a linear correlation of R2 = 0.97 and show that the commercial assay consistently underestimates the concentration of NT-proBNP by an average factor of 4.3 (3.5 - 5.8). Furthermore, results reveal that the concentration measurements between the commercial assay and the LC-MS/MS method converge at higher NT-proBNP concentrations. Method comparison was repeated using the Siemens ADVIA Centaur® and the Siemens IMMULITE Immunoassay systems, confirming the results of underestimation of NT-proBNP in clinically relevant areas. For method robustness investigations, the LC-MS/MS method was set up and used at two geographically different sites by different operators, using two different mass spectrometry systems (Agilent 1200 with triple quadrupole and the nano-LC-ESI-Orbitrap from Thermo Fisher Scientific), resulting in CVs from 13% to 2% for NT-proBNP concentrations ranging from 220 ng/L to 9700 ng/L.

Conclusion:

Gentian AS presents a robust and reliable mass spectrometry method, capable of detecting the total amount of NT-proBNP in a sample, independent of its degree of glycosylation. This method is suitable for serving either as a reference method for calibrating and ensuring the accuracy of existing methods, harmonization of existing methods, or as a novel method in clinical chemistry for accurately determining the concentration of total amount of NT-proBNP.

A-013

Creation of a Commutable Cardiac Troponin I Reference Material Coined 8121 by the National Institute for Science and Technology and the IFCC Workgroup for Cardiac Troponin I Standardization

R. H. Christenson[1], S. Duh[1], R. Payne[2], S. Clapshaw[3], M. S. Lowenthal[4]. *[1]University of Maryland School of Medicine, Baltimore, MD, [2]Siemens Healthcare, Tarrytown, NY, [3]Solomon Park Research Laboratories, Inc., Burien, WA, [4]National Institute of Standards and Technology, Gaithersburg, MD*

Background: Harmonizing cardiac troponin I (cTnI) is needed because this biomarker is central to defining cardiac injury, and is essential for diagnosis and management of myocardial infarction (MI) and other cardiac conditions. cTnI measurements have evolved through several generations; state-of-the-art assays are coined "high-sensitivity cTnI" tests. We describe development and availability of commutable Reference Material (RM)8121, designed and created by the National institute of Standards and Technology (NIST) and the IFCC workgroup for cTnI standardization for availability to stakeholders. **Methods:** RM8121 is a lithium heparinized series of four Levels of increasing cTnI concentration coined Levels A (lowest) to D (highest). Each RM8121 concentration resulted from combining an elevated cTnI pool from sample collections from 58 MI patients with a blending pool comprised of low cTnI samples from 15 healthy females and 15 males, all <40-years old. All subjects donating collections were consented under an IRB-approved protocol at the University of Maryland. All enrolled subjects were confirmed negative for hepatitis A,B,C and HIV infection. Hemolyzed, lipemic and icteric samples were excluded from pooling. RM8121's Levels A-D were homogenized, and 0.71 mL aliquots pipetted into 2-mL vials. hs-cTnI concentrations were measured at critical points throughout the recruitment, sample collection, blending and final aliquoting processes using the Atellica® IM TnIH assay (Siemens Healthineers). **Results:** Approximately 3000 units of RM8121 are available. The table displays the hs-cTnI concentrations of the blending and high pools. Concentrations of RM8121 Levels A and B were designed to bracket the female and male 99th percentile URLs, with RM8121 Levels C and D in the range of 9-fold and 100-fold greater than the female 99th URL, respectively. **Conclusion:** Values for FDA cleared hs-cTnI assays will be assigned for RM8121. Availability of this commutable cTnI RM is intended to assist in harmonizing current and future hs-cTnI assays.

Description	Purpose	Atellica IM TnIH, ng/L	Relative Conc. to Female URL = 34 ng/L	H-Index
TnI Negative Pool	Blending Pool	<2.8	NA	1
TnI Positive Pool	Elevated cTnI pool	10 443	307.2-fold URL	1
LEVEL A	RM 8121-A	17.1	0.50-fold URL	1
LEVEL B	RM 8121-B	76.6	2.3-fold URL	1
LEVEL C	RM 8121-C	299	8.8-fold URL	1
LEVEL D	RM 8121-D	3427	100.8-fold URL	1

A-014

Analytical Outlier Occurrence and False Positivity Rate of the Beckman Coulter Access High-sensitivity Cardiac Troponin I Assay

J. H. Noguez[1], L. Delfin Mendez[2], K. Kulp[1], X. Zhang[1], L. Stempak[1]. *[1]University Hospitals of Cleveland, Cleveland, OH, [2]Case Western Reserve University School of Medicine, Cleveland, OH*

Background: Cardiac troponin (cTn) is the preferred biomarker for the diagnosis of acute myocardial infarction due to its high sensitivity and specificity relative to other available cardiac markers. Accurate measurement is therefore paramount to proper risk stratification of patients. Analytical outliers and false positive results have been previously reported on various platforms though the exact cause remains unknown. These false positive test results may pose a risk for the misclassification of patients and lead to adverse clinical outcomes. We evaluated the performance of the Beckman Coulter Access high-sensitivity cTn I method by measuring the occurrence of analytical outliers between duplicate results and the false positivity rate (i.e. fliers).

Methods: We tested 2,535 samples from 1,879 patients in duplicate on the Beckman Coulter Access hs-cTn I assay. These specimens had initial troponin measurements above the gender-specific 99th percentile of the upper reference limit (URL) cutoff and were immediately aliquoted, re-centrifuged, and re-tested. Non-reproducible results (outliers) were defined as having a concentration difference between the replicates exceeding 10%. Critical outliers were defined as duplicate results with concentrations that differed by greater than 10% from the initial result and the second result was classified in a lower risk stratification category than the original result.

Results: Approximately 12% (305) of the positive samples tested in duplicate had a percent difference that was >10% and therefore did not meet the recommended precision requirements of the assay. Of these specimens, 33 (10.8%) were classified as critical outliers because the repeat test result crossed a critical decision limit threshold. For sixteen samples the risk classification for the patient was downgraded from high (>50 ng/L) to moderate risk (13-50 ng/L (F); 20-50 ng/L (M)). Seventeen false positive results were identified in which the patients were downgraded from the high to low risk category (<13 ng/L (F); <20 ng/L (M)). Nearly 85% of the samples identified as outliers were lithium heparinized plasma.

Conclusion: We evaluated the reproducibility of the Beckman Coulter Access hs-cTn I method across 11 hospitals in an integrated healthcare network to characterize the occurrence of analytical outliers and false positive results. Our data indicated an outlier rate of 12% (305/2,535) for positive hs-cTn I results; however, the risk category only changed for 1.3% (33/2535) of positive troponin results upon repeat analysis. Overall the false positivity rate was determined to be <0.1% (n= 55,560).

A-015

Hemoglobin A1c control is an independent predictor of circulating troponin concentrations using machine learning

H. M. Brown, N. C. Spies, M. A. Zaydman, C. W. Farnsworth. *Washington University in St. Louis School of Medicine, St. Louis, MO*

Background Diabetes mellitus (DM) is associated with multiple comorbidities that may precipitate cardiac damage and elevated cardiac troponin (cTn) concentrations including renal failure, hyperlipidemia (HLD), hypertension (HTN), coronary artery disease (CAD), and congestive heart failure (CHF). Diabetics >70 years old have a higher likelihood of adverse outcomes including major cardiovascular events and cardiac mortality. However, the risk to younger diabetic patients and the precipitating factor(s) that facilitate cardiac injury in those with DM are unknown. The objective of this study was to investigate factors predictive of cTn concentrations in young, relatively healthy diabetic patients. **Methods** We collected 1,533 remnant plasma samples from outpatients between 06/22-09/22 with physician ordered hemoglobin A1c testing. cTn was measured using the Abbott ARCHITECT High Sensitivity Troponin-I assay (limit of detection = 1.7 ng/L, imprecision = 4.76% at 50 ng/L). Demographic information (sex, race, BMI) and pertinent medical history (diabetes, HTN, HLD, CAD, CHF) were collected from the electronic medical record, along with estimated glomerular filtration rate (eGFR) and hemoglobin A1c. Exclusion criteria included: patients with missing laboratory data, those undergoing cancer treatment, or a history of myocardial infarction/cardiomyopathy/cardiac surgery. Troponin results were classified as normal- or high-risk using a cut-off of 10 ng/L for females and 12 ng/L for males, thresholds previously shown to correlate with incidence of cardiovascular disease risk within 15 years. Univariate statistics were calculated using Wilcoxon rank sum tests. An XGBoost model was trained to predict high-risk troponinemia, and summary statistics were calculated on a held-out test set. **Results** Of the 1,135 patients that met inclusion criteria, 621 (54.7%) were female. The median age was 60 years (IQR: 49-69 years) and the median A1c was 6.2% (IQR: 5.8-7.0%). A total of 746 patients (65.7%) had a prior diabetes diagnosis, with 156 patients (13.7%) having prediabetes, 42 patients (3.7%) having Type 1, and 548 patients (48.3%) having Type 2. Median troponin concentration for patients with an A1c <5.7% was 1.6 ng/L (IQR: 0.8-3.4), 5.7-6.4% was 2.2 ng/L (IQR:1.3-4.8), and ≥6.5% was 2.9 ng/L (IQR: 1.6-6.7). Univariate analysis demonstrated significant differences in troponinemia by age (CI of difference: 4.4-8.9 years), A1c (0.2-0.7%), and eGFR (28-38mL/min/1.73m^2). The machine learning model demonstrated strong predictive capacity (sensitivity: 0.74, specificity: 0.86, PPV: 0.5, NPV: 0.92, area under ROC curve: 0.91). The features with the greatest impact on area under the ROC curve when removed were eGFR, Age, A1c, and CHF. **Conclusion** A combination of clinical and laboratory variables can be used to predict circulating troponin concentrations in outpatients. A1c control was an independent contributor to cTn concentrations, both in univariate and multivariate analyses. This study provides evidence that glucose control may be associated with cardiac damage and future cardiovascular events, warranting longitudinal outcome studies.

A-016

Clinical Performance Comparison Between the Vitros Immunodiagnostic Products NT-proBNP II and Roche Elecsys proBNP II Assays to Aid in the Diagnosis of Heart Failure

A. Ott-Vasconi[1], P. Ajongwen[1], S. Alvey[1], J. Herman[2]. *[1]QuidelOrtho, Raritan, NJ, [2]QuidelOrtho, Rochester, NY*

Background The International Collaborative of NT-proBNP (ICON) study established NT-proBNP levels for the diagnosis and exclusion of acute heart failure for patients presenting to the emergency department (ED). Following the development of the Vitros Immunodiagnostic Products NT-proBNP II assay, a multi-center prospective study was conducted at 20 EDs across the United States. The study enrolled subjects presenting with dyspnea and clinical suspicion of heart failure (HF). Clinical performance of the Vitros Immunodiagnostic Products NT-proBNP II assay using the cutoffs established in the ICON study was compared to that of the Roche Elecsys proBNP II assay using samples from the multi-center study. **Methods** Clinical sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) were determined by comparing the assay result interpretation using the corresponding cutoffs against the adjudicated diagnosis of HF/non-HF. Clinical performances were then compared between the VITROS NT-proBNP II and the Roche Elecsys proBNP II assays. Analyses were performed using both the age-independent and age-dependent cutoffs within and across age groups. **Results:** After applying the rule-in cutoffs from the ICON study to the Vitros NTproBNP II and the Roche Elecsys proBNP II assay results, the clinical performance of the Vitros NT-proBNP II assay was comparable to that of the Roche Elecsys proBNP II assay (see Table 1).

Table 1: Clinical Performance Characteristics Within and Across Age Groups: Three Rule-in Cutoffs

Age Group (Years)	Cut-off (pg/mL)	Sensitivity (%) (n/N)	95% CI* (%)	Specificity (%) (n/N)	95% CI* (%)	NPV (%) (n/N)	95% CI* (%)	PPV (%) (n/N)	95% CI* (%)
Vitros NT-proBNP II									
22 - < 50 N=226	450	91.92 (91/99)	84.70 -96.45	87.40 (111/127)	80.35 - 92.62	93.28 (111/119)	87.18 - 97.05	85.05 (91/107)	76.86 - 91.20
50 - < 75 N=1066	900	88.19 (433/491)	85.00 -90.91	81.74 (470/575)	78.33 - 84.81	89.02 (470/528)	86.03 - 91.55	80.48 (433/538)	76.88 - 83.75
≥ 75 N=732	1800	83.93 (350/417)	80.05 -87.33	81.90 (258/315)	77.20 - 86.00	79.38 (258/325)	74.57 - 83.65	86.00 (350/407)	82.24 - 89.22
All Subjects*** N=2024	**	86.79 (874/1007)	84.54 -88.82	82.50 (839/1017)	80.02 - 84.79	86.32 (839/972)	83.99 - 88.42	83.08 (874/1052)	80.67 - 85.30
Roche Elecsys proBNP II									
22 - < 50 N=226	450	92.93 (92/99)	85.97 - 97.11	82.68 (105/127)	74.96 - 88.81	93.75 (105/112)	87.55 - 97.45	80.70 (92/114)	72.25 - 87.49
50 - < 75 N=1066	900	87.78 (431/491)	84.55 - 90.54	81.91 (471/575)	78.52 - 84.98	88.70 (471/531)	85.70 - 91.27	80.56 (431/535)	76.95 - 83.83
≥ 75 N=732	1800	84.41 (352/417)	80.57 - 87.76	84.13 (265/315)	79.61 - 87.98	80.30 (265/330)	75.60 - 84.46	87.56 (352/402)	83.93 - 90.63
All Subjects*** N=2024	**	86.89 (875/1007)	84.65 - 88.92	82.69 (841/1017)	80.23 - 84.97	86.43 (841/973)	84.12 - 88.52	83.25 (875/1051)	80.86 - 85.46
*95% Exact confidence interval		**Using the three age-dependent rule-in cutoffs (450 pg/mL for 22 - < 50 years old, 900 pg/mL for 50 - < 75 years old, 1800 pg/mL for ≥ 75 years old)				***Combining all age groups			

Clinical performance was also comparable using the age-independent ruleout cutoff (300 pg/mL) for both assays. The sensitivity and NPV of the Vitros NT-proBNP II assay to ruleout HF were higher compared to those of the Roche Elecsys proBNP II assay, 99.01% versus 96.23% and 98.21% versus 93.41%, respectively. **Conclusion** The use of the ICON cutoffs resulted in high assay specificity to rulein HF and excellent sensitivity to ruleout HF. The Vitros NT-proBNP II assay demonstrated comparable clinical performance to the Roche Elecsys proBNP II assay.

A-017

Analytical and clinical performance evaluation of high sensitivity Cardiac Troponin I hs-cTnI measurement through point of care

R. F. Paula[1], C. E. Ferreira[2], F. V. Paladino[2], T. C. Lamounier[1], H. P. Silva[1], S. R. Gomes[2], B. B. Caretta[2], P. R. Ferreira[2], T. P. Mota[2], M. B. Melo[2], F. G. Pitta[2], A. D. Midrigal[1]. *[1]Siemens Healthineers, São Paulo, Brazil, [2]Hospital Israelita Albert Einstein, São Paulo, Brazil*

Background: Most cases of death from Acute Myocardial Infarction (AMI) occur in the first hours after the manifestation of clinical symptoms, constituting 40 to 65% in the first hour and approximately 80% in the first 24 hours. For the safe, effective, and rapid diagnosis of a patient with suspected AMI, international guidelines recommend that it be done based on clinical symptoms, together with electrocardiogram and analysis of biomarkers in the context of suspected of non–ST-elevation myocardial infarction (NSTEMI). Troponins are the biomarkers of choice with level A level evidence and the ability to perform screening in patients complaining of chest pain and refer it to the correct clinical approach based on the 0h/1h algorithm. To date, the successful clinical implementation of these rapid algorithms is restricted to hospitals working with well-defined workflows and a robust central laboratory structure. The Atellica® vTLi analyzer is based on the chemiluminescence methodology for the quantification of hs-cTnI levels with low sample volume, low complexity, and may enable the rapid management of patients with chest pain and application of 0h/1h algorithm. **Objectives:** Analytical and clinical performance evaluation of high-sensitivity Cardiac Troponin I (hs-cTnI) measurements through point-of-care on the Atellica® vTLi analyzer in patients with suspected AMI compared to the reference methodologies on the market. **Methods**: The study is being conducted in the department of Laboratory Medicine, Emergency Department and the Cardiology In the first phase of the study, samples from 80 patients were analyzed for analytical comparability between hs-cTnI of the Analyzer Atellica® vTLi, hs-cTnT Cobas® and hs-cTnI Atellica Solution®. A study was then carried out of precision with samples of commercial controls with three evaluation levels, with five replicates, for 5 days in the Analyzer Atellica® vTLi. The final phase of the study will be performed with samples from 100 patients who report to the emergency department with chest pain for a clinical prospective evaluation and correlation between the hs-cTnI assays of the Analyzer Atellica® vTLi, hs-cTnT Cobas® and hs-cTnI Atellica Solution®.**Results:** The preliminary results obtained the correlation between the Atellica® vTLi, hs-cTnT Cobas® platforms in the sample group was greater than 0.75. Samples with values that exceeded linearity (less than 3 pg/mL and greater than 1,250 pg/mL) were excluded from the analysis. . It wasconsidering the reference values of each manufacturer and the biochemical differences of the troponin I and troponin T molecules. The accucary analysis were obtained for QC1 the evaluation range was (13,0 - 29,1 ng/L) which obtained an average of 17.05, %CV of 7.5 and SD of 1.28. For QC2, the evaluation range was (22.6 - 51.5 ng/L), which obtained an average of 28.48 ng/L, %CV of 8.68 and SD of 2.47. For QC3, the evaluation range was (238 - 499 ng/), which obtained an average of 284, 8 ng/L, %CV of 6.65 and SD of 18.90. **Conclusion**: The study to date demonstrated a strong correlation between the troponins of the hs-cTnI Atellica® vTLi and hs-cTnT Cobas® analyzers, in addition to safety and accuracy through studies with commercial controls.

A-018

Biological Variation Estimates for N-terminal pro-B-type Natriuretic Peptide from Eight Healthy Turkish Women

A. Coşkun[1], C. Abou-Diwan[2], J. Rhea-McManus[2], M. Serteser[1], I. Unsal[1], M. Locatelli[3], A. Carobene[3]. *[1]Acibadem Mehmet Ali Aydınlar University, Istanbul, Turkey, [2]Siemens Healthineers, Tarrytown, NY, [3]IRCCS San Raffaele Scientific Institute, Milan, Italy*

Background: B-type natriuretic peptide (BNP) and its precursor N-terminal pro-B-type natriuretic peptide (NT-proBNP) are released primarily by the ventricles in the heart in response to volume or pressure overload. Measurements of NT-proBNP are used as an aid in the diagnosis and assessment of severity of congestive heart failure (CHF). Within- (CV_I) and between-subject (CV_G) biological variation (BV) estimates for NT-proBNP are limited. Here biological variability (BV) estimates from eight apparently healthy Turkish women for NT-proBNP are provided.

Methods: Serum samples were collected weekly from 24 healthy subjects (12 male, 12 female) for 10 consecutive weeks and stored at −80°C prior to analysis. Study participant samples were analyzed in duplicate within a single run using the Atellica® IM NT-proBNP (PBNP) assay* on the Atellica IM Analyzer. Outlier detection, variance homogeneity analyses, and trend analysis were performed followed by CV-ANOVA to determine BV and analytical variation (CV_A) estimates with 95% CI. The asymmetrical reference change values (RCV) using the lognormal approach, index of individuality (II), and analytical performance specification (APS), based on the CV_A estimate derived in our study, were also calculated.

Results: PBNP results from 11 males and 3 females were lower than the limit of quantitation (LoQ) of the assay (<35 pg/mL). The remaining male sample was not included in the analysis. One female sample was identified as an outlier between individuals and was excluded from the analysis. To fulfil criteria for variance homogeneity of the data from eight women, 7% of results were excluded. In total, 104 analytical results were included in the analysis. PBNP data for the female group was normally distributed, and no trends were identified by regression analysis. The BV estimates derived from females were CV_I: 23.7%, (95% CI 19.0-30.4) and CV_G: 8.22%, (95% CI 0.0-24.7). The CV_A obtained from replicate test results was 11.2% (95% CI 9.4-13.9), which was lower than the desirable APS for imprecision. The II, calculated as the ratio of CV_I/CV_G, indicated a low individuality of PBNP (defined as II > 1.6). Thus, use of a population-based reference interval for interpreting serial PBNP measurements is more appropriate than use of the RCV.

Conclusions: This study provides updated BV estimates for NT-proBNP derived from a protocol consistent with European Federation of Clinical Chemistry and Laboratory Medicine recommendations. The availability of BV estimates allows for refined APS and associated II and RCV, which are applicable when using PBNP as an aid in the diagnosis and assessment of severity of congestive heart failure.

*The products/features mentioned here are not commercially available in all countries. Their future availability cannot be guaranteed.

A-019

Integrative analysis of arrythmogenic right ventricular cardiomyopathy of potencial biomarkers through bioinformatic analysis

A. P. Neta[1], V. N. Silbiger[1], O. C. Marques[2], T. Hirata[2], M. Hirata[2], A. D. Luchessi[1]. *[1]Federal University of Rio Grande do Norte, Natal, Brazil, [2]University of São Paulo, São Paulo, Brazil*

Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a disease usually associated with heterozygous or compound mutations in cardiac desmosomes genes. Pathologically, it is a disease characterized by fibrofatty replacement primarily in the right ventricle of normal cardiac tissue and clinically by sudden cardiac death in healthy young people. Diagnosis is often difficult due to the nonspecific nature of the disease and the broad spectrum of phenotypic variations, being commonly diagnostic post mostem. Non-coding RNAs are biomarkers that regulate gene expression and participate in various pathophysiological processes, becoming an important research field in cardiomyopathology for their potential as non-invasive clinical biomarkers and therapeutic. In this study, we aim to identify miRNAs that may serve as potentially effective targets in diagnosis and treatment as well as the exploration of regulatory miRNAs-mRNAs networks is beneficial for comprehensively understanding the molecular mechanism of ARVC development.

Methods: This study screened RNAs expression profiling studies from the Gene Expression Omnibus (GEO) database. The keyword ‘arrhythmogenic right ventricular cardiomyopathy’ was used to search for suitable datasets that followed the criteria of being a comparable case-control human study, it was selected the GSE29819 and GSE164490 GEO series. The microarray GSE29819 set was analyzed using GEO2R, an interactive web tool disponibility in GEODataset site, resulting in the differential expression genes (DEGs) of this series, while high throughput sequencing GSE164490 set was preprocessing by miARma-Seq v 1.7.5. and for identifying DEGs was used R software v4.2.2 in R Studio (version: 2022.12.0+353) and Deseq2 package. The 2 sets used $p<0.05$ and $|\log FC|>1,5$ as the cutoff criteria. An online prediction database, DIANA-microT CDS v 5.0 was used to obtain interactions with high confidence levels between miRNAs and target mRNAs. STRING online database v9 was used to predict protein-protein interactions (PPI) and functional enrichment analysis was conducted using the web-based utility Enrichr to uncover the roles of these molecules.

Results: The integrated analysis of the 2 datasets from GEO sequencing data from ARVC, after a criterium analysis, identify 04 common DEGs: hsa-miR-30d-5p, hsa-

miR-200a-3p, hsa-miR-34a-5p and hsa-miR-125a-5p between them. Also, by constructing a PPI network, it was identified 04 genes among the overlapping DEGs, including PIK3R2, RARRES1, SCN2B, and SLCO5A1 (scores ≥0.7). The functional enrichment analyses demonstrated that the DEGs were enriched in some GO biological processes such as regulation of atrial cardiac muscle cell membrane and voltage-gated sodium channel activity involved in cardiac muscle cell action potential.

Conclusion: Our results suggest that these new network gene associations possibly play a potential role in metabolic pathways related to the pathogenesis of ARVC and are good candidates for further functional validation assays to confirm these findings.

A-020

Evaluation of the Analytical Performance of BNP and NT-proBNP Assays on the Atellica CI 1900 Analyzer

J. Rhea-McManus, N. Ozgen, H. Leipold. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, high-throughput integrated chemistry and immunoassay analyzer employing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica IM B-type Natriuretic Peptide (BNP) and NT-proBNP (PBNP) assays* on the Atellica CI 1900 Analyzer.

Methods: The Atellica CI 1900 BNP and PBNP assays use the same reagents and calibrators as the Atellica IM assays. Precision and method comparison were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 and method comparison according to CLSI EP09-A3 using native and contrived human plasma (BNP) and serum (PBNP) samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. BNP and PBNP were evaluated with one reagent lot on two systems. For the method comparison, individual native and contrived human plasma samples (BNP) or native human serum samples (PBNP) were analyzed using the Atellica IM BNP and PBNP assays on both the Atellica IM and Atellica CI 1900 Analyzers.

Results: Representative precision and MC results for each assay are listed in the table. For the two assays tested, repeatability and within-lab %CVs were <2.5% and <3.0%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1.

Conclusion: Evaluation of the Atellica IM BNP and PBNP assays using the Atellica CI 1900 Analyzer demonstrated acceptable precision and equivalent performance compared to the same assays on the Atellica IM Analyzer.*The products/features mentioned herein are not commercially available in all countries.

	Precision				Method Comparison	
	Unit	Sample Range	Repeatability %CV Range	Within Laboratory %CV Range	Sample Range	Regression Equation for Comparative Assay
Assay						
B-type Natriuretic Peptide (BNP)	pg/mL	50.1—3742.9	1.4—2.2	1.8—2.8	3.9 - 4860.3	y= 0.97x - 0.8 pg/mL
NT-proBNP (PBNP)	pg/mL	118—30638	0.9—2.0	1.6—2.9	39 - 30111	y=1.02x - 4 pg/mL

Their future availability cannot be guaranteed.

A-021

Comparing the time between the 1st and 2nd troponin in male and female patients in Singapore and Calgary, Alberta: Evidence of extended pre-preanalytical time in females with low ACS risk

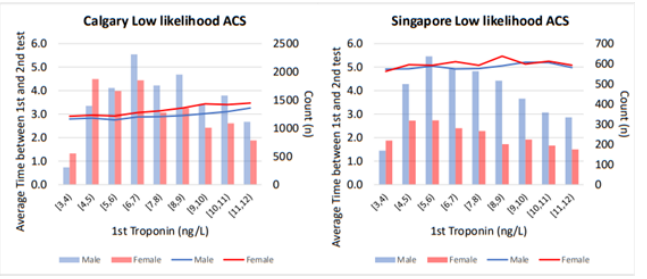
Y. Qiu[1], T. Aw[2], H. Sadrzadeh[3], G. Cembrowski[4]. *[1]University of Alberta, Edmonton, AB, Canada, [2]Changi Hospital, Singapore, Singapore, [3]University of Calgary, Calgary, AB, Canada, [4]Cembrowski & Cembrowski Quality Control Consultants, Edmonton, AB, Canada*

Background: At the 2022 AACC national meeting, in our assessment of reference change values (RCV) in troponins ordered in Calgary and Singapore, we postulated that there were gender differences in troponin-ordering between the two cities.

Methods: Singapore Changi Hospital ED patients [July 2018-July 2020] and Calgary patients [December 2017-December 2021] had hsTnT measured with the Roche Elecsys assay on multiple, local Cobas e801's. The first two troponins of each patient were selected if they were ordered within 12 hours. The troponin pairs were grouped by city, patient sex and whether the troponin pairs fulfilled the criteria of low likelihood of Acute Coronary Syndrome (ACS) according to city specific guidelines.

Results: The figure compares the average time difference between the first and second troponin test of females and males stratified by the initial troponin value. The paired t-test conducted shows that in Singapore there was no significant difference in the time for females and males to receive a second troponin test while in Calgary the time for females to receive a second troponin test was significantly longer (p<0.0001) than males.

Conclusion: The significant difference between Calgary females and males in average time between the first and second troponin tests supports the idea that there may be gender specific differences in troponin ordering timing and patterns. 62%-68% of all laboratory errors originate from pre-pre-analytical processes of which timing is a factor. The well documented atypical presentation of myocardial infarction in females may contribute to the delayed second test in women. This timing difference may be a contributor to the RCV variance observed in our previous study which identified gender differences in the assessment of RCV in troponins ordered in Calgary and Singapore.



A-022

Muscle and Cardiac Marker Levels Impact for SARS-CoV-2 Infection

G. C. Carvalho, R. P. Carvalho, D. R. Ramadan, S. Tufik. *Associação Fundo de Incentivo a Pesquisa, São Paulo, Brazil*

Background: To diagnose cardiac problems, biomarkers such as CK-MB and troponin are effective tools. COVID-19 negatively affects the cardiovascular and muscular systems, potentially causing heart failure and arrhythmias. In addition, COVID-19 can also lead to muscle wasting and muscle weakness and the correlation between CK levels and muscle injury in SARS-CoV-2 diagnosis is not well studied. Our aim is to study the relationship between serum levels of muscle and cardiac injury markers on the same day as SARS-CoV-2 diagnosis. **Methods:** Study population and characteristics: This study enrolled an 11-month retrospective analysis from a large laboratory database in the city of São Paulo, Brazil from January 2022 to November 2022, from a specific patient population. Individuals aged 18 or older with available records for markers such as creatine kinase (CK), creatine kinase-MB (CK-MB), and troponin were selected, including only outpatient results. All individuals in this group, suspected of having COVID-19, underwent SARS-CoV-2 diagnosis by RT-PCR at physician's request. Statistical analysis: The Mann-Whitney U test and Kruskal-Wallis test were used for statistical analysis. Results with p<0.05 were considered significant. **Results:** A total of 1109 samples collected through nasopharyngeal swabs from individuals with or without SARS-CoV-2 infection were analyzed, upon medical request for PCR molecular diagnosis. There were 555 (50.04%) men and 554 (49.96%) women evaluated with a mean age of 60.1 ± 0.54 SEM. Of these, 312 (28.1%) were positive for the virus and 797 (71.9%) were negative. No differences in cardiac markers were observed between the two groups (SARS-CoV-2 positive group compared to non-infected individuals). Significant differences regarding the reduction of CK levels were mainly observed in men compared to women positive for SARS-CoV-2 (p<0.05). Finally, no differences in CK serum levels were observed when the SARS-CoV-2 group was analyzed by age stratification data for the 31-40, 41-50, 51-60, or 61-90-years old groups. **Conclusion:** Identifying a specific group's fingerprint of injury biomarkers, such as creatine kinase (CK), creatine kinase-MB (CK-MB), and troponin, at the same time point when individuals are laboratory-confirmed positive for SARS-CoV-2, could be valuable for risk management strategies. We recognize the significance of evaluating these biomarkers in populations infected with SARS-CoV-2 and acknowledge the need for follow-up studies.

A-023

Performance Evaluation of the Atellica IM High-Sensitivity Troponin I Assay on the Atellica CI 1900 Analyzer

H. ZHANG[1], J. Rhea-McManus[2], G. Caicedo_Montoya[1], L. Halik[1], E. Mahmod[1], S. Askegard[1], H. Leipold[1]. *[1]Siemens Healthineers, Tarrytown, NY, [2]Siemens Healthineers, Neward, DE*

Background: The Atellica® CI 1900 Analyzer is an automated, high-throughput, integrated chemistry and immunoassay analyzer employing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica IM High Sensitivity Troponin I (TnIH) assay* on the Atellica CI 1900 Analyzer*. **Methods:** Precision and method comparison were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 and method comparison according to CLSI EP09-A3. Precision studies used a panel of four native and one contrived serum/lithium-heparin plasma samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days with two reagent lots on two systems. For the method comparison, individual native human lithium-heparin plasma samples were analyzed using the Atellica IM TnIH assay on both the Atellica IM and Atellica CI 1900 Analyzers. Detection capability studies including limit of blank (LoB), limit of detection (LoD), and 20-day functional sensitivity (LoQ) were performed according to CLSI EP17-A2 guidelines. Expected values of normal healthy individuals and hook effect were also verified on the Atellica CI 1900 Analyzer. **Results:** Repeatability and within-lab %CVs ranged from 0.7-4.4% and 1.3-5.1%, respectively, at TnIH concentrations ranging from 13.73-23197.12 ng/L. Method comparison between the Atellica IM TnIH assay on the Atellica CI 1900 Analyzer vs. the Atellica IM Analyzer showed a regression slope of 1.00 and an intercept of 0.04 ng/L (n = 170). In detection capability studies, four lots of the Atellica IM TnIH assay on two Atellica CI 1900 analyzers showed a LoB of 0.35 ng/L and an LoD of 0.76 ng/L. LoQ, defined as the lowest amount of analyte in a sample at which within-lab %CV = 20%, was 1.10 ng/L; %CV was approximately 10% at a TNIH concentration of 2.57 ng/L. In addition, >50% of healthy individuals tested had TnIH values >LoD. The 99th percentile for the Atellica IM TnIH assay (45.20 ng/L) was verified on Atellica CI 1900 Analyzer and was consistent with the Atellica IM Analyzer. No interference from biotin was observed up to a concentration of 3500 ng/mL, and no hook effect was observed up to a concentration of 500,000 ng/L. **Conclusion:** Evaluation of the Atellica IM TnIH assay using the Atellica CI 1900 Analyzer demonstrated reproducible assay precision, sensitive detection capabilities, and equivalent performance compared to the same assay on the Atellica IM Analyzer. This evaluation shows that the Atellica IM TnIH assay on Atellica CI 1900 Analyzer meets the definition of a true high-sensitivity troponin I assay as defined by the International Federation of Clinical Chemistry (IFCC) Task Force on Clinical Applications of Cardiac Biomarkers. *The products/features mentioned here are not commercially available in all countries. Their future availability cannot be guaranteed.

Endocrinology and Metabolism

A-024

Evaluation of the Analytical Performance of Thyroid-stimulating Hormone, Free Triiodothyronine, and Free Thyroxine Assays on the Atellica CI 1900 Analyzer

H. Zhang, M. Quintanilla, M. Guo, J. Rhea-McManus, H. Leipold. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput integrated chemistry and immunoassay analyzer utilizing both Atellica CH and Atellica IM assays. This study evaluated the analytical performance of the Atellica IM Thyroid Stimulating Hormone 3 Ultra™ (TSH3-UL), Free Triiodothyronine (FT3), and Free Thyroxine (FT4) assays* on the Atellica CI 1900 Analyzer.

Methods: The Atellica CI 1900 TSH3-UL, FT3, and FT4 assays use the same reagents and calibrators as the Atellica IM assays. Precision and method comparison studies were performed according to CLSI EP05-A3 and CLSI EP09-A3 using native and contrived human serum (TSH3-UL, FT3, and FT4) and plasma (TSH3-UL) samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. FT3 and FT4 were evaluated with one reagent lot on two systems. TSH3-UL was evaluated with two reagent lots on two systems. For the method comparison, individual native human serum samples were analyzed using the Atellica IM TSH3-UL, FT3, and FT4 assays on both the Atellica IM and Atellica CI 1900 Analyzers.

Results: Representative precision and MC results are listed for each assay in the table. Slopes determined by the Deming linear regression model were approximately equal to 1.

Conclusion: Evaluation of the Atellica IM TSH3-UL, FT3, and FT4 assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on the Atellica IM Analyzer.

		Precision			Method Comparison	
Analyte (Assay)	Unit	Sample Range	Repeatability %CV Range	Within Laboratory %CV Range	Sample Range	Regression Equation for Comparative Assay
Thyroid Stimulating Hormone (TSH3-UL)	µIU/mL	0.017—120.833	1.1–1.4%	2.9–3.0%	0.013—144.030	y = 0.96x−0.001 µIU/mL
Free Triiodothyronine (FT3)	pg/mL	0.75—17.21	0.8–13.2%	1.7–16.8%	1.26—18.89	y=1.00x-0.10 pg/mL
Free Thyroxine (FT4)	ng/dL	0.37—11.15	1.1–2.7%	3.1–6.3%	0.43—11.51	y =0.97x+0.02 ng/dL

*The products/features mentioned herein are not commercially available in all countries. Their future availability cannot be guaranteed.

A-025

Principal Component Analysis as a Clustering Method for the Identification of Populations with Risk of Diabetes Development

G. C. Carvalho, F. S. Siqueira, D. R. Ramadan, S. Tufik. *Associação Fundo de Incentivo a Pesquisa, São Paulo, Brazil*

Background: Obesity is a chronic disease, defined by the World Health Organization as the abnormal or excessive accumulation of fat in the body. Overweight populations have grown epidemically in all age groups in recent decades and represent a major public health issue globally. Excess weight is one of the potential factors for insulin resistance, thus increasing the risk of developing type 2 diabetes. This study aimed to investigate the urea/creatinine ratio as a marker of renal function in individuals with different body mass index (BMI) levels and examine clusters with increased risk for diabetes development. **Methods:** Study population and characteristics: This study was a 34-month retrospective analysis from a large laboratory database in the city of São Paulo, Brazil (January 2019-November 2022). Individuals ranging between 19-60 years old were selected based on the availability of BMI data and results for creatinine, urea, serum glucose, and glycated (A1C). Only outpatient results were included in the current analysis. The following parameters were included in this study: BMI (kg/m2): 18.5-24.9 (healthy), 25-29 (overweight), 30-34.9 (overweight level 1), 35-39.9 (overweight level 2), and ≥40 (overweight level 3). The definition of diabetes risk was as follows: (i) without diabetes risk: serum glucose>100 mg/dL and A1C<5.7; (ii) transition to diabetes risk: serum glucose 101 to 125 mg/dL and A1C 5.7-6.4%; (iii) diabetes risk: serum glucose>126 mg/dL and A1C>6.4%. Statistical analysis: Principal component analysis (PCA) was performed resulting in a division of four subpopulation clusters based on the risk of diabetes development. One-way ANOVA with the Tukey test was used for intergroup comparison. For all results *p<0.05 was considered statistically significant. **Results:** A total of 667 participants met the inclusion criteria. From all BMI profiles, the overweight population in diabetes risk situation showed a significant difference in the ratio of urea/creatinine compared with the same BMI population without diabetes risk [33±11.5 vs 38±11.8 Mean ± SD] (p<0.05)**.** PCA analysis formed four clusters: PC1+ (cluster 1) with increased creatinine (1.9 mg/dL) & urea (63.5 mg/dL) and transition to diabetes; PC2+ (cluster 2) with normal creatinine (0.7 mg/dL) & urea (28.1 mg/dL) and diabetes risk; PC1+PC2+ (cluster 3) with increased creatinine (1.4 mg/dL), increased urea (50.44 mg/dL), and diabetes risk; PC1-PC2- (cluster 4) with normal creatinine (0.7 mg/dL), normal urea (28.7 mg/dL), and transition to diabetes. PCA analysis found that Cluster 1 had a higher urea/creatinine ratio (34.7±10.6 vs 40±12.3, Mean ± SD) compared to Cluster 2 (p<0.01). Cluster 1 was made up of mostly men (80.5%) with an average age of 54±8.9 years, of which 22% were normal weight and 34.2% were overweight. Cluster 2 had more women (65.10%) with an average age of 47±11.7 years, 20.6% normal weight and 32.6% overweight. A total of 17.9% of overweight individuals with a transition to diabetes risk were found in PC1 (14/78) and 8.1% in PC2 (4/49) **Conclusion:** Urea/creatinine ratio could be a useful indicator of diabetes risk in overweight individuals. PCA selection method identifies diabetes risk indexes for early care management by clinicians.

A-027

The Clinical Indications of Low or Non-reportable HbA1c Results

S. Lu, T. Neibauer, E. Saylor, B. Dewasse, M. Straub, Y. Zhu. *The Pennsylvania State University, Hershey, PA*

Background: Hemoglobin A1c (HbA1c) is the major non-enzymatic glycation product of hemoglobin A (HbA) and usually measured as a percentage of total hemoglo-

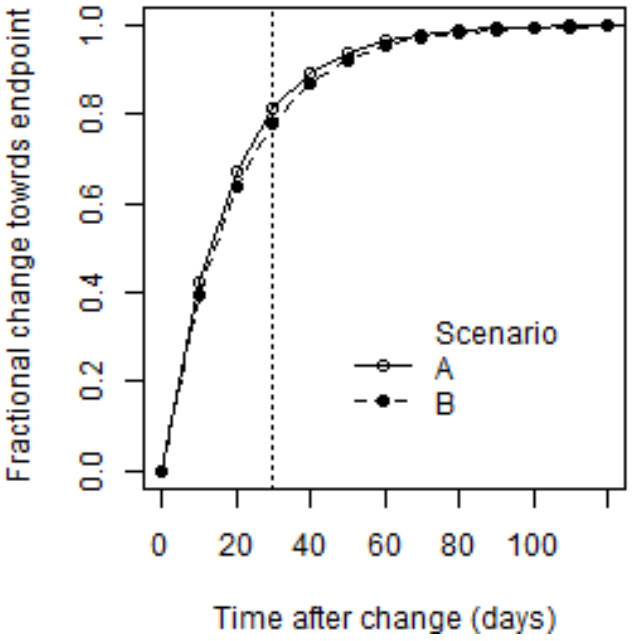
bin. It is widely used in the screening, diagnosis, and monitoring of diabetes because HbA1c correlates with the average blood glucose level over the past 8-12 weeks in patients with a normal RBC lifespan. However, many factors can cause inaccurate HbA1c measurements or invalidate this correlation. While a high HbA1c raises the concern of inadequate glycemic control, a low or non-reportable HbA1c may also indicate diverse clinical conditions or technical issues. This study analyzes the pathophysiological conditions and technical limits leading to a low or non-reportable HbA1c value and discusses the use of other lab values in HbA1c result interpretation. **Methods:** HbA1c was measured by cation exchange HPLC (Tosoh G8, v5.24F). 37 cases with a low or non-reportable HbA1c (< 4.0%) from 1/1/2022 to 11/30/2022 were reviewed. The relevant diagnoses, medical history, medications, and recent (± 1 week from the HbA1c test) lab results (CBC, blood glucose, fructosamine, haptoglobin, LDH) were examined to identify the causes of low HbA1c values. The causes were grouped by pathological mechanisms or technical issues to calculate the frequency, and the association with other lab values was also explored. **Results:** Low or non-reportable HbA1c cases accounted for 0.1% of total HbA1c tests (37/42771), and about 30% (11/37) had been diagnosed with diabetes by the end of the review period. From the chart, 41% (15/37) cases had elevated blood glucose (> 109 mg/dL), 73% (27/37) were anemic (Hb < 13.0 or 11.7 g/dL for male or female), and 46% (17/37) showed a high MCV (> 96 fL). Among those cases, a minor portion was caused by method-specific technical issues (8%, 3/37) or unknown reasons (5%, 2/37). The majority (86%, 32/37) was attributed to the following mechanisms: intravascular hemolysis (51%, 19/37), hepatosplenic RBC destruction (27%, 10/37), and increased erythropoiesis (8%, 3/37). For those cases with intravascular hemolysis (51%), the causes were categorized as inherited RBC disorders (19%, 7/37, including hemoglobinopathy and G6PD deficiency), acquired hemolytic diseases (19%, 7/37, including autoimmune hemolytic anemia), and adverse effects of medications (13%, 5/37, including dapsone and hydroxychloroquine). For those cases with hepatosplenic RBC destruction (27%), most of them were caused by cirrhosis (19%, 7/37). In addition, increased erythropoiesis (8%) was observed in patients with recent hemorrhage or EPO treatment. Some hemoglobin variants like homozygous HbE resulted in non-reportable HbA1c (8%), which represented a technical limit of the HPLC method. **Conclusion:** Although low or non-reportable HbA1c cases are rare (0.1%), a significant portion (30%) of them are diabetic patients, and their HbA1c results can be misleading. The majority of low HbA1c cases (86%) are associated with clinical conditions causing a shortened RBC lifespan, and the most common causes include inherited RBC diseases (19%), cirrhosis (19%), medication adverse effects (14%), and autoimmune diseases (11%). Because increased RBC destruction and compensatory erythropoiesis are the underlying mechanisms, hemoglobin, MCV, and markers assessing hemolytic anemia (haptoglobin, LDH, reticulocyte%) can aid in HbA1c interpretation.

A-029

Modeling the Effect of Step Changes in Glucose on %Glycated Albumin vs. Time

D. F. Stickle[1], G. J. DiNatale[1], R. Molinaro[2]. *[1]Jefferson University Hospital, Philadelphia, PA, [2]Siemens Healthcare Diagnostics, Inc., Tarrytown, NY*

Background: Glycated albumin (GA) is often referred to as being a reflection of glucose exposure over the past 20-30 days. We examined this premise from a theoretical standpoint using a model for formation of GA and simulation of the effects of step changes in glucose concentration ([G]). **Methods**: A model for glycation of albumin parallels established models for formation of hemoglobin A1c, differing only in characterization of the age distribution for albumin. Probability of survival (P(s)) of albumin at a given age (t) is based on a first-order rate constant (ke) for elimination: P(s) = exp(-ke t) [Eqn.1], where ke=4.08e-2/d (t(1/2)=17 d). Probability of glycation (P(g)) at a given age is given by: P(g) = (1-exp(-k[G] t)) [Eqn.2]. There is a simple analytical solution for %GA for constant [G]: %GA=100(1-w/z), where w=ke and z=(ke+k[G]). k=9.77e-4/d/(mmol/L) reproduces reference values for %GA [PMID: 29436378], and for which %GA=2.41(%A1c)-0.004 (r=0.999, for A1c range=5%-12%). For non-constant [G], %GA must be determined by simulation of changes in P(g) using Eqn.2, calculated in small time increments, dt. Using dt=0.01 d, we calculated changes in %GA by simulation as a function of time for two scenarios involving step changes in [G] at time = 0: A. from 10 mmol/L to 15 mmol/L (%GA ultimately moves from 19.3% to 26.4%); B. from 15 mmol/L to 10 mmol/L (%GA ultimately moves from 26.4% to 19.3%). **Results**: **Figure** shows the *fractional transition of %GA between respective starting points and ultimate endpoints vs. time* for scenarios A and B. In both cases, transition at 30 days was approximately 80% of the ultimate full transition. **Conclusions**: Physiological model-based calculations support that description of %GA as a reflection of [G] over the past 30 days is a very reasonable one under conditions in which [G] has changed.



A-030

Indicators Generated from Salivary Cortisol Measurement to Assess Circadian Rhythm: Comparison of Immunoassay and Liquid Chromatography-Tandem Mass Spectrometry

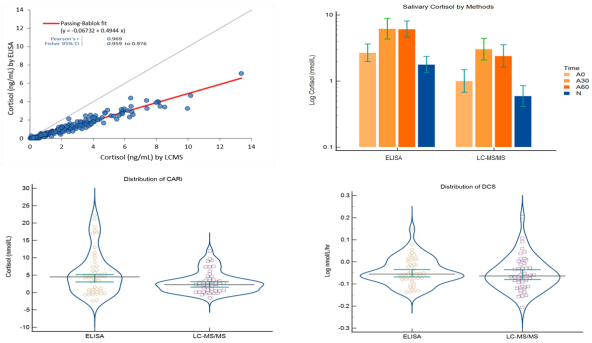
A. Lee[1], S. Jang[2], J. Kim[3], J. Jang[1], I. Kim[2], S. Lee[3], J. Seok[2]. *[1]Seoul Clinical Laboratories, Yong-In, Korea, Republic of, [2]Research Institute of Minds AI, Co. Ltd., Seoul, Korea, Republic of, [3]Center for Companion Biomarker, Seoul Clinical Laboratories Healthcare Inc., Yong-In, Korea, Republic of*

Background: Salivary cortisol is considered a reliable alternative to serum-free cortisol for evaluating hypothalamic-pituitary-adrenal axis rhythmicity. Its advantages include free hormone measurement, non-invasiveness, precision, and cost-effectiveness. Several cortisol indicators in serially collected saliva have been used in research studies to evaluate the circadian rhythm. We aimed to find if the distribution of those indicators is affected by the specific assay platform used.

Methods: Four-time points of saliva specimens were obtained immediately after awakening, 30 minutes and 60 minutes post-awakening, and in the nighttime from 49 subjects (aged between 19 and 25, mean±SD: 28.9±8.4 years). Salivary cortisol was analyzed by ELISA method using the Cortisol Saliva ELISA kit (IBL, Hamburg, Germany). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay was performed on the API 4000 triple quadrupole mass spectrometer coupled with an Agilent 1200 liquid chromatography system. Four cortisol indicators [DCS (diurnal cortisol slope), CARi (cortisol awakening response by an absolute increase within 30 minutes of awakening), AUCg (area under the curve with respect to ground), and AUCi (AUC with respect to increasing)] were calculated from each patient by the method. Correlation between the two methods was evaluated for overall cortisol concentrations, DCS, CARi, AUCg, and AUCi.

Results: The cortisol concentrations between the two methods strongly correlated (r=0.973) but with a mean bias of 3.4 nmol/L (95% CI -3.8 to -2.9). Four cortisol indicators strongly correlated between the two methods, but showed significant differences. DCS data generated from LC-MS/MS measurement was more broadly distributed than those from ELISA. On the contrary, CARi data generated from ELISA measurement dispersed more than those from LC-MS/MS.

Conclusion: The concentrations of salivary cortisol strongly correlated between ELISA and LC-MS/MS, though considerable proportional bias between the two methods. The cortisol indicators generated from different methods can affect its distribution, therefore possibly affecting its discrimination power.



A-031

Global Discovery of Serological Metabolome Uncovers Unique Molecular Signature for Early Onset of Type 2 Diabetes Mellitus: A Retrospective Study in Chinese Population

L. Zhu, Y. Wu. *Tianjin TEDA Hospital, Tianjin, China*

Background Type 2 diabetes mellitus (T2DM) is a multifaceted disorder affecting epidermic proportion at global scope. Defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond appropriately to insulin are the primary causes of T2DM. Early detection of people at high risk of developing T2DM is of great importance as interventions such as dietary control and intentional weight loss can effectively improve the insulin sensitivity and prevent the disease incidence. However, circulating biomarkers that are indicative of early diabetic onset at asymptomatic stage have not been well described. Here, we aim to identify new serological markers indicating early asymptomatic onset of T2DM by MS-based metabolomic discovery. **Methods** Three independent cohorts were assembled for the study. The discovery cohort included sera from 25 patients with T2DM and 25 healthy controls. The validation cohort included sera from 15 patients with T2DM and 15 healthy controls. The testing cohort included sera from 111 patients with pre-diabetes and 89 healthy controls. Untargeted approach was used to characterize the serological metabolome by high performance liquid chromatography-high resolution mass spectrometry. **Results** The univariate analysis identified 11 metabolites that were significantly altered in patients with T2DM across discovery and validation cohorts. After evaluated by the testing cohort, 8 of 11 metabolites, such as hexose, N-acetyl-glucosamine, and alpha-ketobutyrate, remained statistically significant in patients with prediabetes as compared to healthy controls. The multivariate analysis based on 8 significant metabolites revealed distinct patterns of metabolic perturbation at the early onset of T2DM. To gain mechanistic insights into the disease pathophysiology, pathway enrichment and correlation network analyses were conducted to unveil the molecular crosstalk underlying the disturbed metabolic network in the pathogenesis of T2DM. **Conclusion** Our study not only identified serological biomarkers detecting early asymptomatic onset of T2DM but also provided additional insights into the disease pathophysiology.

A-032

Comparative Serum Free Thyroxine Measurements between Immunoassays and ID-LC/MS/MS Procedure Based on Equilibrium Dialysis

L. Zhang, A. Ribera, Z. D'Zio, A. Doty, C. Tse, O. Sugahara, D. Spector, N. Vazquez, U. Danilenko, H. Vesper. *CDC, Atlanta, GA*

Background:

Serum free thyroxine (FT4) measurement is one of the most important clinical laboratory tests for diagnosis and classification of thyroid disorders. Currently serum FT4 is commonly measured with Immunoassays (IAs) in patient care. However, there are concerns about the accuracy of FT4 IAs. A reference measurement procedure (RMP) for FT4 based on well-defined equilibrium dialysis (ED) of serum has been established for standardization of routine FT4 IAs. As FT4 RMP is not intended for routine clinical application, in the current study, we aim to develop a high-throughput routine FT4 method based on ED-isotope dilution liquid chromatography tandem mass spectrometry (ED-ID-LC/MS/MS). FT4 RMP was used as a reference to evaluate the measurement accuracy of our ED-ID-LC/MS/MS method and an IA method, respectively.

Methods:

FT4 in dialysate was isolated from protein-bound T4 in serum by using a commercially available micro-ED plate after 18-hours ED. The 13C6 labeled T4 was added into dialysate as an internal standard. FT4 in dialysate was purified with 96-well C18 SPE plate. FT4 in the dialysate samples was analyzed by using LC/MS/MS. The T4 certified primary reference material (IRMM-468) was used for assay calibration. FT4 IA measurement was conducted in clinical analyzers (Roche e411). A set of 40 single donor sera, covering a FT4 concentration range of 11.2 to 32.1 pmol/L were measured by IA and our ED-ID-LC/MS/MS, respectively. Deming regression analysis was performed to compare the results obtained by these two methods with the reference values assigned by FT4 RMP.

Results:

The described high-throughput method based on ED-ID-LC/MS/MS could simultaneously quantified T4, T3, reverse triiodothyronine (rT3) in the dialysate within 4.5 min. The linear range of the routine FT4 assay covered 1-100 pg/mL. The assay sensitivity allowed detection of 0.3 pg/mL FT4 in serum, which is sufficient for FT4 measurement in clinically relevant ranges including hypothyroid patient samples. The throughput of the ED-ID-LC/MS/MS method could be further improved by application of automated liquid handling system. Deming regression analysis showed good agreement between RMP and the routine ED-ID-LC/MS/MS method with a slope close to 1 ($p>0.05$) and an intercept close to 0 ($p>0.05$), indicating that the results generated from the platform of high-throughput ED-ID-LC/MS/MS could be tracible to FT4 RMP. However, proportional bias ($p<0.05$) with slope of 0.74 and constant bias ($p<0.05$) with intercept of 2.6 was observed between RMP and IA tested, indicating calibration bias should be addressed to standardize IA.

Conclusion:

In summary, the accuracy, sensitivity, and throughput of ED-ID-LC/MS/MS method are appropriate for FT4 measurements in clinical laboratories and in large epidemiologic studies to establish accurate reference intervals for FT4. Population data generated by FT4 ED-ID-LC/MS/MS method will support the use of standardized FT4 measurements.

A-033

Development and Validation of a Liquid Chromatography Tandem Mass Spectrometry Bioanalytical Method for Prostaglandin D2 in Serum or Urine

M. Morr, B. Holmquist. *Labcorp, Calabasas, CA*

Background: Prostaglandin D2 (PGD2) is a major prostaglandin produced by the brain and by mast cells, and plays a role in vasodilation ("niacin flush") and in the development of allergic diseases such as asthma and alopecia. We sought to develop a bioanalytical method for multiple specimen types for PGD2 for clinical use.

Methods: An analytical method was developed using a TX-4 HPLC system (ThermoFisher) with Agilent® 1200 pumps (Agilent Technologies, Inc.) and a Sciex® 5500 (Danaher) triple quadrupole mass spectrometer. Calibration curve based on purified PGD2 (Cayman) was prepared in depleted serum (Golden West Biologicals). Sample preparation consisted of an initial isotope dilution using a deuterated heavy isotope internal standard (PGD2-2H4) followed by solid phase extraction, evaporation and reconstitution using a second heavy isotope (PGD2-2H9). Double-isotope dilution allows discrimination between extraction loss and ion suppression as causes of decreased ion current. A reversed phase C18 analytical column was used with an acidified water/acetonitrile/methanol solvent gradient to achieve chromatographic separation of physiological PGD2 from related isobaric metabolites include Prostaglandin E2. Negative mode electrospray ionization (ESI) was used for detection in Multiple Reaction Monitoring (MRM) mode.

Results: Analytical range was 1 - 1000 pg/mL (up to 5,000 pg/mL with dilution). Inter-assay precision ranged from 8.8-10.2% and inter-assay accuracy ranged from 95.7-103.3%. Reference intervals for adults was developed using residual serum and urine specimens. No interference was observed in the presence of hemolysis, icteric or lipemia. No difference in result was observed when using collection tubes containing EDTA, Heparin or gel barriers.

Conclusion: A bioanalytical method for PGD2 has been fully validated for clinical use.

A-034

Multicenter Comparison of Analytical Interference of Vitamin D Immunoassay and Mass Spectrometry Methods by Endogenous Interferents and Cross-reactivity with 3-epi-25-OH-Vitamin D3

J. Lee[1], J. Seo[2], K. Lee[1], E. Roh[3], Y. Yun[2], Y. Lee[4], S. Cho[5], J. Song[1]. *[1]Seoul National University Bundang Hospital, Seongnam, Korea, Republic of, [2]Konkuk University School of Medicine, Seoul, Korea, Republic of, [3]Seoul Metropolitan Government-Seoul National University Boramae Medical Center, Seoul, Korea, Republic of, [4]Soonchunhyang University Bucheon Hospital, Bucheon, Korea, Republic of, [5]Green Cross Laboratories (GC Labs), Yongin, Korea, Republic of*

Background: Vitamin D plays an important role in calcium homeostasis and bone metabolism and is known to increase calcium absorption from the intestinal tract and decrease calcium excretion from the kidneys. Vitamin D deficiency is prevalent in a substantial and continuously increasing proportion of the Korean population, thus highlighting the need for accurate vitamin D measurements. The current problems of vitamin D testing include significant inter-method and inter-laboratory differences in measurements, and interferences by both intrinsic and cross-reactive substances. In this study, the interfering effects by such intrinsic and cross-reactive substances were compared between several vitamin D immunoassays and mass spectrometry methods routinely used in Korea.

Methods: Two different mass spectrometry methods and 4 vitamin D immunoassays with different manufacturers (Abbott, Beckman Coulter, Roche, Siemens), from 5 different institutes were compared. Residual patient samples were utilized to collect either hemolyzed samples, icteric samples (total bilirubin >20 mg/dL), lipemic samples (triglyceride >500 mg/dL), of samples with rheumatoid factor >200 IU/mL, samples from myeloma patients (paraprotein >1.1 g/dL upon protein electrophoresis) or from patients undergoing hemodialysis. Four levels of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 972 were prepared, with each level having different compositions and concentrations of vitamin D. Also, samples spiked with different levels of 3-epi-25(OH)D3 were prepared for interference analysis. The 10% bias limits for concentrations of intrinsic interferents and cross-reactivity percentages, provided by the respective immunoassay manufacturers, were referred to during data interpretation. The collection of intrinsic interferent samples, NIST material, and epimer spiked sample sets were delivered to each institute and measured on the same date.

Results: Consistently significant interference in vitamin D measurements was observed in hemolytic samples (Roche assay), icteric samples (Beckman and Siemens assays) and lipemic samples (all 4 immunoassays). General positive biases were also observed in myeloma samples (Abbott assay),

hemodialysis samples (Siemens assay), and general negative biases were observed in high rheumatoid factor (Roche assay) and hemodialysis samples (Roche assay). Both the level 4 NIST material, which had a high concentration of 3-epi-25(OH)D3, and samples spiked with 3-epi-25(OH)D3, induced significant interference, yielding higher total vitamin D measurements in non-epimer separating MS methods, and the Beckman and Roche immunoassays.

Conclusion: In this study, we were able to observe interferences due to intrinsic substances and cross-reactive substances in common vitamin D tests used in Korea. Clinical awareness of possible causes of interference is important to increase the accuracy of vitamin D measurements. Moreover, due to the high cross-reactivity of 3-epi-25(OH)D3 with common vitamin D assays, particular care is due when interpreting vitamin D results of newborns, infants, and less commonly, pregnant women, who are known to have physiologically high levels of 3-epi-25(OH)D3.

A-035

Association between Obesity and Prostate Specific Antigen level in Diabetes Mellitus Type2

O. C. Ugwunna[1], E. N. Okafor[2], N. C. Ugwunna[3], E. E. Chukwukelu[3], U. A. Eze[4], S. O. Uzoigwe[5], E. C. Aniogo[6]. *[1]College of Medicine, University of Nigeria, Nsukka, Enugu, Nigeria, [2]University of Nigeria Enugu Campus, Enugu, Nigeria, [3]College of Medicine, University of Nigeria Nsukka, Enugu, Nigeria, [4]De Montfort University, Leicester, Leicester, United Kingdom, [5]University of Nigeria Teaching Hospital, Ituku-Ozalla, Nigeria, [6]City of Hope Beckman Research Center, Duarte, CA*

Background: Body mass index (BMI) is a simple index commonly used to classify obesity. It is defined as a person's weight(kg) divided by the square of his height (m). An inverse relationship between obesity and prostate-specific antigen (PSA) has been reported by previous studies with lower PSA levels found in groups with a higher body mass index (BMI). The main objective of this current study is to assess the association between body mass index (BMI) and PSA in male patients with diabetes mellitus. **Method:** This study uses a case-control experimental procedure to assess adult men between 40-75 years old, attending University of Nigeria Teaching Hospital, Enugu State, Nigeria. Eighty-six (86) of the volunteers recruited for this study were patients diagnosed with type II diabetes, and sixty (60) others were non-diabetic patients used as controls. Consent was sorted from these patients, and a questionnaire was given to the participants in which data about their age, family history of diabetes, and prostate condition was collected. Their hospital folders were used to confirm their diabetic status and prostate gland health. Body Mass Index (BMI) was calculated after measuring weight (kg) and height(m). Blood samples were assayed to determine the fasting blood sugar (FBS) using Glucose Oxidase enzymatic method, and total prostate-specific antigen (tPSA) concentration was determined using Enzyme-Linked Immunosorbent Assay (ELISA). The Body mass index (BMI), as a "gold standard" approach for obesity classification, was used to group the patients as; underweight (below 18.5), normal weight (18.5 to 24.9), pre-obesity (25.0 to 29.9), obesity class I (30 to 34.9), obesity class II (35.0 to 34.9), and obesity class III (40 and above). The Statistical Package for Social Sciences (SPSS) version 22.0 was used to analyze data generated and compared between groups using student T-test. Statistical difference was at a *P* value of 0.05 or higher. **Result:** The result of this study demonstrated a significant difference in the BMI of diabetic men (test) and non-diabetic men (control). Moreso, lower tPSA was observed in obese men compared to normal BMI in both test and control. Hemodilution caused by elevated plasma volume, which may reduce serum concentration of soluble tumor markers, may be responsible for lower PSA levels in obese men. **Conclusion**: Obesity may hinder the ability to detect prostate cancer at an earlier stage. Therefore, BMI should be considered while determining a PSA threshold for prostate disorders screening. **Keywords**: Obesity, Prostate-specific antigen, diabetes, body mass index

A-038

An Improved Multiplexed HTLC-HESI-MSMS Method for the Measurement of Total Testosterone in Serum

S. L. Daly, J. Kochuparambi, Z. Wu, R. Abdula, C. Yuan, B. Wei, Z. Wu, M. J. McPhaul, A. K. Ghoshal. *Quest Diagnostics, Secaucus, NJ*

Background: Testosterone is an anabolic steroid produced primarily in the testicles of males and mainly in the ovaries of females. Imbalance of testosterone is the primary cause of hypogonadism in males, hirsutism and virilization in females, osteoporosis, and diabetes mellitus. Accurate and fast-turnaround measurement of testosterone is critical for diagnosis, prevention, and treatment of testosterone-related diseases in adults and children. In a previous study, testosterone was derivatized with methoxyamine and hydroxylamine in a multiplexed high turbulence liquid chromatography with heated-electrospray ionization-tandem mass spectrometry (HTLC-HESI-MS/MS) method. Using these reagents, differential mass-tagging of testosterone allowed different specimens to be combined in a single injection to increase assay throughput. However, interference was observed for hydroxylamine-derivatized testosterone in serum collected in serum separator tubes (SST). In the current study, our objectives were to (1) replace hydroxylamine with a new derivatizing agent not subject to interference and (2) develop an improved HTLC-HESI-MS/MS method for multiplexed total testosterone measurement. **Method**: Healthy male and female donor specimens were collected in SST and red-top tubes. Serum was mixed with stable isotope-labeled testosterone as internal standard (ISTD) prior to protein precipitation. Testosterone in the supernatants was derivatized, enriched by solid phase extraction, dried, and reconstituted. Specimens were injected on the HTLC system for on-line extraction, transferred to analytical columns, and eluted using binary gradient. Testosterone derivatives and ISTDs were detected and analyzed on a Triple Quadrupole Mass Spectrometer (Thermo) equipped with HESI. Ionization and multiple reaction monitoring (MRM) scan parameters were optimized for maximized ion transmission and sensitive, specific, and stable quantitation. MRM transitions were used as quantifiers and qualifiers for both testosterone derivatives. Also assessed were ion suppression using T-column infusion, specimen stability by storage in SST tubes at 4ºC, isobaric interference from dehydroepiandrosterone (DHEA), and carryover. **Results:** Intra- and inter-assay coefficients of variation (CV%) for total testosterone levels were <5% at 12, 80, 250, and 1,200 ng/dL for both testosterone derivatives. Limits of quantitation (LOQs) for both derivatives were <1 ng/dL. Calibration linearity was verified across the measurement range of 2.5-2,000 ng/dL. Both agents were equivalent in derivatizing and recovering testosterone from serum based on a comparison of their quantitative results (R = 0.991, bias=-3.8 ng/dL). No interference was observed in serum collected in SST. Serum specimens collected in SST and red-top tubes from same donors gave equivalent results (R = 0.991, bias= 0.2 ng/dL). Ion suppression was calculated as less than 20% for both derivatives. CV% of the ion-ratios of both derivatives were

less than 15% and were stable across the linear range. Testosterone levels were stable in SST for 72 hours (at 4°C) with the serum not separated from the gel. No isobaric interference and no carry over were observed. **Conclusion** We have developed and validated an improved multiplexed HTLC-HESI-MS/MS testosterone assay using new derivatizing agents. The current method allowed accurate, rapid, specific, and stable analysis of serum total testosterone. SST is an acceptable specimen type for the current method. Precision, accuracy, linearity, ion suppression, and sample stability in SST were validated.

A-040

Night shift work could induce oxidatively generated damage to DNA: A pilot study

Y. Zou, X. Ma, S. Yu, L. Qiu. *Peking Union Medical College Hospital, Beijing, China*

Background: Currently, specific effects of different night shift modes on human oxidative stress still need to be explored in a realistic cohort study. We aimed to evaluate the effect of night shift on human nucleic acid damage status.

Methods: We recruited 16 healthy volunteers (aged, 33 ± 5 years) working the night shifts at the Department of Laboratory Medicine at Peking Union Medical College Hospital. Their matched serum and urine samples were collected at four time points: before, during (twice), and after the night shift period. The levels of 8-oxo-7,8-dihydroguanosine (8-oxoG) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), two important nucleic acid damage markers, were accurately determined based on a robust self-established LC-MS/MS method. The Mann-Whitney U or Kruskal-Wallis test was used for comparisons.

Results: The levels of serum 8-oxodG, estimated glomerular filtration rate corrected serum 8-oxodG, and 8-oxodG in serum to urine ratio significantly increased during the night shift period. These levels were significantly higher than at baseline even after 1 month of discontinuing; but no such significant change was found for 8-oxoG. Moreover, 8-oxoG and 8-oxodG levels revealed a significantly positive association with many routine biomarkers such as total bilirubin and urea levels, and significantly negative association with serum lipids such as total cholesterol levels.

Conclusion: The results of our cohort study suggested that working the night shifts may increase the oxidatively generated damage to DNA even after a month of discontinuing night shift work. Further studies with large-scale cohorts, different night shift modes, and longer follow-up times are needed to clarify the short- and long-term effect of night shifts on DNA damage, and find effective solutions to combat the negative effects.

A-041

Measurement of plasma 3-Hydroxyisovalerylcarnitine by LC-MS/MS to predict marginal biotin deficiency in inflammatory bowel disease

B. Patil[1], A. Bock[1], F. Armbruster[1], J. Stein[2], M. R. Diesner[1], A. Askan[3]. *[1]Immundiagnostik AG, Bensheim, Germany, [2]Institute of Pharmaceutical Chemistry Goethe University, Frankfurt, Germany, [3]Institute of Nutritional Science, Justus-Liebig-Universität, Giessen, Germany*

Background:

Biotin's role in the immune response have emergently revealed that biotin is related to inflammation and that its deficiency leads to an increase in the level of proinflammatory cytokines. A biotin-deficient diet was recently shown to induce a colitis-like phenotype in mice, alleviated by biotin substitution. Mice with dextran sulphate sodium (DSS)-induced colitis showed biotin deficiency and significantly reduced biotin absorption. Oral biotin substitution reversed DSS colitis and induced remission. Diagnosis of biotin deficiency is rather challenging since based solely on measurement of circulating biotin levels has been shown to be insufficiently sensitive for clinical purposes. Increased urinary excretion of biotin metabolites, is suggested as a sensitive indicator of biotin deficiency. However, 24 h urine collection is time consuming, expensive, and the test relies on correct collection of samples by the patient, thus if this is not done properly the results may be inaccurate. 3HIVc increases in the case of biotin deficiency as a response to the decreased activity of biotin dependent enzyme methyl crotonyl-coenzyme. Analysis of circulating 3HIVc levels by LC-MS/MS has been found to be one of the most sensitive markers of biotin depletion.

Methods:

Study cohort conducted with 100 inflammatory bowel disease (IBD) patients (20-60 years, 40 females) whom diagnosed according to standard clinical, radiological and pathological criteria and 100 healthy controls (20-60 years, 40 females). Of the patients 58/100 had Crohn`s Disease (CD), 42/100 had ulcerative colitis (UC) and 29/100 were in inflammatory state (serum high sensitivity C reactive protein (hsCRP) concentrations >5mg/L). Standard serum and citrateplasma samples were withdrawn between 8:00 - 10:00 hours in the clinic after overnight fasting and stored at -20 °C until the analysis. Complete blood count (CBC), high sensitivity C reactive protein (hsCRP), fecal calprotectin (fCal) were determined with standard methods in serum matrix. 3-Hydroxyisovalerylcarntine (3HIVc) levels were determined by a commerical LC-MS/MS Kit in citrate plasma (KM3200, Immundiagnostik AG, Germany).

Results:

Patients with IBD were found to have significantly higher 3HIVc levels than controls (6.1±2.4 ng/mL vs 5.1±2.1 ng/mL respectively, p = 0.004). 3HIVc concentrations were compared according to different disease characteristics within the patient group: circulating 3HIVc levels were found to be similar in patients with CD and UC (5.2±2.1 ng/mL vs. 4.9±2.1, p = 0.451). Patients were also found to have similar circulating 3HIVc concentrations irrespective of whether or not inflammatory activity was present (5.1±2.0 ng/mL in inflammatory vs. 5.7±2.4 ng/mL in noninflammatory conditions, p = 0.131).

Conclusion:

The clinical findings of this study are in line with the existing preclinical evidence indicating that biotin deficiency is more common in patients with IBD in comparison to healthy controls in a clinical setting. However, surprisingly, the biotin status of patients with IBD was not found to differ according to inflammatory status. Therefore, there is still a room for more comprehensive look into the relationship between IBD related inflammation and biotin deficiency. Moreover, LC-MS/MS analysis of plasma 3HIVc has the potential to help clinicians to define biotin repletion and help to clearify the latter relation.

A-042

Comparison of the Second-Generation Parathyroid Hormone Assays: ELSA-PTH and Alinity i Intact PTH

J. Kang[1], J. Park[2], H. Choi[1], S. Kee[1]. *[1]Chonnam National University Hospital, Gwangju, Korea, Republic of, [2]Chonnam National University Hwasun Hospital, Hwasun, Korea, Republic of*

Background: Measurement of parathyroid hormone (PTH) level is essential in diagnosing and treating hyper- and hypoparathyroidism. Because of the various presenting forms of PTH (biologically active full-length PTH and also PTH fragments) in the blood and the lack of standardization, there can be a difference in results between the assays even when the same assay generation is used. We compared the two second-generation PTH assays, ELSA-PTH and Alinity i Intact PTH, and evaluated the analytical performance of Alinity i Intact PTH, newly adopted in the laboratory. **Methods**: For method comparison, 56 remnant samples of routinely ordered sera for the PTH test were collected. ESLA-PTH assay tested on Gamma-10 contains the anti-[39-84, amino acids] PTH antibody coated on the tube, which captures PTH in the sample and is measured by the radioactivity through the bound I^{125}-labelled anti-[1-34] PTH antibody. Alinity i Intact PTH assay performed on Alinity i system uses the anti-[39-84] PTH antibody coated on a paramagnetic microparticle and is analyzed by the emitted light through the captured acridinium-labeled anti-[1-34] PTH antibody. The correlation between the two tests was evaluated. Additionally, precision, linearity, and reference range verification were analyzed to investigate the performance of the Alinity i Intact PTH assay. **Results**: Of the 56 samples, 35 (62.5%) were requested by the Nephrology department for evaluating chronic kidney disease, 16 (28.6%) by the General surgery department during and after parathyroid and thyroid surgery, and 5 (8.9%) by other departments. 52.3% of the patients were female, and the median age was 61.5 years (interquartile range: 45.3 - 71). The median values of ELSA-PTH and Alinity i Intact PTH were 69.0 pg/mL and 101.9 pg/mL, respectively, and the PTH values of Alinity i Intact PTH were significantly higher ($P < 0.0001$) by the Wilcoxon test. The Pearson correlation coefficient (r) was 0.959, and the average difference between the two tests was 78.57 pg/mL. In Alinity i Intact PTH, the coefficient of variation obtained at each low (10.5 pg/mL), medium (66.6 pg/mL), high (254.6 pg/mL) levels were 1.9%, 2.4%, and 1.8% for repeatability, and 2.03%, 2.71%, and 2.65% for within-laboratory precision, respectively. Linearity was observed within the analytical measuring range of 3 - 3,000 pg/mL. The reference range (15 - 68.3 pg/mL) recommended by the manufacturer was validated with 20 normal sera. **Conclusion**: The PTH values of Alinity i Intact PTH were significantly higher than those of the ELSA-PTH, and both assays showed a strong correlation. Additionally, the Alinity i Intact PTH assay demonstrated acceptable analytical performance.

A-044

Using ID-Vit test systems to assess the effect of vitamin B supplementation on fatigue symptoms in professional athletes

K. Erpenbach[1], M. C. Erpenbach[1], D. Maier[2], H. Seidl[2], A. Aksan[3], W. Mayer[4], S. Mücke[1]. *[1]Institut für medizinische Leistungsoptimierung und Trainingssteuerung, Erftstadt, Germany, [2]Immundiagnostik AG, Bensheim, Germany, [3]Institute of Nutritional Science, Justus Liebig University of Giessen, Giessen, Germany, [4]Lab4more GmbH Bavariahaus, München, Germany*

Background: Training and competition stress may result in temporary decrements (muscle damage, impaired immune system) in physical performance and significant levels of fatigue in professional athletes. Evidence suggest a causal association between these prevalent fatigue symptoms, temporary decrements and low serum levels of B vitamins. Therefore, it is suggested that supplementing B vitamins has a positive effect on reducing fatigue symptoms. However existing studies failed to show the connection with the traditional methodology like HPLC or LC/MS where only one analyte is quantified. Thus, the aim of this study was to assess the effects of vitamin B complex supplementation on fatigue symptoms, in professional athletes by measuring bioavailable vitamins with ID-vit test systems, that quantifies the bioavailable B vitamins.

Methods: The serum samples of 57 professional athletes (12 female, 23.6±6.9years) before and after 3 months of daily vitamin B complex supplementation (20mg thiamine, 15mg riboflavin, 30mg niacin, 10mg pantothenic acid, 15mg pyridoxal-5-phosphate, 400µg 5-methyltetrahydrofolate, 1000µg methylcobalamine, 1000µg biotin) were compared. CBC was determined by standard tests. Bioavailable vitamins B_2, B_6, folic acid, B_{12} in serum and B_1 in whole blood were measured by the ID-*Vit*® test kits from Immundiagnostik AG. The test system is based on a microbiological method. The microorganisms used in this test system can metabolize various vitamers that are equally available in vivo. Thus, a functional system is simulated that quantifies the bioavailable B vitamins in comparison to other methods as e.g. HPLC or LC/MS, where only one analyte is quantified. The results of the methodology correlate very well with the observed symptoms and are therefore a promising tool for therapy monitoring. Using 5-point Likert scales, fatigue, sleep quality, muscle damage and impairment of immune system were assessed at the baseline and after 3 months intervention.

Results: Ultimately 29 professional athletes (10 female, 22.6±7.9years) were completed the study. The bioavailable vitamin B levels were significantly higher after the 3 months supplementation period. (for vitamin B_1 35.6±9.4µg/L and 48.1±18.3µg/L, for vitamin B_2 99.9±63.3µg/L and 125.0±66.4µg/L, for vitamin B_6 10.2±13.8µg/L and 30.7±29.2µg/L, for vitamin folic acid 9.3±7.4µg/L and 12.7±9.1µg/L, for vitamin B_{12} 362.9±335.2µg/L and 445.2±367.5µg/L respectively). The level of feeling tired significantly lowered (6.0±2.9 vs 1.9±1.8, $p<0.001$) after 3 months in parallel to higher serum bioavailable vitamin B levels. The professional athletes significantly less troubles with their sleep quality (34.5% vs 6.9%; $p<0.001$), muscle damage (58.6% vs 6.9%; $p=0.033$), and infection rate (44.8% vs 0.0%; $p<0.001$). Performance fatigue was reported in 75.9% and mental fatigue was reported in 79.3% of the athletes before vitamin B complex supplementation and significantly decreased to 0.0% and 13.8% after 3 months of supplementation period ($p<0.001$).

Conclusion: Low serum bioavailable vitamin B values found to be related to fatigue, sleep quality, muscle damage and impairment of immune system. The supplementation of B complex vitamins was effective on reducing fatigue symptoms, infection frequency, muscle damage and helped increasing sleep quality. The ID-vit tests system is an effective and easy to apply tool to quantify the bioavailable B vitamins in clinical settings to assess undersupply.

A-046

Investigating the Role of RNase L in Metabolic Syndrome

A. A. Zame, D. Liu, A. Zhou. *Cleveland State University, Cleveland, OH*

Background: Metabolic syndrome is closely associated with the incidence of heart disease, type 2 diabetes, and stroke. Though the exact cause of metabolic syndrome is not known, many features of the metabolic syndrome are caused by insulin resistance. Recent years, accumulating evidence has shown that dysregulation of insulin signaling leads to insulin resistance. Interestingly, we have observed that RNase L affects the insulin signaling pathway by regulating the activation of insulin receptor subtrate-1 (IRS-1) in primary mouse embryonic fibroblasts (MEFs) and primary mouse hepatocyte. RNase L is an enzyme that plays a critical role in interferon function against viral infection and cellular proliferation. How RNase L affects the insulin signaling pathway is still unknown. **Methods**: Age-matched C57BL/6 background RNase L wild type (WT) and knockout (KO) mice (n = 6 - 8) were fed on a chow diet. Metabolic tolerance tests including glucose tolerance test (GTT) and insulin tolerance test (ITT) were conducted on mice between 12 - 16 weeks old. Mice were subjected to either a morning (4 hr) or an overnight (12 hr) fast, then intraperitoneally injected with glucose (1.5g/Kg body weight) in PBS, pH 7.4, for GTT and insulin (1.0IU/Kg body weight) for ITT. Blood was collected via tail vein at different time intervals over a span of 2 hr and blood glucose was measured using a glucometer. **Results:** We found that RNase L KO mice displayed significantly increased glucose tolerance in compared to RNase L WT mice as measured in the intraperitoneal glucose tolerance test. The glucose level in RNase L KO mice was 150% higher than that in RNase L WT mice after IP injection of glucose in 60 min. In the ITT experiment, the glucose level in RNase L KO mice was 130% higher than that in WT mice in 60 min post injection. Taken together, the results in GTT and ITT indicated that RNase L mediates the metabolism of glucose in vivo. Further investigation of molecular mechanisms is currently being conducted.**Conclusion:** Our results suggests that RNase L plays a role in insulin sensitivity, which affects glucose metabolism.

A-047

Role of the Gut Microbiota in the Efficacy of Dietary Intervention With Flavonoid-Containing Foods in Obesity

S. Alqudah[1], B. DeLucia[2], L. Osborn[2], R. Markley[2], V. Bobba[2], L. Hamidi Nia[2], J. Claesen[2]. *[1]Cleveland State University/Cleveland Clinic, Cleveland, OH, [2]Cleveland Clinic, Cleveland, OH*

Background:

Flavonoids are an active component of plant-based diets that have been investigated for their beneficial antioxidant properties in high fat diet induced obesity animal models and human intervention trials. Certain bacterial members of the gut microbiota have been reported that transform flavonoids into monophenolic acids. Our goal is to characterize the microbiome link between a flavonoid-rich diet and improved metabolic parameters characteristic of obesity. We hypothesize that flavonoid supplemented diets are most effective in presence of microbiota capable of monophenolic acids formation, and that these bacterial metabolites are a main bioactive compound responsible for the improved metabolic parameters.

Methods:

To test our hypothesis, males and females C57BL/6 mice were fed a high fat control diet, or the same diet supplemented with 1% berry extract, or 1% monophenolic acid for 12 weeks. Separate experimental groups received either regular drinking water or antibiotic water to suppress their gut microbial community. We monitored metabolic parameters, including body weight, lean and fat mass, glucose and insulin tolerance, histological assessment of liver cell, quantification of liver injury biomarkers, and transcriptional changes in metabolically active peripheral tissues.

Results:

For insulin tolerance test, mice on high fat diet with regular water, or antibiotic water control mice had a comparable increase in insulin resistance, demonstrated by the increase in glucose levels after injecting insulin. Similarly, the groups of mice on high fat diet with 1% monophenolic acid and regular water, or antibiotic water, had improved insulin sensitivity, presented as a decrease in glucose levels after insulin injection. Consistent with our hypothesis, mice on high fat diet supplemented with 1% berry extract had an improved insulin sensitivity, and this effect was dependent on an intact microbiome. This suggests that the gut microbiota are required to metabolize the berry extract to produce bioactive metabolites that improve insulin tolerance. We also observed a significant decrease in fasting blood glucose, an improvement in the expression for genes involved in lipid and glucose metabolism, and a noticeable reduction in hepatic steatosis in both 1% monophenolic acid and 1% berry extract with high fat diet group, compared to the high fat diet control group.

Conclusion:

By feeding the mice a flavonoid rich diet, with the presence of an intact gut microbiota, we can improve the metabolic parameters associated with metabolic diseases. Our work will open the door for new benign approaches for treatment, such as dietary flavonoid supplementation with concomitant gut microbial probiotic therapy.

A-048

Accurate Measurement of Free Testosterone in Serum using Equilibrium Dialysis Coupled with ID-UHPLC-MS/MS

H. Zhou[1], A. Ribera[2], U. Danilenko[1], H. Vesper[1]. *[1]Centers for Disease Control and Prevention, Atlanta, GA, [2]Battelle, Atlanta, GA*

Background: Assessment of free testosterone (FT) has been recommended in recent clinical practice guidelines as a biomarker for diagnosis and management of testosterone related disorders such as male testosterone deficiency due to hypogonadism and female androgen excess due to polycystic ovary syndrome (PCOS). FT is currently measured using direct commercial immunoassays, assays using equilibrium dialysis (ED) or other separation techniques. Though the ED approach is recognized as the recommended approach, it is laborious and technically challenging for routine clinical use. The CDC Clinical Standardization Program is developing a high throughput automated method using the ED procedure coupled with isotope dilution ultra-high-performance liquid chromatography tandem mass spectrometry (ID-UHPLC-MS/MS) that is suitable for routine patient care and large-scale epidemiologic studies.

Methods: Serum samples are dialyzed in a custom-designed multi-well plate against a protein-free HEPES buffer (pH 7.4) at 37 °C until equilibrium. After isolating endogenous FT from protein-bound testosterone by ED, isotope-labeled internal standard ($^{13}C_3$-testosterone) was added to the dialysate for quantification. Certified pure primary reference material (National Measurement Institute-M914) was used to prepare calibrators, enabling traceability and ensuring measurement trueness. FT was further isolated from the dialysate matrix using supported liquid extraction and a chromatographic separation from interfering compounds and quantitation by tandem MS.

Results: The measurement ranges covered 0.2 - 1000 ng/dL for testosterone, with the bias within ±5% and precision less than 10% CV. A total of 45 samples with a wide range of total testosterone (TT, 21-912 ng/dL) and SHBG (11-129 nmol/L) were analyzed and showed the suitability of this assay to measure the serum free testosterone levels in normal, hypogonadal males as well as in the majority of normal females and females with androgen excess. The application of 48-well format plate and automated liquid handler system significantly improves the throughput of sample preparation. In addition, comparison of the TT results measured with the presented TT method with calculated TT concentrations, suggest that on average calculation overestimates TT concentration by 34%.

Conclusion: The described high throughput method for FT allows for sufficiently accurate and precise measurement for routine applications including large epidemiologic studies to help with establishing reference intervals.

A-049

Inter-assay Variation of Insulin-like Growth Factor-1 Remains an Issue

A. S. Ptolemy, C. MacDonald, V. Browne, R. W. Peake, M. D. Kellogg. *Boston Children's Hospital, Boston, MA*

Background: Human insulin-like growth factor-1 (IGF-1) is the main mediator of the somatotropic effects of growth hormone (GH). Accurate measurement of IGF-1 is required for the diagnosis and management of GH secretion disorders. Several commercial vendors and laboratories have standardized their IGF-1 assays to the WHO International Standard 02/254. This standardization does not prohibit the existence of significant inter-assay variability in IGF-1 measurements. Such concentration variance can lead to inaccurate patient case decision making. The IGF-1 testing performed in our laboratory was recently migrated from an Immulite 2000 platform to a CobasPro system. This shift in vendor necessitated an investigation of the inter-assay variability between these standardized tests. This relative variance was established through a validation of the analytical performance of the CobasPro IGF-1 assay. **Methods:** Reportable range, intra-day precision and accuracy of IGF-1 measurements using the CobasPro (Roche Diagnostics) system were determined. Patient correlation studies between the CobasPro and an Immulite 2000 (Siemens Healthcare Diagnostics) instrument was also performed. The concentration of IGF-1 in a subset of these correlation study specimens was also quantified by liquid chromatography mass spectrometry (LC-MS) assays from two different reference laboratories. **Results:** The CobasPro assay had a verified linear range of 7 to 1443 ng/mL (R^2=0.9998, slope=0.9915). At IGF-1 concentrations of 54 and 345 ng/mL the intra-day precision (%CV, N=10) of the CobasPro assay did not exceed 0.9%. Patient specimen correlations between the CobasPro and Immulite 2000 assays showed significant bias (Deming regression: y=1.543x-48.61, N=128, R=0.9641). This method bias was exacerbated at Immulite 2000 derived IGF-1 concentrations >100 ng/mL (Deming regression: y=1.662x-84.29, N=93, R=0.9492, CobasPro range=77 to 910 ng/mL, Immulite 2000 range=103 to 539 ng/mL). Patient specimen correlations between the CobasPro and LC-MS assay from reference laboratory A showed a positive bias (Deming regression: y=1.375x-64.5, N=19, R=0.9210). An analogous correlation with reference laboratory B demonstrated a higher level of concordance (Deming regression: y=1.005x+10.2, N=16, R=0.9499). The CobasPro and Immulite 2000 assays both produced acceptable result when challenged by multiple external quality assurance (EQA) specimens provided by the College of American Pathologists (CAP). However, the observed relative trends in IGF-1 concentrations from these challenges were opposite to our observations with authentic patient specimens. The CobasPro assay consistently produced lower IGF-1 concentrations relative to the Immulite 2000 assay for these EQA specimens. **Conclusion:** The CobasPro IGF-1 assay offered acceptable analytical performance. Although both assays were traceable to a reference standard, a significant positive concentration bias was observed between the CobasPro and Immulite 2000 assays. Standardization efforts have not eliminated inter-assay IGF-1 variability. A careful evaluation of the impact of this variance on the diagnosis and management of GH deficiency and acromegaly must be considered when IGF-1 testing platforms are changed.

A-050

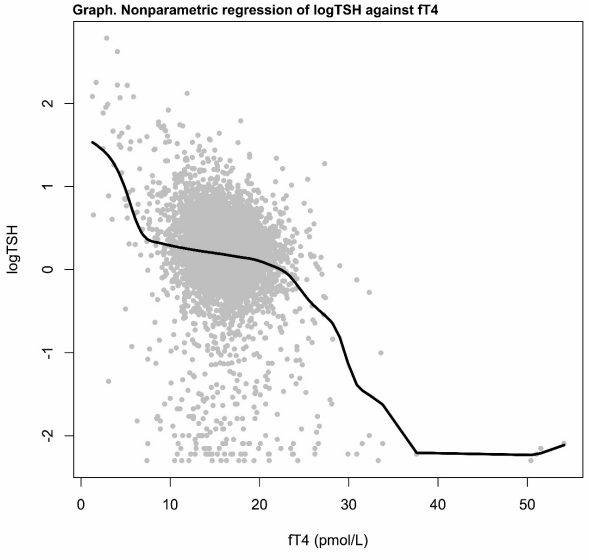
Understanding the complex relationship between TSH and free thyroxine

D. J. Lee[1], N. S. Che Rahim[2], T. C. Aw[1]. *[1]Changi General Hospital, Singapore, Singapore, [2]Kuala Lumpur Hospital, Kuala Lumpur, Malaysia*

Background: Guidelines suggest screening for thyroid disease using thyroid stimulating hormone (TSH) first before free thyroxine (fT4) since there is an inverse log-linear relationship between them; the signal favors TSH over fT4 in a ratio of >100:2. We reassessed this fT4 to TSH relationship following the improved 3rd generation TSH assay from Roche (since 09/2019).

Methods: Consecutive paired TSH/fT4 (n=7,484) from distinct ambulatory non-hospitalized subjects that were assayed on cobas e801 were retrieved from our laboratory information system and combined with similar data from Kuala Lumpur Hospital (n=11,369) that were assayed on cobas e601. Reference intervals and measuring limits for the Roche assays were 0.4-4.0µIU/L and 0.005-100µIU/L for TSH, and fT4 10-20pmol/L and 0.3-100pmol/L for fT4, respectively. Values outside the reportable limits (n=618) were excluded. Descriptive statistics, linear and nonparametric (kernel) regression were performed using the statistical software packages in R 4.2.0.

Results: Linear regression of logTSH against fT4 (n=18,235) gave an r=-0.35. Nonparametric regression revealed two negative sigmoid curves merging into the euthyroxinemic region (see Graph). Three different segments can be discerned; hypothyroxinemic (fT4<10): logTSH = 1.91 - 0.136fT4 (n=422; r=-0.39, P<0.001), euthyroxinemic (fT4 10-20): logTSH = 0.672 - 0.028fT4 (n=15,754, r=-0.13, p<0.001), and hyperthyroxinemic (fT4>20): logTSH = 1.106 - 0.055fT4 (n=2,059, r=-0.33, P<0.001).



Conclusion: The relationship between fT4 and TSH is complex. The widely accepted view of a constant gradient of logTSH against fT4 over the whole range of thyroid test values is inaccurate. Three distinct relationships correspond to each biochemical

thyroid status. In the hypothyroxinemic and hyperthyroxinemic range, TSH increases almost 5-fold and 13-fold with a doubling of fT4, respectively. In the euthyroxinemic range, there is no relationship between logTSH and fT4. While a TSH first strategy is applicable in hypo/hyperthyroxinemia, both TSH/fT4 are still required to confirm diagnoses and for baseline assessment prior to commencing therapy.

A-051

Reference Intervals for Serum T4 and FT4 among Pregnant Women linked to the Mãe Curitibana Program

R. F. Paula[1], T. M. Telles[2], P. C. Felchner[3], K. D. Valdati[4], C. R. Zuanazzi[5], T. M. Panizza[4], T. P. Moraes[3], C. O. Junior[6], G. A. Carvalho[6], G. B. Carlos[1]. *[1]Siemens Healthineers, São Paulo, Brazil, [2]University Federal from Parana, Curitiba, Brazil, [3]Pontifical Catholic University, Curitiba, Brazil, [4]Municipal Laboratory-SMS, Curitiba, Brazil, [5]Municipal Laboratory - SMS, Curitiba, Brazil, [6]Hospital das Clinicas Complex / Federal University of Paraná, Curitiba, Brazil*

Background: Thyroid diseases are frequent in women of childbearing age and can have negative maternal and fetal repercussions when not identified and properly treated. Physiological changes during pregnancy cause changes in thyroid function tests, which is why they must be carefully interpreted and, whenever possible, based on local and specific reference values for pregnant women. Determining the serum level of total (T4T) or free (FT4) T4 is fundamental for defining the diagnosis of thyroid dysfunctions between clinical (TSH and T4 altered) or subclinical (only TSH altered). This definition modifies the treatment recommendations for thyroid disorders in various published guidelines. **Objectives:** To determine the reference intervals for FT4 and T4T of pregnant women, in the first trimester, residing in the city of Curitiba and followed by the municipal public health system. **Methods:** Longitudinal study with a sample of 225 pregnant women aged over 18 years, linked to prenatal care in the public health system (SUS) of Curitiba city, with up to 14 weeks of gestation. Pregnant women were selected proportionally to each health district in the city, to represent the entire population of the city served by the SUS. Pregnant women who had TSH outside the reference range, positive ATPO, family history of thyroid disease, use of antithyroid drugs, use of medications that could interfere with the measurement of the markers under study were excluded. The studied population had a median urinary iodine concentration considered adequate for pregnant women (158 mcg/L). Serum FT4 and T4T were measured by chemiluminescent immunoassay on Atellica IM Analyzer. Continuous variables were expressed as mean and standard deviation and percentiles were determined and variations between 2.5 and 97.5 percentiles were considered as the normal reference interval. The results were analyzed and compared with the reference interval provided by the manufacturer for non-pregnant women. **Results:** Of the 225 pregnant women (with mean ± standard deviation of gestational age 8 weeks ± 2.3; with mean ± standard deviation of age 27 years ± 5.9) the FT4 values of the pregnant women ranged between 0.83 and 1.68 ng/dL, with mean ± standard deviation of 1.23 ± 0.15. The 2.5th and 97.5th percentiles were 0.94 and 1.64 ng/dL. Compared with the kit manufacturer's reference values for non-pregnant women (0.95 to 1.47 ng/dl), we would have 08 pregnant women with a high FT4 result. The T4T values of the pregnant women varied between 9.2 and 12.2 ng/dL, with a mean ± standard deviation of 10.9 ± 2.48. The 2.5th and 97.5th percentiles were 9.2 and 12.2 ng/dL. Compared with the kit manufacturer's reference values for non-pregnant women (4.87 and 11.72 ng/dL), we would have 25 pregnant women with high T4T results. **Conclusion:** The FT4 and T4T reference intervals of first trimester pregnant women are different from the non-pregnant population, however this difference is less relevant in FT4. In T4T, the lower limit of normal in pregnant women is much higher than in the normal population, which makes it less sensitive for the definition of clinical hypothyroidism in first trimester pregnant women.

A-052

The importance of thyroid-stimulating immunoglobulin (TSI) in the diagnosis and follow-up of Marine-Lenhart syndrome

R. Fontes[1], M. M. Negri[2], P. Araujo[1], S. Marui[3], A. Gomes[3], Y. Schrank[1], A. Perozo[1], M. F. Pinheiro[1], D. M. Gomes[1], G. Campana[4], E. K. Tourinho[5], A. Vangelotti[6], J. T. Pena[6]. *[1]Dasa, Brasil; Instituto de Ensino e Pesquisa Dasa, Brasil, Rio de Janeiro, RJ, Brazil, [2]Instituto Estadual de Diabetes e Endocrinologia Luiz Capriglione (IEDE), Rio de Janeiro, RJ, Brazil, [3]Dasa, Brasil; Instituto de Ensino e Pesquisa Dasa, Brasil, São Paulo, SP, Brazil, [4]Instituto Estadual de Diabetes e Endocrinologia Luiz Capriglione (IEDE), São Paulo, SP, Brazil, [5]Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil, [6]Private Clinic, Rio de Janeiro, RJ, Brazil*

Introduction: Marine-Lenhart syndrome is a rare cause of hyperthyroidism, occurring in 0.8 to 2.7% of patients with Graves' disease (GD). In this syndrome, GD, which is an autoimmune disease, coexists with one or more hyperfunctioning nodules - Toxic nodular goiter (TNG), or toxic multinodular goiter (TMNG) - which are usually thyroid adenomas. Both induce clinical hyperthyroidism, with suppressed thyroid-stimulating hormone (TSH) and elevated thyroid hormones (TH). Radioactive iodine uptake and thyroid scintigraphy are indicated when there is a nodule on ultrasonography (US) with suppressed TSH, for the differential diagnosis between GD and toxic nodules. However, in this syndrome, scintigraphy does not always define the diagnosis, as the aspects observed do not show the usual pattern. In these cases, it is necessary that a reliable autoimmune thyroid disease marker be performed in order to prove the concomitant autoimmunity. **Objectives**: The aim of this study was to demonstrate the usefulness of thyroid-stimulating immunoglobulin (TSI) for diagnosing hyperthyroidism due to Marine-Lenhart syndrome. **Methods**: The authors prospectively followed 19 patients with suspected Marine-Lenhart syndrome between 2018 and 2020. TSH and TH were measured by Electrochemiluminescence, Roche Diagnostics, and TSI was measured by Chemiluminescence, Siemens Diagnostics. **Results**: All patients had TSH ≤ 0.01 mIU/L, free thyroxine (FT4) was 3.1±0.9 ng/dL, and triiodothyronine (TT3) 312±42.1 ng/dL. All of them had increased radioactive iodine uptake, but the scintigraphy showed nodules sometimes with increased, normo, or low uptake in relation to the adjacent tissue, a finding that is explained by the fact that, both, the nodule and the rest of the thyroid tissue, uptake the radioidine in excess and the pattern varies according to the activity of the DG and nodules in the period in which the test was performed. TSI was 3.98±0.9 IU/L (Negative if < 0.55). All patients had positive TSI, confirming concomitant GD with TNG/TMNG. During treatment monitoring, in 14 cases the hyperfunctioning nodules were treated by local sclerosis procedure, with percutaneous injection of ethanol. Despite complete nodular sclerosis, with normal TH but still suppressed TSH, the TSI still remained elevated. This demonstrated that, despite the successful treatment of the nodule, the underlying GD was still active, requiring complementary treatment with antithyroid drugs until TSI was negative, after 18±4 months of treatment. **Conclusion**: In atypical cases of hyperthyroidism, the TSI measurement is decisive for the diagnosis, as in the rare Marine-Lenhart syndrome. This contributes in a relevant way to the definition of the treatment, which can be different between the two causes that occur concomitantly. Likewise, during treatment follow-up, the TSI was essential in defining the need to maintain clinical treatment, even if the nodule had been already adequately treated.

A-053

Poor clinical utility of free and bioavailable testosterone is underscored by overutilization

C. D. Koch. *Sanford Health, Sioux Falls, SD*

Background Symptomatic androgen deficiency has an estimate prevalence of 2-12% in males over 40 years of age and is associated with several comorbidities such as hypertension, obesity, dyslipidemia, metabolic syndrome, diabetes, and COPD. Diagnosis relies heavily on the measurement of testosterone concentrations. While total testosterone (tT) is often sufficient to diagnose deficiency, free testosterone (fT) or bioavailable testosterone (baT; non-sex hormone binding globulin (SHBG)-bound) may be helpful in assessing patients with suspected hormone binding abnormalities. Consensus guidelines recommend relying on tT measurement and only using fT or baT testing when indicated. Despite these recommendations, requests for measured fT and baT account for a large percentage of testosterone orders in our institution. Here we examined the utilization of testosterone orders, assessed the clinical utility of measured fT and baT relative to tT alone, and devised a reflex algorithm to optimize fT and baT utilization.

Methods Testosterone data was collected over a 12 month period, September 2020 - August 2021. Only data from adult males with established reference intervals was

included in the final cohort; ≥20 and 20-69 years of age for fT and baT, respectively. Logistic regression was used to determine the probability of low fT and baT based on tT concentration. An algorithm based on initial tT concentration was established to reflex to fT/baT when most likely to provide additional benefit.

Results Of 9754 testosterone tests performed on male patients during the study period, 4514 (46%) included a measured fT and/or baT. After excluding data without established reference intervals, the final cohort consisted of 1851 fT and 2514 baT measurements. Overall concordance with tT was 70.0% and 74.8% for fT and baT, respectively. However, only 0.2% (3/1479) were found to have low fT and 8.4% (166/1988) low baT when tT was greater than the reference interval lower limit (i.e., normal or high). The probability of a patient having low fT or baT with normal tT peaked at 0.01 and 0.21, respectively, and was greatest at the tT reference interval lower limit (240 ng/dL). An algorithm was devised in which only samples with tT concentrations of 240 - 500 ng/dL would reflex to baT testing. In this cohort, 90% of discordant (low baT with normal tT) samples would have triggered the baT reflex while reducing the overall utilization of baT by 55%. Applying the same reflex algorithm would have also reflexed 100% of discordant fT samples.

Conclusions Despite being performed with nearly half of all testosterone orders, baT, and especially fT, provided little additional clinical utility to tT alone. By determining the probability of a low baT or fT when Tt is above the reference interval lower limit, a reflex algorithm can be devised to help optimize baT and fT utilization. While this algorithmic approach has been proposed by others, the reflex values in our cohort differ significantly from previously published values suggesting that laboratories should determine limits based on their individual testosterone assays. The logistic regression method utilized here provides one such example laboratories can follow for undertaking such an analysis.

A-054

Comparison of a Reformulated Vitamin D Immunoassay, Its Predecessor, and Two Contemporary Formulations

K. A. Geno[1], Y. F. Alvarez[1], M. A. Cervinski[1], J. A. Hubbard[2], R. D. Nerenz[3], R. J. Singh[4]. *[1]Dartmouth-Hitchcock Medical Center, Lebanon, NH, [2]Three Rivers Diagnostics, Pittsburgh, PA, [3]Medical College of Wisconsin, Milwaukee, WI, [4]Mayo Clinic, Rochester, MN*

Background: We took advantage of our verification of a new biotin-resistant 25-hydroxyvitamin D (vitamin D) immunoassay formulation to compare analytical performance of the outgoing formulation and assays from Roche, Diasorin and Beckman. Liquid chromatography with tandem mass spectrometry (LCMS) was an independent arbiter of vitamin D concentration and provided quantification of vitamin D_2 and vitamin D_3.

Methods: Twenty-five specimens from patients on high-dose vitamin D_2 supplementation (50000 IU/week) and fifty additional specimens spanning our measurable range were identified by the Dartmouth Hitchcock Analytics Institute. Aliquots were tested by the Roche cobas Vitamin D Total II and Vitamin D Total III assays, deidentified, split, and shipped to other study sites for measurement by LCMS, the Diasorin Liaison 25 OH Vitamin D Total assay, and the Beckman Coulter Access 25(OH) Vitamin D Total assay. Estimated vitamin D_2 was calculated by subtracting the LCMS-measured vitamin D_3 result from the immunoassay total vitamin D result. Bias calculations assume LCMS measurements as the true concentration. Statistical analyses were performed in R. This study was approved by the Dartmouth Hitchcock Medical Center Institutional Review Board.

Results: No significant differences were observed between platforms in absolute or relative bias in total vitamin D or estimated vitamin D_2. Clinical classification was unanimous in 62/75 specimens. For thirteen specimens in which at least one disagreement was observed, immunoassay results for discrepant classification were no more than 10.2 ng/mL from the classification cutoff (median 2 ng/mL). Two specimens with 68 and 70 ng/mL vitamin D were classified as toxic (>100 ng/mL) by one immunoassay each.

Conclusion: Current vitamin D immunoassays continue to improve over their predecessors and offer good agreement across platforms. Vitamin D_2 recovery no longer represents a clinically-concerning obstacle. These immunoassays display positive bias for total vitamin D relative to LCMS but generally result in correct clinical classification.

	Total Measurement[a,b]		Estimated Vitamin D_2[a,b,c]		
Platform	**Bias (ng/mL)**	**Percent Bias (%)**	**Bias (ng/mL)**	**Percent Bias (%)**	**Classification Concordant with LCMS, n (%)**
Beckman	5.1 (2.9 – 7.3)	12.8 (7.5 – 18.0)	-2.2 (-3.4 – -1.1)	-1.6 (-10.6 – 7.4)	67/75 (89.3)
Diasorin	4.4 (2.3 – 6.6)	10.7 (6.0 – 15.4)	-1.2 (-2.7 – -0.3)	5.5 (-5.3 – 16.2)*	68/75 (90.7)
Roche Total II	3.8 (1.8 – 5.9)	9.5 (5.1 – 14.0)	-5.9 (-7.6 – -4.2)	-18.7 (-25.6 – -11.8)*	70/75 (93.3)
Roche Total III	4.3 (2.4 – 6.3)	8.7 (4.8 – 12.5)	-3.2 (-4.3 – -2.1)	-11.5 (-18.9 – - 4.0)	70/75 (93.3)

[a]Data are presented as means with 95% confidence intervals for the 72 samples with measurable values across all platforms. Bias calculated as the LCMS measurement subtracted from the immunoassay measurement.
[b]Bias and percent bias were analyzed by one-way ANOVA with repeated measures and were not statistically significant unless noted
[c]Estimated vitamin D_2 is calculated by subtracting the LCMS vitamin D_3 measurement from the immunoassay total
*, $P < 0.05$ by one-way ANOVA with repeated measures and subsequent pairwise Bonferroni-corrected t-tests

A-055

Clinical Performance Evaluation of the ADVIA Centaur AMH Assay

K. Freeman[1], J. Snyder[1], C. Brown[1], R. Singleton[1], R. Christenson[2], S. Donald[2]. *[1]Siemens Healthineers, Tarrytown, NY, [2]University of Maryland, Baltimore, MD*

Background: Anti-Müllerian hormone (AMH) is a transforming growth factor beta protein synthesized by granulosa cells of small growing follicles in ovaries in females and by Sertoli cells of the testes in males. AMH measurements are increasingly being used in the assessment of ovarian reserve, prediction of response to controlled ovarian stimulation in women undergoing assisted reproductive technologies, as an aid in the diagnostic evaluation of polycystic ovary syndrome, and prediction of menopause. The objective of this study was to evaluate the clinical performance of the ADVIA Centaur® AMH assay in the assessment of ovarian reserve and compare performance to the Beckman ACCESS 2 AMH assay. In addition, an exploratory analysis evaluated ADVIA Centaur AMH assay performance in distinguishing women with expected poor ovarian response per published criteria. **Methods:** Clinical specimens were collected from 577 women at 11 U.S. fertility centers. Women enrolled were 22–45 years old and possessed both ovaries. They underwent blood draw and transvaginal ultrasound to measure the antral follicle count on days 2–4 of menstrual cycle. Women with BMI ≥40; pregnancy within 3 months; on contraceptives, hormonal medications; undergoing treatment for malignancy, ovarian abnormalities, surgery in the past 6 months; or with endocrine or metabolic disorders (including PCOS) were excluded. **Results:** Results were considered positive (high ovarian reserve) if the AMH concentration was >1.77 ng/mL and AFC was >15 and negative (normal-to-diminished ovarian reserve) if the AMH concentration was ≤1.77 ng/mL and AFC was ≤15. The estimated sensitivity, specificity, and two-sided 95% Wilson score confidence intervals (CI) of each parameter were determined to be 90.76% (86.97–93.53) and 52.19% (46.29–58.03) using the ADVIA Centaur AMH assay, and 90.10% (86.22–92.98) and 52.19% (46.29–58.03) with the ACCESS 2 AMH assay, respectively.A secondary analysis was completed to investigate poor ovarian response (POR) according to the Bologna criteria, published by the ESHRE. These criteria define poor ovarian response as an AMH measurement of less than 1.1 ng/mL and AFC of less than 7. Of the 577 women included in the study, 32 women had an antral follicle count less than 7, of whom 26 also had a AMH concentration of less than 1.1 ng/mL. Of the 545 women with an antral follicle count greater than or equal to 7, 480 also had AMH measurements of greater than or equal to 1.1 ng/mL. Using the Bologna criteria, the ADVIA Centaur AMH assay performs with a sensitivity of 81.25% and specificity of 88.07%. **Conclusions:** In this clinical population, the sensitivity and specificity of AMH assays on the ADVIA Centaur XP and Beckman ACCESS 2 systems were almost identical when using an AMH cutoff ≤1.77 ng/mL and AFC ≤15 to identify normal-to-diminished ovarian reserve. Using published criteria for POR resulted in a sensitivity and specificity of 81.25% and 88.07% for the ADVIA Centaur AMH assay.

Not available for sale in the U.S. Future availability cannot be guaranteed. Product availability varies by county.

HOOD#05162003331923

A-056

Data driven approach to ensure accurate total testosterone results in consumer initiated, home-collected capillary samples

A. R. Naranjo[1], C. Carter[1], J. Chen[1], A. Hamdan[1], M. D. Krasowski[2], K. O'Connor[1], D. N. Greene[1]. *[1]LetsGetChecked Laboratories, Monrovia, CA, [2]University of Iowa, Iowa City, IA*

Background: Home-collected direct access testing (DAT) has become increasingly attractive as telehealth utilization advances. With DAT, consumers initiate their lab order and have access to clinical consulting staff. Steroid hormone concentrations are uniquely positioned for DAT due to their inherent stability and non-acute indications. Total testosterone (TT) is used to screen for or monitor treatment of hypogonadism. Once diagnosed, testosterone is administered by intramuscular or subcutaneous injection or via transdermal patch or topical gel. TT in adult men is typically between 200-

900ng/dL. However, in our DAT lab we were encountering a significant percentage of samples with supraphysiological TT concentrations (>1500ng/dL). The objective of this study was to design a quality assurance workflow to differentiate whether the supraphysiological results were accurate or erroneous.

Methods: TT was measured using the Roche Cobas e801 immunoassay; basic assay parameters were verified per general CAP guidelines (precision, accuracy, linearity, method comparison, interferences, carryover, dilution). Additional validation experiments were performed to verify that capillary plasma was an acceptable sample type (n=30 paired capillary/venous samples) and to determine if extreme summer temperature fluctuations would influence stability (n=46 participants; samples subjected to temperature cycling for 72 hours before re-evaluation). Self-collected capillary samples received for clinical testing with a TT concentration >1500ng/dL were run on a 1:5 dilution (extending the clinical reportable range to 7500ng/dL) and the sample was reflexed to luteinizing hormone (LH) and follicle stimulating hormone (FSH). Reference intervals in adult men for LH and FSH are 1.7-8.6mIU/mL and 1.4-13.0mIU/mL, respectively. When available, clinical notes were reviewed for additional information on exogenous hormone usage.

Results: All general assay parameters were within acceptable metrics. TT concentrations between capillary and venous samples had an average absolute bias of -9.2ng/dL or -3.8% (n=30; range= <20-823ng/dL; y=0.954x + 1.915; R=0.995). Baseline and post-summer temperature cycling TT concentrations had an average absolute bias of -8.1ng/dL or -3.4% (n=46; range= <20-716ng/dL; y=0.934x + 7.728; R=0.999). Samples (n=506) with a TT concentration >1500ng/dL were reflexed to TT dilution and LH/FSH. Most (n=413; 82%) had undetectable (<0.3mIU/mL) LH/FSH and a mean TT concentration of 3,356ng/dL (SD=1,972). For 18.6% (n=77) of this subset, we had medication information; 68 were using injectable testosterone. Elevated TT was likely a true result caused by overuse of anabolic steroids. A smaller subset of samples (n=21) had detectable LH/FSH with mean concentration/SD of 6.1/5.2mIU/mL for LH, 5.3/5.4mIU/mL for FSH, and TT >7500ng/dL. Medication notes were available for 5 of these samples and indicated that the TT results were likely caused by topical contamination (false positive). A final subset (n=40) had detectable LH/FSH and TT between 1500-7500ng/dL. These samples could not be binned into simple categories and instead were sent to the lab director for review.

Conclusion: Self-collected capillary specimens are acceptable for TT measurements. A quality assurance reflex to LH/FSH can support or refute the validity of supraphysiological results in a consumer initiated/DAT population.

A-057

Cerebrospinal fluid prolactin in patients with neurological disorders

I. A. Hashim, E. Abraham. *UT Southwestern Medical Center, Dallas, TX*

Introduction: Prolactin a 23 kDa glycoprotein produced by the anterior pituitary among other cells with possible immunological properties. The presence and significance of prolactin in cerebrospinal fluid (CSF) is not widely known. This study examined prolactin levels in CSF from patients being investigated for neurological disorders.

Methods: CSF samples (leftover) from patients being investigated for neuropathy were obtained and stored at 4C or at -20C if not analyzed within one week of collection. Prolactin levels were measured using a high sensitivity prolactin ELISA (R and D Systems, Inc., MN, USA) according to manufacturer's instructions. CSF samples reported total protein and glucose levels were obtained and correlated with measured prolactin levels. Statistical analysis were performed using NCSS statistical software,

Results: A total of 53 CSF (leftover samples) were analyzed for prolactin content. Prolactin was detectable and levels ranged from 2.68 ng/mL to 12.1 ng/mL (mean 4.49 ng/mL). Total protein ranged from 21 mg/dL to 219 mg/dL (mean 67.5 mg/dL). CSF glucose levels ranged from 16 mg/dL to 114 mg/dL (mean 63.9 mg/dL). Although there was poor (r= 0.66) but significant (P< 0.003) correlation between CSF prolactin and total protein levels, there was no correlation (r=-0.1) between CSF prolactin and CSF glucose levels (P=0.6).

Conclusion: Prolactin levels was detected in CSF from patients being investigated for neurological dysfunction. There was correlation with total protein and glucose levels. The clinical significance of CSF prolactin is unknown and remains to be elucidated.

A-058

Frequency of biochemical assessment of risk for heart failure among diabetic population

J. Joseph[1], J. Neeley[2], I. A. Hashim[2]. *[1]University of Texas at Dallas, Dallas, TX, [2]UT Southwestern Medical Center, Dallas, TX*

Background: Diabetes remains a prevalent chronic health condition. Patients with diabetes have nearly twice the risk of developing heart failure as the general population and the American Diabetes Association recommends annual assessment by measuring N-terminal pro-B-type natriuretic peptide (NT-proBNP) or BNP among diabetic patients. We evaluated the frequency of biomarker assessment among our diabetic population. Method: Retrospective analysis of glycated hemoglobin (A1c) and NT-proBNP measured over a period of 21 months were performed. The frequency of NTproBNP assessment among patients with diagnostic A1c values were determined. Statical analysis were performed using Microsoft® Excel and NCSS® statistical software. Results: A total of 74,973 A1c and 23,540 NT-proBNP measurements were performed during the study period. Among diabetics, 16% of patients received NT-proBNP testing with about 38% exhibiting elevated values. Conclusion: This preliminary and retrospective study indicated that diabetic patients are receiving biochemical assessment for heart failure and that a significant number are of diagnostic value. It is not clear if other clinical modalities for heart failure assessment are in effect and if increased utility of biochemical assessment is required. Further studies are required to assess the utility among our patient's population and on the best protocol to apply.

A-059

Evaluation of Free Thyroxin levels in Patients with Low Thyroxin Binding Globulin Concentrations: is there interference in the FT4 indirect method?

M. F. Pinheiro, A. C. Lopes, T. S. Souza, R. Fontes, Y. Schrank, A. F. Perozo, D. M. Gomes, P. B. Araujo. *DASA, Rio de Janeiro, Brazil*

Background: Thyroxine-binding globulin (TBG) is the major carrier of thyroid hormones in serum. Variations in the serum concentration of TBG determine proportional variations in the total serum concentrations of T4 and T3, without implying changes in function, as long as the free fraction remains normal. Several common clinical conditions lead to significant alterations in TBG levels, the most important factor behind low levels being genetic defects. As TBG is encoded by a gene located on the X chromosome, the defects are more easily manifested in males. Differences in FT4 concentrations were observed in sera from subjects with TBG abnormalities, when FT4 are measured by different immunoassays, specially at extremes of TBG concentrations. The objective of this study was to evaluate FT4 levels by indirect method in patients with low TBG concentrations.

Methods: We analyzed blood samples requesting serum TBG, TSH and FT4 levels from patients admitted to a private reference clinical laboratory in Brazil, from 01/01/2017 to 12/31/2022. Anonymized data on laboratory tests was available from a database of the local Laboratory Information System. All the patients included had TSH within the reference range, 0.4 to 4.3 mUI/mL (ECLIA, Modular, Roche). FT4 was measured by ECLIA (0.7 to 1.8 ng/dL) and TBG by ICMA, Immulite, Siemens, 13 to 39 mcg/mL.

Results: 357 patients with low TBG levels were evaluated, 220 (62%) men, 137 (38%) women; mean age 35 yrs (19 d to 88 yrs). They were divided in six groups according TBG levels (mcg/mL): <3.5 - 17 (M 15, F2); 3.5 to 6.0 - 26 (M 20, F 6); 6.1 to 8.0 - 30 (M 25, F 5); 8.1 to 9.9 - 59 (M 32, F 27); 10.0 to 11.9 - 139 (M 78, F 61); 12.0 to 12.9 - 86 (M 50, F 36). Three patients had FT4 levels above 1.8 (1.85; 1.87 and 1.96) ng/mL, all them male, with TBG levels < 3.5; 5.79 and 9.53 ng/mL, respectively.

Conclusion: FT4 Indirect methods are developed taking into account the presence, in the serum, of a thyroxine binding capacity within the normal range, a fact that does not occur in individuals with extreme TBG deficiency. In this study, we found only one slightly elevated FT4 value among 17 patients with TBG values below 3.5 mcg/mL, suggesting little interference of very low TBG levels in this indirect free T4 method.

A-060

Thyroid Laboratory Database Assessment Shows that Total Triiodothyronine Significantly Decreases with Age

R. Fontes[1], P. Telles-Dias[1], T. C. Sousa[2], P. Araujo[1], B. S. Santos[3], A. C. Lopes[3], F. S. Lopes[4], M. F. Pinheiro[1], D. M. Gomes[1], C. O. Sabino[3], A. N. Cury[3], M. C. Castelo[5], L. L. Cavalcante[5], Y. Schrank[1], T. C. Veloso[4], A. F. Perozo[1]. *[1]Dasa, Brasil; Instituto de Ensino e Pesquisa Dasa, Brasil, Rio de Janeiro, RJ, Brazil, [2]Dasa, Brasil; Instituto de Ensino e Pesquisa Dasa, Brasil, Recife, PE, Brazil, [3]Dasa, Brasil; Instituto de Ensino e Pesquisa Dasa, Brasil, São Paulo, SP, Brazil, [4]Dasa, Brasil; Instituto de Ensino e Pesquisa Dasa, Brasil, Brasilia, DF, Brazil, [5]Dasa, Brasil; Instituto de Ensino e Pesquisa Dasa, Brasil, Fortaleza, CE, Brazil*

Introduction: The effects of aging on human thyroid function are well studied in relationship to thyroid stimulating hormone (TSH) and free tiroxine (FT4); TSH increases, while FT4 decreases with age. Although animal studies show that total triiodotironine (TT3) is lower in aged male rats, these changes are still not clear enough in humans. The National Academy of Clinical Biochemistry (NACB) suggests that TT3 levels would be lower in subjects over 60 years (y.), but the number of elderly assessed was too small for a robust statistical analysis. In search on PubMed, cross-checking the terms elderly/age/aging, with triiodothyronine/T3/total T3, there are a few studies available using current assays with a significant number of patients. Therefore, the demonstration of how the TT3 performs with age is still an opened question. **Objective:** The aim of this study was to observe if TT3 changes significantly with age. **Methods:** We, retrospectively, analyzed blood samples requesting serum TT3 of unique patients, who had TSH measured on the same day; and thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TGAb), anti TSH receptor antibodies (TRAb) or TSH receptor-stimulating immunoglobulin (TSI) at any time since patient admission. Subjects were adults ≥20 y., admitted to a private reference clinical laboratory in Brazil, from 01/01/2014 to 12/31/2022, using anonymized data. Serum TT3 was measured in Cobas Roche, by electrochemiluminescence assay, reference interval 70-210 ng/dL. Exclusion criteria were (and/or): tests performed in hospitals, use of drugs that could interfere on TT3 or TSH, TSH ≤ 0.1 mIU/L or ≥ 10.0 mIU/L, and any positive antibody at any time. The level of statistical significance was $p < 0.05$. **Results:** Initially, 959,297 records from the database were surveyed. After applying the exclusion criteria, 141,819 subjects were eligible: mean age = 45 y.; median = 42 y., 62% female (F). Statistically significant differences - $p < 0.05$ for all comparisons between groups - were found by age and gender in TT3 mean values (in ng/dL). Regarding age: from 20 to 59 y. = 120±26.6; 60 to 79 y. = 109±20.7; and 80 y. and over = 97.2±20.0. By gender: F = 120±28,9; male = 114±20.4. It was also observed that, in elderly, while TT3 decreases, TSH increases (results not shown). Comparing TT3 means by age groups, it is 9% lower in subjects aged 60 to 79 y. and 19% lower in those aged 80 y. and older, compared to younger (20 to 59 y.) subjects. **Conclusion:** TT3 results is influenced by several factors, including age, gender and laboratory assay. As far as we know, searching in PubMed, this is the largest collection of data on the relationship between TT3 and age. The data obtained showed a statistically significant decrease in TT3 in elderly, and mainly in very elderly, and higher levels in women than in men. This finding draws call attention to the fact that these parameters should taken into account when evaluating TT3 levels.

A-061

Performance evaluation of improved versions of Norudia GA reagents that reduce negative interference during glycated albumin measurements

H. Park, B. Lee, S. Kim. *Samsung Medical Center, Seoul, Korea, Republic of*

Background: Glycated albumin (glycoalbumin, GA) is a biomarker that represents the glycemic status of a short period. While the Norudia GA assay kit (Sekisui Medical Co., Ltd., Tokyo, Japan) demonstrated a performance comparative to that of the Lucica GA-L (Asahi Kasei Pharma Corporation, Tokyo, Japan), we have experienced a few cases with extremely low GA levels measured with Norudia GA, inconsistent with the clinical status. Hence, we have evaluated the analytical performance of the improved version of the Norudia GA that claims to have solved the false negative issue.

Methods: Precision and linearity was evaluated following the guidance of the CLSI EP05-A3 and EP06-A, respectively. Comparative study was performed against the Lucica GA-L and the prior version of Norudia GA with respect to the CLSI EP09-A3. Temperature stability of GA was also assessed. Sample stability under 4°C and -70°C was evaluated using 7 specimens including 1 false negative specimen. Along with the initial result, the results after 7, 14, and 28 days of storage were evaluated. To verify the current GA reference interval of our institute, samples from 20 healthy controls (10 males and 10 females) were used according to the CLSI EP28-A3C.

Results: Precision analysis showed acceptable results. The ranges of repeatability and within-laboratory CV were 1.5-2.2% and 3.1-5.3%, respectively. In the linearity analysis, it was demonstrated that the best fit was first order in the polynomial regression with a slope of 1.040 (95% CI, 1.004-1.075) and a coefficient of determination (R^2) of 0.998. The method comparison with respect to the Lucica GA-L assay and the original version of the Norudia GA assay exhibited a Pearson's correlation coefficient (r) of 0.985 and 0.973, respectively. The Bland-Altman plot revealed that the samples with falsely low results obtained using the original Norudia GA resulted in a higher GA result when measured with the improved version of the Norudia GA. Sample stability revealed that there is a tendency of increasing GA upon storage duration in both 4°C and -70°C. Transference of the reference interval was verified. Negative interferences were reduced in the improved version of the Norudia GA.

Conclusion: The new improved version of the Norudia GA assay showed comparable performance in measuring GA as well as reduced interference in samples that had shown false negative results in the original version of the Norudia GA.

General Clinical Chemistry and Laboratory Medicine

A-062

Development of quality control indicators in the pre-analytical phase and a survey on the current status of clinical laboratories in Korea

H. Park[1], S. Kim[1], S. Kim[2], K. Lee[3], Y. Lee[4], S. Chun[2]. *[1]Samsung Medical Center, Seoul, Korea, Republic of, [2]Asan Medical Center, Seoul, Korea, Republic of, [3]Seoul National University Bundang Hospital, Seongnam, Korea, Republic of, [4]Soonchunhyang University Bucheon Hospital, Bucheon, Korea, Republic of*

Background: Pre-analytical errors, which account for 50% to 75% of all laboratory errors, include various processes handled by various occupations such as prescription, data input, sample collection, sample transport, and sample reception, making it difficult to present standardized quality indicators. Internationally, efforts are being made to present pre-analytical quality indicators and standardize/harmonize them, but in Korea, the status of pre-analytical errors in laboratories has not been identified. Therefore, this study attempted to contribute to improving the quality of domestic clinical laboratories by presenting pre-analytical quality indicators that can be easily applied and effectively managed in domestic clinical laboratories.

Methods: Domestic and foreign programs for pre-analytical quality indicators were investigated, and a pre-analytical quality indicator survey was conducted for domestic clinical laboratories. The survey results were analyzed to identify the current status and problems of quality management in the pre-analytical stage of the domestic clinical laboratories, and the appropriateness of the pre-analytical quality indicators in the application of the domestic clinical laboratories was reviewed.

Results: We prepared 22 pre-analytical quality indicators by referring to the 'Quality indicators of the Pre-analytical phase' presented by the International Federation of Clinical Chemistry Working Group (2017) and 'Development of an external quality control program in the pre-analytical stage for reliable laboratory implementation' presented by the Korean Association of External Quality Assessment (2021). A survey was conducted on 90 institutions nationwide, including tertiary hospitals, secondary hospitals, and professional consignment laboratories. As a result of the survey, 42 institutions (47%) responded, of which 64% were tertiary hospitals, 22% were secondary hospitals, and 14% were professional consignment laboratories. Among the quality indicators, the rate of complete blood counts accompanied by blood clots (93%) and the insufficient sample volume rate (90%) showed the highest response rate. On the other hand, the sample recollection rate (17% in the out of laboratory, 21% in the laboratory), the ratio of samples carried at nonconforming temperatures (24%), and the ratio of delayed samples (24%) showed the lowest response rate, respectively. The median value of the clinical laboratory error rate for all quality indicators of pre-analytical phase was generally very good with a sigma value of 5 or higher.

Conclusion: In this study, 22 pre-analytical quality indicators applicable to domestic clinical laboratories were prepared. Using this, the status of quality management in the pre-analytical phase of the domestic clinical laboratory was identified, and the appropriateness of quality indicators was reviewed. This is an important cornerstone for the standardization of quality management in the pre-analytical phase, and it is expected that it will greatly contribute to improving the quality of clinical laboratories in Korea by establishing a pre-analytical quality management system in the future.

A-069

Atellica® Ci 1900 sample carryover performance evaluation

T. Pham, K. Kolewe, M. Ho, S. Lewisch, J. Snyder. *Siemens Healthcare Diagnostics Inc., Newark, DE*

Background: The Atellica® CI 1900 Analyzer uses independent integration and random-access sampling to process chemistry (CH) and immunoassay (IM) tests. A single-use, disposable sample tip is used for IM assays, whereas a reusable dilution probe is used for CH assays. When a sample tube is first accessed by the CH dilution probe to process CH assays, then accessed by the IM disposable tip to process IM assays, there is the potential for sample carryover that could affect IM assay results. The purpose of this investigation was to evaluate sample-to-sample carryover performance on the Atellica® CI 1900 Analyzer.

Methods: Hepatitis B surface antigen (HBsAg) was selected as the test analyte, given the extreme HBsAg concentrations that can exist in clinical samples. High HBsAg pools ranging from 25 to 55 million Index values were prepared by spiking purified HBsAg into serum. Eleven low (L) samples containing 600 µL of HBsAg-negative control QC and 10 high (H) samples containing 300 µL of the high HBsAg serum pool were created. The samples were first processed with one replicate per sample with the Atellica® CH ALT assay on the Atellica® CI 1900 Analyzer so that the CH dilution probe would aspirate 50 µL of sample. Sample-to-sample carryover testing was performed in a defined high (H)- and low (L)-sample consecutive sequence: L1, L2, L3, H1, H2, L4, H3, H4, L5, L6, L7, L8, H5, H6, L9, H7, H8, L10, H9, H10, L11. All low (L) samples were next assayed for HBsAg using the Atellica® CI 1900 Analyzer. The sample carryover study was conducted on three Atellica® CI 1900 Analyzers. Carryover in parts per million (ppm) was determined by multiplying the difference of the "high-low" mean (L4, L5, L9, L10, L11) and "low-low" mean (L2, L3, L6, L7, L8) by 1,000,000 and dividing by the high HBsAg sample concentration.

Results: The measured sample carryover results were 0.00, 0.00, and 0.00 ppm. All three Atellica® CI 1900 Analyzers produced sample-to-sample carryover of <0.1 ppm using HBsAg as the analyte.

Conclusions: The Atellica® CI 1900 Analyzer* demonstrated the capability to ensure sample-to-sample carryover of <0.1 ppm into primary sample containers with a minimum of 600 µL sample volume.

*Not available for sale in the US. Product availability varies by country.

A-070

A fully automated HbA1c Assay on the DxC 500 AU Clinical Chemistry Analyser

C. Stokes[1], S. Frost[2], L. Frost[1], N. Mcinerney[1], S. O'Dwyer[1]. *[1]Beckman Coulter Ireland, Clare, Ireland, [2]Beckman Coulter, Clare, Ireland*

Background Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type-1 diabetes, the pancreas no longer makes insulin and blood glucose cannot enter the cells to be used for energy. In Type-2 diabetes, the pancreas does not make enough insulin or the body is unable to use insulin correctly.

According to the World Health Organization (WHO), 422 million adults were living with diabetes globally in 2014 with an estimated 1.6 million deaths directly associated with diabetes annually.

HbA1c is the major species of glycohemoglobin in human blood. HbA1c formation occurs through a non-enzymatic reaction, called glycation, which occurs between glucose and the n-terminal valine of the hemoglobin â-chain of HbA.

A Schiff base intermediate product is formed, which rearranges to form a stable ketoamine in an irreversible reaction. The rate of glycation is proportional to the glucose concentration in the bloodstream and is an accurate reflection of average blood glucose over a period of eight to twelve weeks. HbA1c testing is recommended for the diagnosis of diabetes by the International Expert Committee (IEC), the American Diabetes Association (ADA), and the WHO, who recommended a diagnostic threshold of =6.5% (=48 mmoL/moL) HbA1c and a range for pre-diabetes of 5.7%-6.4% (39-46 mmoL/moL) HbA1c.

Method The HbA1c Advanced assay on the DxC 500 AU* utilizes automatic sample pre-treatment, has batch and random access capability. No manual pre-treatment of the whole blood sample or additional washing steps are required. Firstly the red blood cells are hemolyzed automatically, total hemoglobin and glycated hemoglobin are then measured colorimetrically and immunoturbidimetrically respectively.

Results Precision studies were conducted according to CLSI EP15-A3. Commercial controls and four native K2 EDTA whole blood samples ranging from 5.1% to 12.0% HbA1c, were run twice daily, over twenty days using three lots of reagent on three DxC500AU Clinical Chemistry analyzers at a single site. Repeatability ranged from 0.86 to 1.44% CV and Total Precision ranged from 1.55 to 2.43% CV.

Linearity studies were conducted according to CLSI EP06-A, and verified an analytical measuring range of 4.0 - 15.0% (NGSP) and 20 - 140 mmol/mol (IFCC). Method comparison and bias estimation was evaluated using CLSI EP09-A3. K2 EDTA patient samples (n=150) across the analytical range were run versus a Secondary Reference method and yielded a slope of 1.031, intercept -0.147% HbA1c, correlation coefficient R=0.997 for Weighted Deming regression, and slope 1.000 and intercept 0.06% HbA1c for Passing Bablok regression.

Interference studies carried out demonstrated no significant interference from common endogenous interferences, a large panel of drugs, common Hb variants (HbC, HbD, HbE, HbA2 and HbS) and cross reactants (HbA0, HbA1a+b, acetylated hemoglobin, carbamylated hemoglobin, glycated hemoglobin, glycated albumin and labile HbA1c).

Conclusion The HbA1c Advanced assay on the DxC 500 AU is a precise and accurate assay, requiring no manual pretreatment and can be used for monitoring and diagnosing diabetes. *Pending clearance by the United States Food and Drug Administration and achievement of CE compliance. Not currently available for *in vitro* diagnostic use.

A-071

Comparison of eGFR Calculated by the MDRD, the 2021 CKD-EPI Creatinine and the 2021 CKD-EPI Creatinine-cystatin C Equations in Taiwanese

C. Lai, H. Lee, H. Ho. *Taipei Veterans General Hospital, Taipei, Taiwan*

Background:

The new race-free 2021 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Creatinine and the 2021 CKD-EPI Creatinine-cystatin C equations are endorsed by the National Kidney Foundation. This study aimed to evaluate the difference between the different equations in the results of eGFR <60 and ≥60 mL/min/1.73m^2 in different genders and ages in Taiwan.

Methods:

We retrospectively collected 1,403 laboratory data from adults with both serum creatinine and cystatin C tested results from January 2022 to October 2022, including 870 males and 533 females. We calculated the eGFR by MDRD, 2021 CKD-EPI Creatinine, and 2021 CKD-EPI Creatinine-cystatin C equations and divided the results into two groups according to $eGFR_{MDRD}$ <60 or ≥60 mL/min/1.73m^2, and then analyzed the bias and concordance between different equations according to age (18-29, 30-49, 50-69, ≥70) and gender, and used pair T-test or Wilcoxon sign rank test, *P* values less than 0.05 were considered statistically significant.

Results:

(1) Compared with the $eGFR_{MDRD}$, the results of $eGFR_{2021\ CKD\text{-}EPI\ Creatinine}$ and $eGFR_{2021\ CKD\text{-}EPI\ Creatinine\text{-}cystatin\ C}$ showed a positive bias of 2.2 (p<0.001) and 1.8 (p<0.001) mL/min/1.73m^2 in the $eGFR_{MDRD}$ <60 group, 5.4 (p<0.001) and 2.4 (p=0.010) mL/min/1.73m^2 in the $eGFR_{MDRD}$ ≥60 group.

(2) In addition, in the $eGFR_{MDRD}$ <60 group, there were 7.0% (42/601) male and 8.1% (28/345) female would be reclassified to the ≥60 group by the 2021 CKD-EPI Creatinine, and including 8.8% (53/601) male and 4.9% (17/345) female would be reclassified to the ≥60 group by the 2021 CKD-EPI Creatinine-cystatin C.

(3) In $eGFR_{MDRD}$ ≥60 group, there was no patient would be reclassified to the <60 groups by the 2021 CKD-EPI Creatinine, and including 3.0% (8/269) male and 3.2% (6/188) female would be reclassified to the <60 group by the 2021 CKD-EPI Creatinine-cystatin C.

Conclusion:

Compared with MDRD, most of the 2021 CKD-EPI Creatinine and 2021 CKD-EPI Creatinine-cystatin C equations showed positive bias. It also reflected that about 5% of patients whose $eGFR_{MDRD}$ <60 mL/min/1.73m^2 would be reclassified to the ≥60 group by the 2021 CKD-EPI Creatinine and 2021 CKD-EPI Creatinine-cystatin C. Clinical laboratories should fully understand the differences between different equations to ensure the quality of clinical testing.

A-072

An All-Purpose Particle Enhanced Immunoturbidimetric Assay for the Comparable, Instrument-Independent Monitoring of Procalcitonin in the Management of Sepsis

M. Grimmler. *DiaSys Diagnostic Systems GmbH, Holzheim, Germany*

Introduction: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host immune response to infection. It is a global health concern and a leading cause of death worldwide, affecting an estimate of 48.9 million people every year.Procalcitonin (PCT) revealed to be an indispensable parameter for the management of sepsis. However, recent analysis of EQA survey results suggested crucial deviations of PCT measurements in Germany and studies by the IFCC Working Group on standardization of PCT stated the need for a higher-order reference measurement procedure.Here we present the evaluation of the all-purpose particle enhanced immunoturbidimetric assay (PETIA) of DiaSys, which can be easily applied to every high-throughput clinical chemistry analyzer. The high diversity of applications and datasets match current diagnostic and regulative needs (IVDR). Procalcitonin FS enables the equivalent and instrument-independent monitoring of PCT in the management of sepsis. Methodology: Evaluation of Procalcitonin FS was performed on seven clinical chemistry analyzer platforms (Jeol BioMajesty® JCA-BM6010/C, DiaSys respons®920 and respons®940, Roche cobas c501, Abbott Architect c8000 and Alinity c, Beckman Olympus AU680). Measurements were performed according to Clinical and Laboratory Standards Institute (CLSI) protocols. Results: The onboard stability reaches 8 weeks (with chimney) on BioMajesty® JCA-BM6010/C, 20 days on respons®920 and respons®940, 21 weeks on cobas c501, 9 weeks on Architect, 12 weeks on Olympus AU680 and 13 weeks on Alinity c. The calibration stability is 4 weeks (with chimney) on BioMajesty® JCA-BM6010/C, 2 weeks (with chimney) on respons®920 and respons®940, 21 weeks on cobas c501, 3 weeks on Architect, 5 weeks on Olympus AU680 and 10 weeks on Alinity c. The intra-assay precision (CV%) ranges from ≤4.57% on respons®920 up to ≤7.19% on respons®940. The Total Precision (CV%) ranges from ≤3.99% on cobas c501 up to ≤9.62% on Olympus AU680. LOB ranges from 0.07 ng/mL on BioMajesty® JCA-BM6010/C up to 0.130 ng/mL on Olympus AU680. LOQ ranges from 0.20 ng/mL on Architect up to 0.277 ng/mL on Olympus AU680. The reagent displayed a linear recovery up to 50 ng/mL and no prozone effect was detected up to 1000 ng/mL on all seven analyzers. The Passing & Bablok regression versus the reference test showed an excellent comparability/alignment between the methods (slope ≤1.11, intercept ≤0.156 ng/mL, R ≥0.986). Conclusion: Evaluation work proves that DiaSys PETIA reagent Procalcitonin FS shows excellent performance on all seven clinical chemistry analyzers. In addition, the high comparability between the seven analyzers enables the reliable and instrument-independent monitoring of PCT in the management of sepsis, and thus offering a solution to the current landscape of deviating PCT measurement results.

A-073

Specimen Storage and Onboard Stability of Serum Ionized Calcium Using the Nova Prime ES Comp Plus Analyzer

J. J. Hunsaker[1], S. L. La'ulu[1], K. Doyle[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]University of Utah Health, Department of Pathology, Salt Lake City, UT*

Background: Ionized calcium refers to the calcium ions that are present in extracellular fluid which facilitate a multitude of metabolic and physiological processes. As such, ionized calcium may be measured in patients with suspected abnormality in calcium metabolism, including a variety of bone diseases, chronic renal disease, and tetany. Ionized calcium is known to be unstable when exposed to air and many factors including specific handling procedures after specimen collection are known to affect measured analyte concentrations. The present study was conducted to assess the stability of serum ionized calcium in various storage conditions and evaluate stability of specimens when using the sample tray available on the Nova Prime ES Comp Plus analyzer. **Methods:** Studies were performed to evaluate ambient (20-25°C), refrigerated (2-8°C), frozen (-20°C) and freeze/thaw stabilities for ionized calcium using the Prime ES. Residual specimens stored refrigerated for up to 3 days were collected to create pools (n=3-4). Each pool was then split into single-use aliquots to be stored and measured in the following conditions (temperature [timepoints]): Ambient (8, 24 hours), Refrigerated (1, 2, 4 weeks), Frozen (1, 2, 4, 9 weeks), Freeze/Thaw (1, 2, 3 freeze/thaws). An aliquot of each pool was analyzed to obtain a baseline value. All aliquots were tested in duplicate, and the average was used to calculate % change for each pool and for all pools combined at each time point. Additionally, as ionized calcium is known to be unstable in specimens open and exposed to air, onboard specimen stability using the sample tray available on the analyzer was evaluated. Residual specimens used for previous stability studies were used to create pools (n=4) on the same day. Each pool was split into sample cups (0.5mL and 2.0mL) and tested immediately using all the positions available on the sample tray (n=10). For each pool, the first sample that was tested on the tray was used as a baseline value to calculate % change for the remaining cups on the tray. For all experiments, ionized calcium, pH, and pH-normalized ionized calcium results reported on the instrument were analyzed. Acceptable criteria was no greater than ±15% change from the baseline result. **Results:** For the assessment of specimen stability at various storage conditions, the overall average % change of all pools combined at all time points ranged -3.9 to 2.1% for ionized calcium, -0.2 to 1.5% for pH and -1.4 to 5.9% for pH-normalized ionized calcium. For the sample tray stability evaluation, the % change in each sample tray position compared to baseline ranged -2.4 to 5.2% for ionized calcium, -0.1 to 1.6% for pH and 0.0 to 3.9% for pH-normalized ionized calcium. It took up to 15 minutes to test all positions available on the sample tray. **Conclusions:** Ionized calcium, pH and pH-normalized ionized calcium remained stable for 24 hours ambient, 4 weeks refrigerated, 9 weeks frozen and up to 3 freeze/thaws. The sample tray available on the Prime ES was also found to be acceptable for use.

A-075

Development and Validation of Image-based Cell Counter Microscanner C3 for CD34-positive Hematopoietic Stem Cell Enumeration

C. Lee[1], I. Park[2], S. Yoon[1], C. Lim[1], J. Kwon[1]. *[1]Department of Laboratory Medicine, Korea University Guro Hospital, Seoul, Korea, Republic of, [2]College of Medicine, Korea University, Seoul, Korea, Republic of*

Background Accurate measurement of CD34-positive hematopoietic stem cells (HSCs) is critical for successful peripheral blood stem cell transplantation (PBSCT). Flow cytometry is by far the gold standard for measuring CD34-positive stem cells. However, its complicated procedure requires time, cost and highly-trained operators, thus the necessity of simpler and cheaper alternative method is emerging. We have developed a new image-based cell counter, Microscanner C3 (MSC3, Biozentech, South Korea) as an alternative method for enumeration of CD34-positive HSCs and evaluated its performance.

Methods 100ul of apheresis product was stained with CD45-BB515 and CD34-PE antibodies (Becton Dickinson, USA) at room temperature in the dark. Red blood cell lysis with 500ul of OptiLyze C solution (Beckman Coulter, USA) was followed by centrifugation. After the removal of supernatants, the sample was resuspended with 500uL PBS, which then infused to filter paper imbedded BZ-1 microchip (Biozentech, South Korea). Thirty-fields of the loaded microchip was auto-scanned by MSC3 and its dedicated software analyzed the images and yielded CD34-positive cell fraction out of CD45-positive cells as percentage. Results of thirty-six samples were compared to that of FacsLyric (Beckton Dickinson, USA) flow cytometry and accuracy and imprecision (pentaplicate measurements for three samples), repeatability (two experimenters performed triplicate measurements for three samples each), linearity (triplicate measurements for five levels), limit of detection (hexaplicate measurements for five levels) and specificity (using whole blood specimen from three donors with monoclonal gammopathy, thrombocytosis or monocytosis) was evaluated.

Results CV% values of inaccuracy, imprecision and repeatability did not exceed 15%. The method was found to be linear across the measuring range. LOD was evaluated to be 7cells/uL and no background interference was detected. The difference between the measured fraction of MSC3 and FacsLyric was within allowable limit, with an R-value greater than 0.98.

Conclusion We developed a reliable method for quantifying CD34-positive HSCs based on image analysis. The limitation involved in MSC3 applications in CD34-positive HSCs is that, like dual-platform flow cytometry, the results should be combined with the results of conventional hematology analyzer in order to define absolute HSC numbers. However, with its simpler and faster procedures, MSC3 exhibited a great potential to be used as an alternative to conventional flow cytometry in CD34-positive HSCs enumeration and other applications as well.

A-076

Abbott Alinity ci system automated Sample Interference Indices for immunoassays

M. Berman[1], M. Anderson[2]. *[1]Abbott Labs, North Chicago, IL, [2]Abbott Labs, NORTH CHICAGO, IL*

Background: The Alinity ci system Sample Interference Indices (HIL) provides a more accurate and consistent method for interpretation of interferents than time con-

suming visual interpretation. The Alinity c system uses specific wavelength pairs and an algorithm to provide a Sample Interference Index that can correlate with sample interference due to hemolysis, bilirubin and turbidity present in serum/plasma samples. Using just a single aspiration from a sample, the individual or all 3 indices can be selected to be reported. These values in combination with assay specific endogenous interference can be used to determine the potential for HIL interference in the Alinity i immunoassays.

Methods: The HIL methodology will be reviewed and discussed. The study followed Clinical and Laboratory Standards Institute (CLSI) protocol EP7-A2. Interferences were studied up to concentrations of 1000 mg/dL for hemoglobin, 30 mg/dL for bilirubin (unconjugated) and 1000 mg/dL for triglycerides in serum. Serial dilutions of the sample pools were analyzed in replicates of 4 on the Alinity c system. A cumulative summary was compiled of the Hemolysis, Icterus and Lipemia indices for 100 Alinity immunoassays.

Results: Using known concentrations of hemoglobin, bilirubin and Intralipid, the Abbott Semi-Quantitative Index (concentration range in mg/dL) and Qualitative Index (Blank, 1+, 2+, 3+ 4+) were confirmed on the Alinity c system. Correlation studies show a linear relationship (r = 1.0) of the indices with increasing concentration of analyte. An easy-to-use guide was created that combines the HIL Qualitative and Quantitative Index scores with the assay specific interference results to provide a guide to potential interferents for the Alinity i immunoassays. This guide provides background information on the causes of HIL interferences, conversion factors from Conventional to SI units and denotes the specific concentrations of interferents which could lead to an over or under estimation in the presence of hemoglobin, bilirubin and/or lipemia. In addition, the effects of the influence of two or more HIL interferents on assay reported results is discussed. The majority (96%) of the 100 immunoassays had no significant interference using samples with elevated H, I or L index values. The remaining 3 assays showed slight interference.

Conclusion: The Alinity ci system provides a simple and automated procedure for determining the sample indices (HIL) for patient specimens. These HIL values in combination with assay specific endogenous interference results can be used to determine the potential for HIL interference in the Alinity i immunoassays to avoid misdiagnosis.

A-078

Accuracy Based Sigma Metrics of Alinity c System as Benchmarked against Roche c701

L. Cheng[1], Q. Guan[1], H. Li[1], J. Miao[2], P. Sun[2], C. Chen[3]. *[1]Department of Clinical Laboratory, Tongji Hospital, Tongji Medical College of HUST, Wuhan, China, [2]China R&D Center, Core Diagnostics, Abbott Laboratories, Shanghai, China, [3]Medical & Scientific Research, Core Diagnostics, Abbott Laboratories, Singapore, Singapore*

Background: Assay performance is dependent on the accuracy and precision of a given method. Sigma metric is a well-established tool for objectively assessing such performances. The objective of the study is to evaluate the Alinity c system using a representative set of clinical chemistry assays in an independent central laboratory setting. The analytical performance of the Alinity c assays was compared with the Roche c701 assays using accuracy-based Sigma metrics.

Methods: Analytical performance of 7 clinical chemistry assays, including sodium, potassium, chloride (Integrated Chip Technology or ICT), Glucose, ALT, AST and creatine kinase (CK), was assessed on the Alinity c System as benchmarked against the Roche c701. The accuracy of the assays was evaluated by testing reference standard materials sourced from Reference Material Institute for Clinical Chemistry Standards (ReCCS)) in Japan or National Institute of Standards and Technology (NIST) in America. The 5-day precision evaluation per CLSI EP15-A3 was performed using the Bio-Rad Lyphochek® Assayed Chemistry Control and two patient sample pools. Linearity was assessed using 9 levels of patient sample pools covering the measuring interval of each assay. Method comparison with Roche c701 assays was performed per CLSI EP9-A3 using 60 patient samples spanning the measurement range. Sigma metrics for each assay were determined relative to the total allowable errors according to the Clinical Laboratory Improvement Amendments (CLIA) specifications.

Results: Within-lab (total) imprecision for the 7 assays on Alinity c and Roche were all < 3.5% CV for both the Bio-Rad Control and patient pools. Alinity c assays showed a better precision performance in all of assays tested. Bias of the 7 assays in reference to the assigned values was all below 7.5% for both platforms. Alinity c exhibited a good correlation with Roche c701 on all 7 assays in the method comparison evaluation. Linearity of the Alinity c assays was validated for the measuring intervals as claimed by the manufacturer. Majority of the assays on both analzyers demonstrated at least 6 sigma performance with Alinity c showing better sigma scores.

Conclusion: The Alinity c System demonstrated excellent accuracy, precision and linearity in an evaluation of 7 clinical chemistry assays. A majority of the assays demonstrated at least 6 sigma performance, supporting the fitness for clinical use.

A-079

Analytical Assessment of Abbott's SigmaSTRONG assays on the Alinity *c* and ARCHITECT c Systems

S. Cox[1], T. James[1], K. Allott[1], R. Ervankai[1], G. H. Potter[1], G. Allen[1], I. Lapić[2], S. Komljenović[2], A. Bogić[2], D. Rogić[2]. *[1]Oxford University Hospitals, Oxford, United Kingdom, [2]KBC Zagreb, Zagreb, Croatia*

Introduction: Clinical chemistry assays are responsible for 57 % of all core lab testing [1] as a result there is an ever-increasing demand to improve the accuracy and reliability of results in clinical laboratories. Sigma metrics have proven to be powerful tools for quantitatively evaluating clinical assay and instrument performance. The Sigma performance of an assay is calculated from the formula (TEa-Bias)/CV. To address modern clinical laboratory performance requirements Abbott has developed and launched a portfolio of clinical chemistry assays under the brand name *Sigma* STRONG ™ which claim to achieve world class performance with a Sigma Metric of ≥ 6 for 86% of products within this portfolio [2]. The purpose of this study is to assess these assays against Abbott's products requirement (PR) for precision and method comparison **Method**: Assay precision (%CV) studies were performed based on guidance from CLSI EP05-A3 using 2-3 levels of commercially available serum-based control material. Method Comparison (MC) studies were performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method. Assays under investigation were assessed for performance on either the Abbott Alinity c or Abbott ARCHITECT c systems. **Results:**

Assay	List Number	Instrument	%CV	PR	MC	PR
Creatinine2	04T91	Alinity	1.8-2.4	≤5.0	Slope: 0.99 R: 1.00	Slope 1.0 ± 0.10 R: ≥ 0.98
Urea2	04T12	ARCHITECT	1.4-2.1	≤4.0	Slope: 1.00 R: 1.00	Slope: 1.0 ± 0.10 R: ≥ 0.975
BCP2	04U45	ARCHITECT	0.8-1.1	≤3.0	Slope: 1.00 R: 1.00	Slope: 1.0 ± 0.08 R: ≥ 0.975
Total Protein2	04U44	ARCHITECT	0.8-1.3	≤2.5	Slope: 0.98 R: 1.00	Slope: 1 ± 0.05 R: ≥ 0.975
Total Bilirubin2	04U05	ARCHITECT	3.3-5.0	≤6.0	Slope: 0.96 R: 1.00	Slope: 1.0 ± 0.10 R: ≥ 0.975
Uric Acid2	04T13	ARCHITECT	0.9-1.6	≤3.5	Slope: 0.99 R: 1.00	Slope: 1.0 ± 0.10 R: ≥ 0.975
ALP2	04T83	Alinity	3.6-4.0	≤5.0	Slope: 1.09 R: 1.00	Slope: 1.0 ± 0.10 R: ≥ 0.975
GGT2	04T96	Alinity	2.6-2.8	≤4.0	Slope: 0.98 R: 1.00	Slope: 1.0 ± 0.14 R: ≥ 0.975
ALT2	04T84	ARCHITECT	0.8-2.5	≤4.0	Slope: 0.93 R: 1.00	Slope: 1.0 ± 0.10 R: ≥ 0.975
AST2	04T86	ARCHITECT	2.0-2.7	≤4.0	Slope: 1.05 R: 1.00	Slope: 1.0 ± 0.10 R: ≥ 0.975
LDH2	04T99	Alinity	1.6-3.5	≤4.5	Slope: 0.98 R: 1.00	Slope: 1.0 ± 0.10 R: ≥ 0.975
Amylase2	04S89	ARCHITECT	0.9	≤3.0	Slope: 1.09 R: 1.00	Slope: 1 ± 0.10 R: ≥ 0.975
Iron2	04T98	ARCHITECT	1.1-1.7	≤4.0	Slope: 1.07 R: 1.00	Slope: 1.0 ± 0.10 R: ≥ 0.975
Triglycerides2	04U06	Alinity	3.5-3.7	≤4.0	Slope: 1.01 R: 1.00	Slope: 1.0 ± 0.08 R: ≥ 0.975
Cholesterol2	04S92	ARCHITECT	0.9-1.0	≤3.0	Slope: 0.98 R: 1.00	Slope: 1.0 ± 0.10 R: ≥ 0.975

Conclusion: This study has confirmed the *sigma* STRONG ™ assays, under assessment, have met Abbott's product requirement for both precision and method comparison. **References:** [1] Clinical Laboratory Services Market, 5th Edition. Global IVD Procedure Volumes, 2019 and 2024. Science and Medicine Group Jan 2020.[2] Westgard S, Petrides V, Schneider S, *et al.* 2017. Assessing precision, bias and sigma-metrics of 53 measurands of the Alinity ci System. Clin Chem. 50: 1216-1221. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0009912017306616 Accessed 23rd June 2022.2.

A-080

Overcoming Challenges of Equilibrium Dialysis-Based Free Thyroxine Measurements

N. Vazquez, S. Knoblock, J. Thonkulpitak, A. Ribera, L. Zhang, U. Danilenko, H. W. Vesper. *CDC, Atlanta, GA*

Background Thyroid function tests are widely used to detect diseases of the thyroid. Concerns about the accuracy and reliability of free thyroxine (FT4) measurements

have been stated by the clinical laboratory community. To address these concerns, CDC established a reference measurement procedure (RMP) for FT4 based on equilibrium dialysis (ED). ED-based methods have advantages over traditional direct immunoassays. As with all analytical methods, factors affecting accuracy and reliability of ED-based methods need identification and understanding. To investigate potential sources of error, the CDC FT4 RMP was used to evaluate T4 stability at different of storage conditions, adsorption of FT4 to common labware surfaces, and the effect of dialysis membrane materials on the measured FT4 in serum.

Methods FT4 was measured in serum using the IFCC RMP [1]. FT4 was separated from the protein-bound form during the equilibrium dialysis step of the RMP. Dialysates were spiked with internal standard ($^{13}C_6$-T4) and the analyte was further isolated by C18 solid phase extraction (SPE) and liquid-liquid extraction (LLE) in ethyl acetate prior to injection on an UHPLC system coupled with a triple quadrupole mass spectrometer. Bracketed calibration and primary reference materials were used to determine FT4 concentration in serum. This procedure was used to evaluate stability of serum FT4 during storage at -70 to 5°C. Short- and long-term T4 stability of calibrator solutions at -70°C to ambient temperature and the extent of adsorption of T4 calibrator solutions to the commonly used labware surfaces were evaluated. Furthermore, the suitability of 4 different dialysis membranes was assessed. In addition, three alternatives to the currently used SPE and LLE extraction steps were tested: LLE using cyclohexane and ethyl acetate, LLE with ethyl acetate, and supported liquid extraction with cyclohexane and ethyl acetate.

Results T4 calibrator solution was stable in 1.7% ammonium hydroxide in ethanol for at least 2.9 years at -70°C and up to 7 days at ambient temperature. Exposure of various calibrator solutions such as T4 in ethanol, ethanol with 1.7% ammonium hydroxide, 10% acetonitrile with 0.1% formic acid to common labware resulted in up to 10.1% loss of the analyte. FT4 in serum and T4 in dialysates and extracts were stable at -70 to 5 ^{0}C. Use of alternative dialysis membranes resulted in up to -8.8% mean bias to the reference membranes. Recoveries of the analyte using four extraction methods were 85.4-95.0%. None of the tested extraction conditions significantly changed the results obtained with the original FT4 RMPs.

Conclusion Although FT4 RMP must strictly adhere to the ED conditions described for the RMP, other parts of the sample preparation procedures can deviate from the originally published procedure. Careful selection of procedure for calibrator solvents, dialysis membranes, labware, and storage conditions is required to ensure accurate and reproducible FT4 measurements. **References** [1] Clin. Chem. Lab. Med. 2011, 49, 1275-1281.

A-082

Measuring Peroxynitrite as an Early Biomarker for Cystic Fibrosis Using Organoselenide-Modified Glassy Carbon Electrode

M. Ibrahim[1], H. Kalil[2], M. Bayachou[1]. *[1]Cleveland state university, Cleveland, OH, [2]Cleveland State University, Cleveland, OH*

Background: Cystic fibrosis (CF) is a genetic disease that affects the respiratory, digestive, and reproductive systems. One of the issues associated with CF is oxidative stress, which is an imbalance between the production of reactive oxygen species (ROS) and the ability of the body to neutralize them. Peroxynitrite, a member of ROS, is a very noxious molecule formed from the reaction of nitric oxide (NO) and superoxide and can damage cellular components such as proteins, lipids, and DNA. Studies have shown that peroxynitrite levels are elevated in people with CF and that this increased oxidative stress can contribute to the development of lung damage, airway obstruction, and other symptoms associated with the disease. Therefore, understanding the role of peroxynitrite in the pathogenesis of CF is important for developing new therapeutic strategies to reduce oxidative stress and improve the quality of life for people with this disease. **Method:** In this study, we have quantified peroxynitrite levels, which could serve as an early biomarker for CF. A modified glassy carbon electrode (GCE) with a series of organoselenide compounds was adopted using the electro-grafting method to measure peroxynitrite levels in situ. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) were used to examine the structure and surface chemistry of organoselenide. Quartz Crystal Microbalance (QCM) analysis was used to determine the thickness of the electrodeposited selenium on the GCE surface. **Results:** Our organoslenide GCE allowed for the application of low positive potential, which enhances the separation of peroxynitrite from other analytes sensitive to higher potentials. Cyclic voltammetry and amperometry showed sensitivity at 110 nA/mol, and the detection limit was recorded at 10 micromolar. This electrochemical technique of our modified electrodes can be miniaturized to detect peroxynitrite at the cell line. The sensitive probe, which was constructed, can be utilized to target the ratiometric changes in several lung slices, from healthy to lung inflammation and cystic fibrosis, via peroxynitrite fluctuation. In addition, ratiometric alterations and the prevalence of cystic fibrosis have a strong linear association, resulting in good potential in predicting the progression of cystic fibrosis in the early stage and improving effective treatment. **Conclusion:** The study aimed to quantify peroxynitrite levels in cystic fibrosis (CF) patients as a potential early biomarker for the disease. We have used a modified glassy carbon electrode with organoselenide compounds and adopted an electro-grafting method to measure peroxynitrite levels. The modified electrodes showed sensitivity and a limit of detection of 10 micromolar, which can be miniaturized to detect peroxynitrite in the cell line. The results suggest that peroxynitrite's sensors could serve as an early biomarker for CF by understanding its role in the pathogenesis, which can improve the quality of life for people with this disease.

A-085

Analytical Performance of BÜHLMANN sCAL® turbo, a New, Reliable and Precise High-Throughput Assay to Assess the Inflammatory Status of Patients

A. Melone, D. Vogt, A. Ohmann, C. Moniz, M. Eichmann, D. Guschin, C. Gerhold, T. Schuster. *BÜHLMANN Laboratories AG, Schönenbuch, Switzerland*

Background:

Serum calprotectin plays an important pro-inflammatory role in the innate immune system and is a promising marker with several potential applications in rheumatoid arthritis, juvenile idiopathic arthritis and systemic onset juvenile idiopathic arthritis. Serum calprotectin can be a useful tool to monitor disease activity and predict treatment response in these patients.

Methods:

The aim of this work was to evaluate the analytical performance of BÜHLMANN sCAL® turbo, a new turbidimetric test on clinical chemistry analyzers for the determination of calprotectin levels in serum. Assay characteristics were established according to CLSI guidelines (CLSI C28-A3, EP05-A3, EP07-A3, and EP09-A3). 111 samples were measured using 3 lots over 3 days and compared to another commercially available assay.

Results:

The analytical measuring range from 0.23 to 15 µg/mL, extended to 225 µg/mL with an additional dilution in an automated rerun on a clinical chemistry analyzer, is suitable to address the described pathologies. 160 self-declared healthy adults (m/w) aged 18 to 83 years exhibit reference limits of 1.77 and 1.10 µg/mL when sampled either in a native tube or a gel separator tube and processed within 3.4 hours after collection, respectively. With a within-laboratory precision up to 5.1% and a reproducibility up to 11.1% the method is precise, reliable and suitable for routine use. Tested concentrations of oral and injectable pharmaceuticals showed no interference. Hemolyzed samples are not recommended. It shows good comparison to an existing commercial assay with a bias of 12.5% (Bland-Altman) and a slope of 1.16 (Passing-Bablok).

Conclusion:

A high-throughput test on clinical chemistry analyzers is now available with the turbidimetric BÜHLMANN sCAL® turbo allowing reliable and precise measurements of serum calprotectin levels.

A-086

Vitamin D and its variation in a pandemic context: Pre-pandemic - Intra-pandemic - Post-pandemic analysis in the province of Buenos Aires - Argentina.

P. N. Canala, M. D. Canala, M. Spiccia, G. Ghioni. *Labint, Moron, Argentina*

Background: The determination of 25 OH Vitamin D3 is a frequently requested measurement for the purpose of evaluating its deficiency, which would be related to various diseases. The behavior of monthly mean values ($\bar{X}$) was analyzed in 984,514 patients from Buenos Aires (Argentina) in the period from January 2014 to December 2022, in order to evaluate how seasonality influences and verify the effect of the mandatory isolation due to the SARS-CoV-2 virus pandemic in 2020.Methodology: We used the Chemiluminescence Assay (QLIA) ADVIA Centaur Vitamin D Total Assay (Siemens) on an Advia Centaur (2014 to 2020) and Aliniti I 25 OH VITAMINE D3 (Abbott Diagnostics) on an Aliniti I (2020 to 2022), reference values are Optimal: > 30.0 ng/ml, Insufficiency: 20.0 to 30.0 ng/ml Deficiency: < 20.0 ng/ml.

Results:

	2014 $\bar{X}$	2015 $\bar{X}$	2016 $\bar{X}$	2017 $\bar{X}$	2018 $\bar{X}$	2019 $\bar{X}$	2020 $\bar{X}$	2021 $\bar{X}$	2022 $\bar{X}$
Ene.	27,72	25,34	29,47	28,24	26,34	26,36	27,83	29,85	27,17
Feb.	25,21	26,31	28,62	28,16	26,37	29,68	31,10	29,51	28,49
Mar.	27,50	28,58	25,54	28,22	25,11	28,89	29,99	29,18	30,49
Abr.	26,06	24,73	27,54	26,76	24,76	26,75	30,41	28,61	27,71
May.	21,50	22,23	24,62	22,83	25,98	27,03	27,50	27,27	24,11
Jun.	23,18	21,43	22,47	22,01	23,45	24,92	25,15	25,40	22,49
Jul.	20,80	21,23	22,64	22,01	21,95	24,53	23,43	23,02	20,64
Ago.	23,30	21,94	21,42	20,90	21,71	23,18	22,72	21,36	19,73
Sep.	24,72	19,86	22,50	21,33	20,69	22,89	23,69	21,39	20,42
Oct.	23,08	21,83	22,82	24,43	20,63	24,33	23,85	22,26	20,17
Nov.	23,40	22,01	22,19	24,52	20,70	25,92	25,62	24,27	23,54
Dic.	23,59	24,78	25,20	25,96	24,28	26,63	25,54	26,06	27,96
AV	24,17	23,36	24,59	24,61	23,50	25,93	26,40	25,68	24,41

Conclusions: From the analysis of the data, a decrease in the winter months is observed, as already described, caused by less exposure to sunlight. In 2020, it was also observed that due to confinement, there was a slight increase in mean values (26.40 ng/ml ($p<.001$)), which was the highest of the years analyzed. One hypothesis is that this could be caused by the increased free time of the population resulting in greater exposure to sunlight. At the same time, the insufficiency of Vitamin D3 in the studied population is evident, as only in 2 out of the 108 months analyzed did it slightly exceed the optimal level of 30.0 ng/ml. We believe that it would be important to evaluate the implementation of a Vitamin D3 supplementation policy for the general population and subsequently conduct further studies of this type to verify the effectiveness of such implementation.

A-087

Relationship among different inflammatory markers in patients with COVID-19 infections

R. Yadav, P. Chopra, T. Sehgal, S. K. Datta, A. Ningombam, A. Ahirwar. *AIIMS New Delhi, New Delhi, India*

Introduction Acute phase reactants (APRs) are inflammation biomarkers that exhibit significant changes in their serum concentrations due to infectious and noninfectious conditions. APRs such as C-reactive protein (CRP), Interleukin-6 (IL-6), Ferritin, Lactate dehydrogenase (LDH); coagulation biomarkers such as D-dimer, Fibrinogen; cardiac biomarkers such as Creatine kinase-MB (CK-MB), and hematological biomarkers such as Neutrophil to Lymphocyte ratio (NLR) have been used extensively during the COVID-19 pandemic for early diagnosis, monitoring of response to treatment and prognostication. Amongst these, CRP, although a non-specific marker of inflammation has been widely used in COVID-19 patients. **Objectives:** In this study, we tried to evaluate the relationship of the other markers like IL-6, Ferritin, LDH, D-dimer, Fibrinogen, CK-MB and NLR with CRP levels in COVID-19 patients. **Methods:** This cross-sectional study was carried out in a tertiary care hospital, New Delhi India, July 2020 to Dec 2020. Total 282 adults, confirmed COVID positive by RT-PCR were included in the study. The investigations were performed on day 0 or day 1 of COVID positivity. Correlation of CRP with other markers was studied by regression analysis using Minitab ver.17. **Results:** Among the 282 patients, gender distribution was 190 males to 92 females with an average age of 52.5 ± 15.1 years. All the markers were observed to have non-parametric distribution. CRP was raised in 92.5% cases (n=239), IL-6 in 83.2% (n=279), Ferritin in 66.8% (n=238), LDH in 99.1% (n=233), D-dimer in 96.6% (n=30), Fibrinogen in 68.4% (n=19), CK-MB in 84.6% (n=98) and NLR of more than 4 in 82.8% (n=280). The median and range for the different markers were as follows: CRP (44.8 U/L; 15.6-736), IL-6 (67.3 pg/ml; 0.8-1624), LDH (608.0 U/L; 161-6705), Ferritin (643.5 ng/mL; 7.9-11932), D-dimer (7 µg/ml; 0-5250), NLR (9.43; 0.05-157.17); Fibrinogen (475 mg/dL; 285-759) CK-MB (9.9 mg/L; 0.1-46.7) respectively. Significant correlation was not observed between CRP and any of the inflammatory markers. **Conclusion:** Inflammatory markers are raised in most patients with COVID-19, however, all markers are not raised in all patients. Sequential evaluation of these markers may be beneficial rather than at one time point.

A-088

Establish laboratory Limit of Blank and Limit of Detection for LH Assay on the Ortho VITROS 3600 system

D. Su, H. Wu, Q. Zhao. *Maternity & Child center of Qinhuangdao, Hebei, China*

Background: In our lab, Luteinizing hormone (LH) was tested on VITROS 3600 system with manufacture's declared limit of detection (LoD) 0.216 mIU/mL, limit of Blank (LoB) 0.0367 mIU/mL. It's determined with NCCLS guideline EP17,false positives (α) less than 5% and false negatives (β) less than 1%, based on more than 700 determinations on several systems. Chinese government launched industry standard WS/T 514-2017, defining how a clinical laboratory establish its detection capability with stated proportions α=β=5% and some other differences from EP17. Here we set up the LoB, LoD of VITROS 3600 LH assay in our lab based on Chinese industry standard. **Method:** a)LoB was designed to run 4 blank samples, LoD with 4 low positive samples, respectively with two VITROS LH kit lots, on one VITROS 3600, over two runs per day, for 3-4 days using two different calibrations, therefore we got 120 results (outliers omitted) for LoB and LoD respectively.b)Negative (blank) samples were matrix fetal bovine serum (FBS), low positive samples were in house produced, VITROS 3600 System was well maintained, fully calibrated and quality controlled.c)Data analysis: LoB and LoD sample data are not Gaussian, according to WS/T 514-2017, the LoB and LoD were computed with nonparametric statistics. **Results** Perform steps described in method for each lot reagents. LoB data distribution were non-Gaussian (as when truncated at zero). therefore the LoB can be determined by ranking the data in order from lowest to highest concentration and using the following equations, LOB=$Pct_{100-\alpha}$, here α is 0.05, P is 0.95, 60 determinations, each reagent lot arrangement position=0.5+60*0.95=57.5, result was not an integer, LOB should be re-calculated=$X_{57}+0.5(X_{57}-X_{58})=0.5(X_{57}+X_{58})$. The final result for LoB is 0.0105.with proportions α=β=5%, each reagent lot LoD determinations more than 50, thereof, K-S test is used for normality analysis. All results of both reagent lots have p-value less than 0.05,which means that the samples were not been generated from a normal distribution. According to WS/T 514-2017, the LoD is estimated with nonparametric statistical derivation method. All results are greater than LoB 0.0105, 0% results are lower than LoB which is less than stated probability 5%. The median value for lot 1990 is 0.0898, and lot 2010 0.0899, so LoD is 0.0899. **Conclusion:** The limit of detection (LoD) for VITROS LH assay in our lab is 0.0899 mIU/mL , the limit of Blank (LoB) is 0.0105 mIU/mL, determined with WS/T 514-2017, and with proportions of false positives(α)=false negatives (β)= 5%. In our lab the detection capability is surpass to what manufacture claimed in the IFU. It could be as following factors: singleton platform in our lab, with greater stated proportions, less determinations, and consistently assigned operator. So we can conclude that this system is fit for a more sensitive detection scenario, like pediatric patients.

A-090

Impact of Changing Total Calcium Methodology on Observed Hypercalcemia Prevalence at an Academic Health System

C. K. LaValley, K. Dagang, J. J. Lado Abeal, P. Fu Teng, N. Tran. *UC Davis Health, Sacramento, CA*

Background: The UC Davis Health clinical chemistry laboratory implemented a new total laboratory automation (Roche Diagnostics, Indianapolis, IN) system in February 2022 - replacing a prior system (Beckman Coulter, Brea, CA) that was in place since 2007. In August 2022, primary care and endocrinology providers contacted laboratory leadership to report an increase in patients with calcium values flagged as abnormally elevated, resulting in increased communications from patients to primary care providers and increased referrals to endocrinology. This change in testing methodology produced a shift in the upper reference interval limit from 10.5 mg/dL to 10.0 mg/dL. The new testing methodology determined total calcium photometrically versus by indirect potentiometry via the outgoing method. We hypothesize photometric-based total calcium measurements to be more sensitive. **Methods**: We conducted a retrospective study to investigate the reported increase in hypercalcemic values—our electronic health system's (EHR) EPIC's self-service reporting tool ("SlicerDicer") was utilized for data acquisition and visualization. Although the reference interval had already been verified prior to implementation, a "normal study" study was conducted to re-confirm manufacturer derived intervals. One hundred thirty-six samples from apparently healthy adults (age ≥ 18 years) were banked for this study. Parathyroid hormone (PTH) values were then measured to confirm levels were within normal limits. Additional observational data was also collected for PTH levels pre- and post-new automation line implementation. Quality control (QC) and proficiency testing (PT) data was also reviewed during this time. **Results**: In the four months preceding

implementation of the new automation line, 1,211 patients had total calcium values flagged as elevated, compared to 5,146 patients during a similar post implementation time period (302.8/month vs. 1,286.5/month), or an increase of 324.9%. During these same time periods, patients with abnormal PTH were observed at 270.8/month pre-implementation compared to 393/month post (45.2% increase). An increase in patients with concurrently performed calcium and PTH was also observed (30.3%; 583.8/month vs. 760.8/month), though a greater increase was seen in patients with concurrently elevated calcium and abnormal PTH (294.5%; 22.8/month vs 89.8/month). One hundred twenty samples with normal PTH values were included in the total calcium normal study, which reported a mean (SD) value of 9.3 (0.33) mg/dL, confirming the manufacturer-derived intervals of 8.7 to 10 mg/dL. No biases, shifts, or trends were observed in PT or QC data in the four months preceding and post analyzer change. **Conclusion**: Increased hypercalcemic events post-automation line implementation is likely not due to analytical error or inappropriate reference intervals, but rather due to increased sensitivity of methodology. Hypercalcemic cases determined by our new methodology were corroborated by corresponding abnormal PTH values. The perceived increased sensitivity could be due to known limitations of indirect potentiometry (*i.e.,* lack of selectivity, difficulty detecting all forms of calcium, etc.). Further investigation is warranted to determine the overall impact of changing this one assay on the health system.

A-091

Assessing the Reliability of Creatinine-Estimated Glomerular Filtration Rate in Living Kidney Donor Candidates: Does it Measure up to the Measured Rate?

S. Lahorewala, X. Yi, R. Bertholf. *Houston Methodist Hospital, Houston, TX*

Background: A living donor kidney transplant is the best therapy option for patients with end stage renal disease (ESRD); however, it is associated with an increased risk of ESRD development in the donor. Thorough evaluation of the donor candidate's kidney function status is essential for the appropriate selection of candidates with minimal risk of post-donation kidney dysfunction.

Methods: Glomerular filtration rate (GFR) values measured by iohexol plasma clearance (mGFR) and estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) creatinine equations (eGFR), with and without the race factor (2009 and 2021 CKD-Epi equations, respectively), were compared in a cohort of 303 kidney donor candidates. GFR values were analyzed using Bland-Altman plots, linear regression, Mann Whitney U tests, and p10 and p30 values.

Results: Bland-Altman analysis revealed a lack of agreement between the 2009 and 2021 CKD-Epi eGFR and corresponding mGFR values, with both equations showing a negative bias of 10 - 11 mL/min/1.73 m^2 and indicating an underestimation of creatinine-eGFR. eGFR underestimation was confirmed by Mann Whitney U analysis; median GFR values were significantly lower for both CKD-Epi equations as compared to corresponding iohexol-measured values (2009: 85.86; 2021: 87.29; mGFR: 96.58). Despite the overall underestimation, over 14% of donor candidates with eGFR >90 mL/min/1.73 m2 had an mGFR value <90 mL/min/1.73 m^2. Conversely, 36% - 38% of donors with normal kidney function of >90 mL/min/1.73 m^2 as determined by iohexol-mGFR had corresponding eGFR values <90 mL/min/1.73 m^2. eGFR showed a significant but poor correlation with mGFR, with R^2 values of 0.2775 and 0.2803, respectively. While the 2021 eGFR equation had better accuracy as compared to the 2009 equation (p30 of 81% versus 76%), it displayed low overall accuracy with a p10 of <43%. Separate analysis of Black donor candidates' eGFR and mGFR values revealed similar trends as observed for the total cohort. While exclusion of the race factor in the 2021 CKD-Epi equation slightly improved accuracy (p30 of 77% versus 72%), the correlation with mGFR remained poor for both equations (R^2: 0.1455 and 0.1377 for the 2009 and 2021 equations, respectively).

Conclusion: The results of this study confirmed that creatinine-eGFR values, regardless of the race factor, differ significantly from iohexol-mGFR. Our data shows that even potential kidney donors with eGFR >90 mL/min/1.73 m^2 cannot be presumed to have normal kidney function, while eGFR <90 mL/min/1.73 m^2 cannot accurately exclude donor candidates. We conclude that creatinine-eGFR cannot reliably serve as a substitute for mGFR in the context of living donor kidney function evaluation.

A-092

Trends in Laboratory Communications Utilized in the Implementation of the Race-Free CKD-EPI 2021 Estimated Glomerular Filtration Rate (eGFR) Equation.

S. Luby. *University of North Carolina-School of Medicine, Chapel Hill, NC*

Background:

The National Kidney Foundation (NKF) established a Laboratory Engagement Working Group (WG) that issued strategies for implementing the race-free CKD-EPI 2021 eGFR equation following the recommendation from the National Kidney Foundation/American Society for Nephrology Joint Task Force on Reassessing the Inclusion of Race in Diagnosing Kidney Diseases. These guidelines covered four areas of implementation including communication, assay selection, programming, and reporting. Regarding communication, the NKF-WG recommendations identified six categories of stakeholders that should be included in communications: physicians and advance practitioners, pharmacists, clinical researchers, patients, patient advocates, and dietitians and nutritionists. This research evaluated the alignment of laboratory communications and inclusion of specific stakeholders during implementation of the CKD-EPI 2021 equation with recommendations from the NKF Laboratory Engagement Working Group. Additionally, the research sought to identify if concerns of racial bias associated with previous equations were communicated to stakeholders.

Methods:

An anonymous, web-based, cross-sectional survey of laboratory directors in the United States was conducted from November 1st through November 29th, 2022. The call for participants was distributed widely to the membership of the American Society for Clinical Laboratory Science (ASCLS) and the American Association for Clinical Chemistry (AACC). Participants were asked to denote if they had implemented the CKD-EPI 2021 equation, to identify communication methods utilized during implementation, and to identify key stakeholders included in communications. Participants were also asked to indicate if communications included information regarding concerns of racial bias associated with previous eGFR equations.

Results:

In total, there were 34 survey participants, with 32 completed submissions. Participants represented 19 states, with 5 states having 2 or more participants. Of the completed submissions, 87.5% (n=28) of respondents reported implementing the CKD-EPI 2021 equation. Of those respondents, 100% reported issuing communications to physicians and advance practitioners. 71.4% (n=20) of respondents reported issuing communication to pharmacists, 32.1% (n=7) included clinical researchers, and 14.3% (n=4) included dieticians and nutritionists. The most common method of communication directed to the patient care teams was through an official laboratory memo 82.1% (n = 23), followed by direct email 64.8% (n=18). Notably, only 10.7% (n=3) of respondents reported issuing communication to patients, however 25.0% (n=7) of respondents stated that the equation change was noted in the patient's medical record upon receiving an eGFR value calculated using the updated equation. Of the participants who implemented the equation change, 64.8% (n=18) reported addressing concerns of racial bias with previous equations in communications with physicians and advanced providers. 42.8% (n=12) included this information in communications with pharmacists, and only 10.7% (n=3) reported sharing this information with patients.

Conclusion:

A majority of participating laboratories did not include patients as stakeholders in communications during CKD-EPI 2021 implementation, highlighting a lack of alignment with NKF-WG recommendations. Further, and in contrast to physician communications, a majority of laboratories declined to include information regarding potential racial bias associated with previous eGFR equations in their patient communications.

A-093

Clinical Performance of Biomarkers for Traumatic Brain Injury

K. B. Grasso[1], J. L. Yen[1], A. Rutkowski[1], R. S. Ostro[1], D. R. Johnston[1], A. C. Buss[2], S. A. Datwyler[1], J. Marino[1], R. H. Christenson[3], Y. Zhu[4], C. deFilippi[5], K. L. Caudle[6]. *[1]Abbott Laboratories, Lake Forest, IL, [2]Abbott Laboratories, Abbott Park, IL, [3]University of Maryland- Baltimore, Baltimore, MD, [4]Penn State Milton S Hershey Medical Center, Hershey, PA, [5]Inova Health System, Falls Church, VA, [6]US Army Medical Materiel Development Activity, Fort Detrick, MD*

Background: An estimated 5 million people are evaluated in emergency departments (ED) for Traumatic Brain Injury (TBI) in the United States each year. The vast majority (80%) of patients evaluated for TBI in the ED will receive head CT scans, however

less than 10% of those will have any findings of acute traumatic abnormalities. This highlights the need for objective, rapid, accurate tools to help clinicians evaluate and triage TBI patients and reduce unnecessary radiation exposure. The Alinity i TBI test is a panel of in vitro diagnostic chemiluminescent microparticle immunoassays used to measure glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in human plasma and serum. TBI performance was evaluated in a multi-center study by performing reproducibility and a pivotal study.

Methods: A pivotal study using prospectively collected archived (frozen) plasma specimens was conducted to establish the clinical performance of the TBI test on the Alinity i system. The testing of the archived specimens was performed at three clinical sites in the United States. The specimens tested were originally collected in a prospective, multi-center clinical study and were from individuals 18 years of age or older who presented to a health care facility or emergency department with suspected traumatic brain injury. These subjects had an initial Glasgow Coma Scale (GCS) score of 13-15 and had a computed tomography (CT) scan of the head performed per the clinical site's standard of care.

Reproducibility was assessed using multiple panel/control members for 5 days based on guidance from Clinical and Laboratory Standards Institute EP05-A3. Testing was conducted using 3 lots of GFAP and UCH-L1 reagents, and 1 Alinity i system at each of 3 testing sites.

Results: Of the 1899 mild TBI subjects, 120 had positive head CT scan results; 116 of the 120 specimens had a positive TBI interpretation (Sensitivity 96.7%;95%CI:91.7%,98.7%). Of the 1779 subjects with negative CT scan results, 713 had a negative TBI interpretation (Specificity 40.1%;95%CI:37.8%,42.4%). The negative predictive value (NPV) of the test was 99.4% (713/717, 95%CI:98.6%,99.8%). Within-Laboratory imprecision (includes repeatability, between-run, and between-day variance components) results ranged from 2.2 to 3.5% CV for GFAP and 1.8 to 3.2 %CV for UCH-L1. The Overall Reproducibility (includes repeatability, between-run, between-day, between-lot, between-site, and site-lot interaction variance components) results ranged from 2.5 to 4.7% CV for GFAP and 2.6 to 6.6 %CV for UCH-L1. **Conclusion:** The Alinity i TBI clinical performance results demonstrated that the TBI test is characterized by high sensitivity and high NPV, which supports the clinical utility to assist in determining the need for a CT scan of the head in subjects presenting with suspected mild TBI. The GFAP and UCH-L1 assays demonstrated acceptable reproducibility across a wide dynamic range. These assays can report results within 18 minutes and be conveniently loaded into the Electronic Health Records to aid clinicians in their triage (or imaging) decisions.

Funding: The study was funded by Abbott Laboratories and in collaboration with the US Army Medical Materiel Development Activity, US Army Medical Research and Development Command CRADA 20-1266-CRA.

A-094

Performance Evaluation of GFAP and UCH-L1 Biomarkers for Traumatic Brain Injury in the Alinity i TBI Test (*in development*)

S. A. Datwyler[1], R. Chandran[1], J. A. Marino[1], D. R. West[1], H. N. Syed[1], Z. Al Sahouri[1], J. C. Badciong[1], K. L. Caudle[2], B. McQuiston[1]. *[1]Abbott Laboratories, Abbott Park, IL, [2]US Army Medical Materiel Development Activity, Fort Detrick, MD*

Background: Traumatic Brain Injury (TBI) has been recognized as an important cause of death and disability and is a growing public health problem. An estimated 69 million people globally experience a TBI annually. Blood-based biomarkers such as glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase L1 (UCH-L1) have shown utility to predict acute traumatic intracranial injury on head CT scan after TBI. **Methods**: The Alinity i TBI test is a panel of in vitro diagnostic chemiluminescent microparticle immunoassays for the measurement of GFAP and UCH-L1 in plasma and serum. Performance characteristics such as detection limits, imprecision, linearity, measuring interval, expected values, and interferences were established following CLSI guidance. A feasibility cohort of 354 subjects was used to establish cutoffs which assist in determining the need for a CT (computed tomography) scan of the head. A prospective study was conducted to assess the clinical performance of the TBI test. Fresh plasma specimens from 97 mild TBI subjects presenting to Level I trauma centers were collected within 12 hours of injury from subjects ≥18 years of age who presented with suspected TBI and had a head CT scan performed. The TBI interpretation is considered positive if either GFAP or UCH-L1 is greater than or equal to the cutoffs. The TBI interpretation is considered negative if both GFAP and UCH-L1 are less than the cutoffs. **Results**: The analytical measuring interval (AMI) extends from the limit of quantitation (LoQ) to the upper LoQ and is determined by the range that demonstrates acceptable performance for linearity, imprecision, and bias. The AMI is 6.1 to 42,000.0 pg/mL for GFAP and 26.3 to 25,000.0 pg/mL for UCH-L1. Within-laboratory imprecision (20 day) ranged from 2.8 to 5.3% CV for GFAP and 2.1 to 5.6% CV for UCH-L1. Cutoffs for GFAP and UCH-L1 of 35.0 pg/mL and 400.0 pg/mL, respectively, were selected using both 10-fold cross validation and bootstrapping methods and applying a selection criterion of negative predictive value (NPV) (prevalence adjusted) ≥99% and Sensitivity ≥96%. Of the 97 mild TBI subjects evaluated, 14 had positive head CT scan results. All 14 of the subjects with positive head CT results had a positive TBI interpretation (Sensitivity 100.0%; 95% CI:78.5%,100.0%). There were 23 subjects with a negative TBI interpretation, all 23 had a negative head CT scan result. The NPV of the test was 100% (95% CI:85.7%,100.0%). **Conclusion**: The Alinity i TBI test shows robust analytical performance across a broad concentration range of GFAP and UCH-L1 and may serve as a valuable tool to help evaluate TBI patients across the spectrum of mild to severe injury. The established cutoffs were evaluated in a prospective cohort of mild TBI (Glasgow Coma Scale score of 13-15) subjects and demonstrated high sensitivity and NPV relative to CT scan results, supporting the utility to rule out the need for CT scan in mild TBI subjects presenting to the emergency department. **Funding**: This work is in collaboration with the US Army Medical Materiel Development Activity, US Army Medical Research and Development Command CRADA 20-1266-CRA.

A-096

Novel Gastrointestinal Testing: Comparative Performance of the ALPCO Five-Plex Gastrointestinal Marker Assay compared to Conventional ELISA/CLIA testing for Human Stool Samples.

J. M. Cacicedo, S. Papkov, J. Petzke, W. Schmidt, H. Vedrani, K. Veenstra, C. Wisherd. *ALPCO, Salem, NH*

Background:

All modern gastrointestinal (GI) testing laboratories face a similar challenge: How to test a panel of analytes from the same stool or plasma/serum sample and deliver clinically accurate results in a timely fashion. GI analytes are tested primarily on ELISAs that while accurate, manual ELISA testing is time-consuming and work intensive. In this study, we utilize the automated ALPCO five-plex assay (AutoPlex™) to measure five key GI biomarkers: Calprotectin (CP), Pancreatic Elastase (PE), Lactoferrin (LF), Eosinophil derived neurotoxin (EDN), and Hemoglobin (Hgb). These biomarkers are widely recognized indicators of gut inflammation and disease, including inflammatory bowel disease, pancreatic insufficiency, and colorectal cancer. The assay employs a simple and efficient sample preparation protocol and utilizes multiplexed bead-based technology to quantify each biomarker at the same dilution and time point in an automated fashion. In this study, we determine how this new assay performs against gold standard ELISA/CLIAs that measure the same analytes.

Methods:

Capture and detector antibodies were optimized for each of the analytes to perform on beads, and buffers were optimized to allow for all the analytes to be measured simultaneously. A stool extraction device was utilized to extract 386 human stool samples from both healthy controls and populations with suspected GI impairment. Samples were analyzed using the ALPCO 5-Plex (AutoPlex) assay run on the Multiplier® - a fully-automated chemiluminescence analyzer (DYNEX® Technologies, Chantilly, VA), and conventional Chemiluminescence ELISA/CLIA methods.

Results:

Comparative analysis of 386 human stool samples run on both the 5-Plex and ELISAs for each respective analyte, showed strong clinical correlation between the two platforms. With a clinical cut-off of 50 ug/g calprotectin had a 91% clinical correlation between the two platforms. Similarly, with a cut-off of 200 ug/g PE was at 91%, LF with a cut-off of 7.24 ug/g at 83%, EDN with a cut-off of 4.6 was at 97%, and Hgb with a cut-off of 20 ug/g was at 98% clinical correlation between the two platforms.

Conclusions:

The results generated by ALPCO five-plex assay demonstrated on average >90% clinical matching for all 5 GI analytes tested when compared to their gold standard ELISA/CLIA counterparts. Since this assay allows for the simultaneous measurement of five common GI analytes that occur from a single stool extract in one concurrent and automated workflow, this saves time as it eliminates the need for multiple ELISA/CLIA tests and reduces processing time for stool samples.

A-097

Stability study of 21 common plasma biochemistries in unspun whole blood

M. Ospina Romero[1], T. Robakowski[2], A. Nelson[2], K. Grant[2], Z. Jin[1], K. Galior[1]. *[1]UW Madison, Madison, WI, [2]UWHealth, Madison, WI*

Background: Most blood collection tube manufacturers typically recommend separating serum/plasma from red blood cells within 2 hours of collection prior to biochemical analysis to minimize cell contact and thus maintain stability of analytes. This can lead to rejected testing when uncentrifuged samples are transported from ambulatory sites to core laboratories over long distances, leading to delayed patient care and decreased patient satisfaction from having to be redrawn. Few studies are available to make recommendations of specimen acceptance with delayed centrifugation time. Our study aimed to assess whole blood stability of routinely ordered chemistry tests. **Methods:** Between 11/30-12/1/2022, venous blood from 20 non-fasting healthy adults(30% male) with ages ranging from 23-57 years were collected into 3-ml plastic Becton Dickinson(BD) Vacutainer™plasma separated tubes(PST). For each person, 6 PST were collected. One whole blood sample was centrifuged immediately(T0) at 4000g for 3 min at room temperature(RT) and analyzed. Remaining 5 whole blood samples were stored at either RT or 4°C and the subsequent centrifugation of the samples occurred at 2, 4, 6, 12 and 24 hours after collection. The analytes studied using Abbott Alinity instrumentation were comprehensive metabolic panel(CMP), lipid panel, TSH and FT4. Absolute and percent bias at various time points for each participant were calculated using the reference sample(T0). Observed biases were compared to allowable total error based on desirable biological variation(BV) from European-Federation-of-Laboratory-Medicine(EFLM) database and Clinical-Laboratory-Improvement-Amendments(CLIA) 2024 upcoming limits. **Results:** Analytes stable in the whole blood at both RT and 4°C up to 24 hours based on EFLM-BV and CLIA were ALP, ALT, AST, BUN, cholesterol, creatinine, HDL, total bilirubin, triglycerides, and TSH. Analytes with variable stability times are shown in the table below:

		Biological Variation (desirable)			CLIA 2024		
Analyte	Concentration, mean	Allowable Total Error	Stability at 21C (hr)	Stability at 4C (hr)	Allowable Total Error	Stability at 21C (hr)	Stability at 4C (hr)
Albumin	4.26 g/dL	3.4%	24	12	8%	24	24
Total Calcium	9.73 mg/dL	0.2	24	6	1.0	24	24
Chloride	106.0 mmol/L	1.3%	6	12	5%	24	24
Bicarbonate	25.78 mmol/L	5%	2	4	20%	24	24
FT4	0.88 ng/dL	6.3%	12	12	20%	24	24
Glucose	96.12 mg/dL	6.5%	<2	4	8%	<2	4
K	4.04 mmol/L	0.20	2	<2	0.3	6	2
MG	1.97 mg/dL	4%	12	24	15%	24	24
NA	139.6 mmol/L	1.0	6	<2	4.0	24	12
PO4	3.20 mg/dL	9.7%	4	24	10.0%	4	24
Total Protein	7.59 g/dL	3.5%	24	12	8%	24	24

Conclusion: This study provides a guideline of acceptable times for whole blood samples sent for routine chemistry tests when centrifugation was not performed within 2 hours from collection.

A-098

Preliminary tests of cell viability, proteins and mRNA after freezing PBMC for the study of chronic and acute lymphoproliferative leukemias

M. C. Feres[1], L. V. Soares[2], S. d. Roz[3], O. F. Souza[4], P. Vicari[3], C. C. Cabral[3], D. R. Ramadan[3], S. Tufik[3], A. F. Popi[2]. *[1]Associação Fundo de Incentivo - Afip, Sao Paluo, Brazil, [2]Universidade Federal de Sao Paulo - Unifesp, Sao Paulo, Brazil, [3]Associação Fundo de Incentivo - Afip, Sao Paulo, Brazil, [4]Universidade Federal de Sao Paulo - Unifesp, Sao Paulo, British Indian Ocean Territory*

The peripheral blood mononuclear cells (PBMC) cryopreserved are widely used in human immunology. However, PBMC, like other cell types, are sensitive to the freezing process. The impact of cryopreservation on T and B cell subsets of PBMC remains a challenge. For human PBMC, some reports have not observed significant changes between fresh and frozen cells in terms of proportions, phenotype and functionality, as well as antigen-specific responses or response to mitogens. Others, however, report that cryopreservation alters the proportions of human PBMC subsets in various immune responses. With the interest of studying chronic and acute lymphoproliferative leukemias (CLL and ALL), the authors' objective was to test the viability of PBMC cells obtained from patients with CLL and ALL before and after freezing, following a simplified protocol. Methods: On blood samples collected in EDTA, readings and cell counts were performed. Samples with a suggestive diagnosis of CLL and ALL were selected for the separation of PBMC whose analyzes were performed before and after freezing. The freezing of the PBMC was carried out in several stages and the whole process is carried out with care and in a gradual and simplified way of freezing until reaching the liquid nitrogen phase. Cells were kept frozen in freezing medium (RPMI plus 3% DMSO and 20% fetal bovine serum) for 1 week. Afterwards, staining with Trypan blue and counting in a Neubauer chamber were carried out. Student's t test for independent variables and a significance level of 5% were considered. Results: Table 1. Conclusion: The description of this technique allows its use for analysis of gene expression profile, being able to contribute with the description of the molecular signature of leukemias.

Table 1: Represents the viability, protein and RNA levels in the two conditions of the studied sampl

Tests	without freezing Mean ±SD	after freezing Mean ±SD
Viable cells - (x10⁶)	0.931 ± 0.047	0.858 ± 0.674
Protein levels - (ug/mL)	106.825 ± 6.741	106,8621 ± 15,038
RNA levels - (ng)	51.40 ± 7.210	50.95 ± 7.302
No significant difference < 5 %		

A-099

Assessment of stability of routine clinical biochemical results in 24-hour urine specimen under conditions simulating various preanalytical variables

H. Chae, H. Jang, K. Cha, E. Oh. *Seoul St. Mary's Hospital, Seoul, Korea, Republic of*

Background: The preanalytical phase has long been recognized as a source of substantial variability for 24-hour urine samples. Hence, we examined the stability of analyte concentrations of 14 clinical biochemistry parameters commonly requested in a 24-h urine sample. The effects of various preanalytical variables simulating preexamination processes of the testing process were assessed.

Methods: We aimed to examine the effect of time delay in sample processing (up to 7 days), storage temperature (at 4℃ and room temperature), storage container (24-h urine bag, styrofoam box), and the effect of addition of toluene as preservative. The effect of the variables on clinical biochemistry parameters (uric acid, calcium, amylase, LDH, total protein, urea nitrogen, magnesium, phophorus, glucose, creatinine, sodium, potassium, chloride, and micro-albumin) in 24-h urine samples were assessed.

Results: Most of the analytes (uric acid, calcium, amylase, total protein, urea nitrogen, magnesium, creatinine, sodium, chloride, and micro-albumin) showed values not significantly different (≤± 5%) from baseline after 7 days of storage regardless of the storage temperature or addition of preservative. Three analytes were stable ≤24-hrs: phosphorus and glucose were stable up to 24-hrs and potassium up to 4-hrs. The addition of toluene had significant positive interference with LDH measurements.

Conclusion: According to our results, major analytes (uric acid, calcium, amylase, total protein, urea nitrogen, magnesium, creatinine, sodium, chloride, and micro-albumin) were stable up to 7 days in ambient storage conditions and toluene as a preservative was not necessary. However, a few analytes (phosphate, glucose, and potassium) were much more unstable and allowed ≤24-hrs or ≤4-hrs of storage in ambient or refrigerated conditions.

A-101

Assessing Glycemic Control in Potential Blood Donor at the Blood Bank of the University College Hospital, Ibadan, Nigeria

T. D. Ogunleye. *University College Hospital, Ibadan, Nigeria*

Background: Voluntary blood donation exercise remains one the safest means of sourcing for blood product made available for patients. However, there are several eligibility criteria put in place before a prospective donor can be accepted for donation exercise. One of the eligibility criteria not commonly considered is the glycemic control of a donor. This is because pooly controlled diabetes being treated with insulin or a patent with type 1 Dm is considered unfit for blood donation. This study carried out was to determine gycemic control of the prospective donors by assaying their HBA1C levels before blood donation at the blood bank of the university college hospital, Ibadan, Nigeria.

Methods: Venous blood of the prospective donors was collected during the screening exercise that precedes blood donation to determine eligibility. The blood was for the

assay of HBA1C was collected into EDTA bottle and analysed using spectrophometric method. A total number of 184 prospective donors were screened within a period of three weeks. Any result that falls between 4.0-5.6% was considered normal, 5.7-6.4% was considered Pre-diabetic while results that are greater than 6.5% were considered diabetic and likely not fit for blood donation.

Results: A total number of 184 apparently healthy young adult between the ages of 19 and 45 years were screened. Out of the 184 prospective donors, 42 (22.8%) of the population were female, the remaining 142 (77.2%) were male. I56 (84.7%) of the population screened had normal gycemic control between 4.0-5.6% while 20 (15.2%) of the population had a pre-diabetic results (5.7-6.4%). The reaming 2 (1.1%) prospective donors had poor glycemic control (>6.5%). This suggests that most of the donors had good glycemic control.

Conclusion: As much as a good attention is placed on other eligibility criteria for blood donation attention should also be placed on the glycemic control of donor for the safety of both the donor and the recipients of blood products.

A-102

Development and validation of turbidimetric Retinol Binding Protein (RBP) assay

M. Jaquemar, A. Safdari, T. Nilsen, T. Knüttel, A. Havelka. *Gentian AS, Moss, Norway*

Background: Retinol-binding protein (RBP) is a non-glycosylated protein synthesized by the liver. The role of RBP is to transport retinol (vitamin A) from the liver to the target tissues. RBP is also a sensitive marker of undernutrition. Serum/plasma RBP levels decrease with Vitamin A deficiency, hepatocellular insufficiency, and severe inflammation. Serum/plasma RBP levels increase with renal failure, type-2 diabetes, and steatosis.

Methods: The immunoassay was developed and validated on the turbidimetric instrument Cobas c501 from Roche Diagnostics. The antibodies against RBP in the assay react with RBP in the sample, and the turbidity formed by formation of antibody-antigen complexes is measured at 340 nm and 700 nm. The analytical sensitivity was tested in a study including 4 samples, analysed in 3 replicates over 3 days using a protocol based on the CLSI guideline EP17. The limit of quantification (LoQ) is defined as the lowest concentration of an analyte that can be reliably detected and at which the total error meets the requirements for accuracy. Interference with triglycerides, haemoglobin and bilirubin was tested using a protocol based on the CLSI guideline EP07. Precision of the assay was tested in a 22-day precision study using a protocol based on the CLSI guideline EP05. 3 controls were measured 2 times a day in 2 replicates each time.

Results: The assay has a calibration range from 7.5 to 120 mg/L. Samples with higher concentrations are automatically diluted and reanalysed. No antigen excess was observed up to 600 mg/L. Precision achieved a total CV of <4 % for samples with concentrations in the range 34-63 mg/L. Analytical sensitivity of RBP was measured as 7.81 mg/L with a total error (TE) of ≤ 20 %. No clinically relevant interference towards triglycerides (13 g/L), haemoglobin (6.9 g/L) and bilirubin (0.3 g/L) was observed.

Conclusion: The Gentian RBP assay is a turbidimetric assay based on the formation of antibody-antigen complexes and can be used for assessment of undernutrition, vitamin A deficiency, hepatocellular insufficiency, severe inflammation, renal failure, type-2 diabetes, and steatosis. The assay can be installed on high-throughput platforms from different manufacturers and provides accurate, cost efficient and fast results.

A-103

Stability study of the biogenic amines 5-hydroxyindoleacetic acid (5-HIAA), Vanillylmandelic acid (VMA), and Homovanillic acid (HVA) in acidified versus non-acidified human urine samples

L. F. Junior, B. F. Paulo, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Carcinoid tumors and neuroblastomas are disorders in which some neurotransmitters are produced. The first one is characterized by the overproduction of serotonin, which the main metabolite is 5-Hydroxyindoleacetic acid (5-HIAA). In neuroblastomas, approximately 90% of the tumors produce catecholamines, wherein Vanillylmandelic acid (VMA) and Homovanillic acid (HVA) are the main final products of catecholamine metabolism. Due to the great clinical importance of these disorders, our group previously developed a very sensitive method to identify and quantify VMA, HVA, and 5-HIAA by LC-MS/MS. Thus, was performed a study of sample stability to identify the impact of no-acidification of the samples and the optimal storage temperature to perform a high accuracy analysis. The study aimed to evaluate stability of acidified and non-acidified urine samples under refrigerated and frozen conditions to identify and quantify VMA, HVA and 5-HIAA compounds.**b Methods:** Isolated urine samples were collected from 24 volunteers. For each compound, the stability of non-acidified samples was compared with HCl acidified samples, aiming for a final pH among 1 and 4. Independent aliquots of the samples were made to obtain the following cohort: Baseline (day of collection acidification), 24 hours post-collection acidification, 4 days, 8 days and 15 days acidification. Those samples were divided into 4 groups: A - refrigerated storage and non-acidified; B - frozen and non-acidified; C - refrigerated and acidified (default) and D - frozen and acidified. The quantification of the analytes was performed by a previously validated method that employ a simple dilution of the samples followed by the LC-MS/MS analysis. Chromatographic separation and detection were performed on the Waters ACQUITY UPLC system equipped with RP-C18 column and gradient separation and on a Waters XEVO TQ-S Micro mass spectrometer and electrospray ionization (ESI+), respectively. **Results:** The comparison between the basal groups (collection day) was identical for the 3 compounds, in the non-acidified groups (A and B) and also in the acidified group (C and D). Pearson's Correlation was 0.995, 0.,993 and 0.995 ($p < 0.0001$) for VMA,HVA and 5 -HIAA, respectively. Stability for refrigerated samples has been confirmed for up to 7 days and 15 days for compound 5-HIAA. For 15-day samples, one of the differences exceeded the total allowed error (31.3%-Westgard). Frozen samples (non-acidified or acidified) reverse percentage differences when compared to the standard group. In addition, for group B (frozen and non-acidified) the differences exceeded the total allowed error. However, comparing those days after collection and basal groups, it was calculated that the maximum acceptable period to maintain the stability of the samples was up to 7 days in refrigerated condition. **Conclusion:** The stability for the urinary measurement of VMA, ‚HVA and 5-HIAA was considered acceptable in this study. The maximum period allowed for acceptable stability is up to 7 days in the refrigerated condition.

A-104

Clinical Validation of the VITROS® Immunodiagnostics Products B·R·A·H·M·S PCT Test Medical Decision Points and Prediction of Cumulative 28-Day All-Cause Mortality

G. Ogbonna[1], P. Ajongwen[2], S. Alvey[2]. *[1]QuidelOrtho, Rochester, NY, [2]QuidelOrtho, Raritan, NJ*

Background: Procalcitonin (PCT) is a biomarker for systemic bacterial infection and sepsis and may be used to aid in decision-making on antibiotic therapy for patients with suspected or confirmed lower respiratory tract infections (LRTI) and/or sepsis. Procalcitonin medical decision points have been established using the B·R·A·H·M·S PCT sensitive KRYPTOR assay. In this study, we compared the performance of the VITROS® Immunodiagnostic Products B·R·A·H·M·S PCT assay to the performance of the B·R·A·H·M·S PCT sensitive KRYPTOR assay at the established medical decision points. Percent change in PCT level over time can be used to aid in the prediction of cumulative 28-day mortality in patients diagnosed with severe sepsis and septic shock. The 28-day all-cause mortality prediction of the VITROS B·R·A·H·M·S PCT assay was also evaluated in the study.

Methods: Patient sample sets from the Multicenter Procalcitonin MOnitoring SEpsis (MOSES) study conducted in 13 sites in the United States were used in this study. Blood samples were collected on Days 0, 1, 3 and 4 from subjects who were admitted to the ICU with severe sepsis or septic shock. Each patient's vital status was verified at Day 28. The samples were tested on the VITROS B·R·A·H·M·S PCT assay and the B·R·A·H·M·S PCT sensitive KRYPTOR assay. Concordance analysis between the VITROS B·R·A·H·M·S PCT and the B·R·A·H·M·S PCT sensitive KRYPTOR assays was performed at the established clinical decision points of 0.100 ng/mL, 0.250 ng/mL, 0.500 ng/mL, 2.00 ng/mL and 10.0 ng/mL to determine overall agreement between the two assays. For prediction of cumulative 28-day all-cause mortality using the VITROS B·R·A·H·M·S PCT assay results, the change in PCT values (Δ PCT) from Day 0 to Day 4 and Day 1 to Day 4 was calculated and evaluated against 28-day-all-cause mortality.

Results: Overall agreements of 98.5%, 98.0%, 97.4%, 97.8% and 98.0% were observed at 0.100 ng/mL, 0.250 ng/mL, 0.500 ng/mL, 2.00 ng/mL and 10.0 ng/mL, respectively, with the B·R·A·H·M·S PCT sensitive KRYPTOR assay. Δ PCT decline >80% or ≤80% was significantly associated with 28-day-all-cause mortality ($P = 0.006$). Univariate Cox proportional hazards regression analysis showed a 1.93-fold increase in risk for mortality in subjects with a Δ PCT change ≤80%.

Conclusion: The VITROS B·R·A·H·M·S PCT assay demonstrated excellent agreement with the B·R·A·H·M·S PCT sensitive KRYPTOR assay at medical decision points used to aid in decision-making on antibiotic therapy for patients with suspected

or confirmed LRTI and/or sepsis. Clinical performance shows ΔPCT information can be used to classify patients diagnosed with severe sepsis or septic shock as lower and higher risk for cumulative 28-day all-cause mortality.

A-105

Analytical Performance Evaluation of Four General Chemistry Assays on the Atellica CI 1900 Analyzer Used to Assess Kidney Function

S. Lewisch, C. Tyler, K. Kolewe, G. Arrode-Bruses, J. Rhea-McManus, J. Snyder. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput, integrated chemistry and immunoassay analyzer utilizing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica CH Creatinine (CREA_2)*, Enzymatic Creatinine (ECre_3)*, Urinary/Cerebrospinal Fluid Protein (UCFP)*, and Microalbumin_2 (µALB_2)* assays on the Atellica CI 1900 Analyzer*.

Methods: The Atellica CI 1900 CH CREA_2, ECre_3, UCFP, and µALB_2 assays use the same reagents and calibrators as the Atellica CH assays. Precision and method comparison (MC) were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 using native and contrived human urine samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. MC studies were performed according to CLSI EP09-A3. Individual native and contrived human urine samples were analyzed using the Atellica CH assays on both the Atellica CH and Atellica CI 1900 Analyzers.

Results: Representative precision and MC results observed from one reagent lot across indicated sample ranges are listed for each assay in the table below. Over the four assays tested, repeatability and within-lab %CVs were <5.1% and <6.0%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1.

Conclusion: Evaluation of the Atellica CH CREA_2, ECre_3, UCFP, and µALB_2 assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on the Atellica CH Analyzer.

	Precision				Method Comparison	
	Unit	Sample Range	Repeatability %CV Range	Within Laboratory %CV Range	Sample Range	Regression Equation for Atellica CH 930 Comparative Assay
Urine Analyte (Assay)						
Creatinine (CREA_2)	mg/dL	61.48–190.68	0.3–0.4	1.6–3.2	4.37–239.13	y = 0.97x – 0.64 mg/dL
Enzymatic Creatinine (ECre_3)	mg/dL	43.01–155.37	0.2–0.3	1.0–2.2	3.22–242.92	y = 0.99x – 0.43 mg/dL
Protein (UCFP)	mg/dL	21.1–145.8	0.9–5.1	1.0–5.6	7.0–224.0	y = 0.93X + 1.9 mg/dL
Albumin (µALB_2)	mg/dL	3.3–36.8	0.6–1.2	1.8–5.9	0.3–37.3	y = 1.03x + 0.1 mg/dL

*The products/features mentioned here are not commercially available in all countries. Their future availability cannot be guaranteed.

A-106

Sigma Metric Performance of Chemistries on the Abbott Alinity c Analyzer

E. T. Laryea, J. H. Nichols. *Vanderbilt University Medical Center, Nashville, TN*

Background: The analytical performance of the Abbott Alinity c (Abbott Park, IL) was compared to our current Abbott Architect analyzers using sigma metrics.

Methods: Sigma metrics of 28 chemistry analytes on our six Abbott Alinity analyzers was compared to three Abbott Architect 16000 analyzers and one Architect 8000 analyzer using Bio-Rad Multiqual quality control (QC) (Hercules, CA). Method imprecision was estimated for each assay using the cumulative coefficient of variation (CV) of 12 months data for each analyzer at three QC concentrations and calculating the average sigma. Method bias was estimated by comparing each analyzer QC mean to peer group QC means. Sigma values were calculated for each method as (TEa - Bias%)/CV% using allowable total error (TEa) from three sources: the CLIA proficiency testing evaluation limits, Australasian Association of Clinical Biochemistry allowable limits of performance (RCPA quality assurance programs Pty limited, Adelaide, Australia) and desirable biological variation (Ricos C et al, *Scand J Clin Lab Invest. 1999,* 59(7):491-500.). Average sigma values were generated for each analyte and graded as optimal >6 sigma; good 5-6 sigma; marginal 3-5 sigma; or poor <3 sigma.

Results: Assay sigmas varied across QC concentrations and between analyzers of the same platform. Na, Cl, and CO_2 had the lowest sigma performance. Since the sigma calculations are dependent on TEa, tighter TEa limits (Ricos and RCPA) generated lower sigmas compared to the wider CLIA TEa limits especially for Ca, Cl, total bilirubin, CO_2, Na, total protein, and HDL.

Conclusion: None of the chemistries met optimal six sigma performance for all analytes, concentrations of QC and sources of TEa. However, the assay performance between analyzer platforms was comparable.

	Sigma metric (CLIA)			
	>6 (Optimal)	5 – 6 (Good)	3 – 5 (Marginal)	<3 (Poor)
Abbott Alinity c	8	11	6	3
Abbott Architect	15	6	5	2
				Total analytes = 28

A-107

Estimating Glomerular Filtration Rate by the Serum Creatinine and Cystatin C-plus-Creatinine Equations: Concordance and Effect on GFR Categorization.

R. C. Faught, M. R. McGill, H. Hagrass. *UAMS, Little Rock, AR*

Background: Estimating the glomerular filtration rate (GFR) is essential for evaluating kidney function, which is part of routine medical care. Most clinical laboratories report an estimated glomerular filtration rate (eGFR) based on serum creatinine, but cystatin C is a potential alternative marker. Evidence suggests that using the creatinine-plus-cystatin C (the 2021 CKD-EPI creatinine-cystatin C equation) eGFR gives more accurate results than the eGFR based on creatinine alone. Here we compared two 2021 CKD-EPI equations for estimating GFR — one using standardized creatinine alone (Cre) and the other using cystatin C combined with standardized creatinine (Cre-Cys). We also explored the effect of the equations on the GFR category. Methods: We retrospectively analyzed 777 simultaneous values for eGFR calculated by the two 2021 CKD-EPI equations over a period of nine months, from the time our laboratory initiated the Cre-Cys equation to the present.Cystatin C was measured turbidometrically on the Optilite (Binding Site, Birmingham, UK) and the creatinine was measured by the DxC method (Beckman Coulter, California, USA). We excluded any patient with Cre eGFR >90 mL/min/1.73 m2 because we do not result any value above 90 for that parameter.

Results: Out of 777 patients (52% female and 48% male), we excluded 82 results with Cre GFR>90. The mean±SD for Cre-Cys eGFR and Cre eGFR were 37.3±23.8 and 37.1±23.1, respectively, with a non-statistical difference between both groups using the paired T-Test (t= -0.8249, p-value= 0.4097). The mean difference (Cre-Cys eGFR minus Cre eGFR) was 0.28, with a standard deviation of 9.027. The correlation coefficient between Cre eGFR and Cre-Cys eGFR was r = 0.93. Two hundred and seventy-four (35%) samples had a difference between the numerical value of the two eGFR equations significant enough to change the GFR classification. 128 (34.5%) of the males had different categories when both equations calculated the eGFR versus 146 (36%) females. 36.3% of the white and 34% of the black patients had a discrepancy. The calculations matched for the other 502 patients. Of the 274 samples with discordant category classifications, the Cre-Cys equation gave a lower result and a higher (worse) category in 144 (53%), and a higher result and lower (better) category in 130 (47%).

Conclusion: The use of Cystatin C is growing and has moved beyond the nephrology community. Furthermore, the 2012 KDIGO guidelines suggest using additional tests (such as cystatin C or a clearance measurement) for confirmatory testing in specific circumstances when eGFR based on serum creatinine is less accurate. Currently, cystatin C testing is only available M-F, 8 am - 4 pm at our institute, but increasing availability and decreasing cost will likely expand Cre-Cys eGFR testing in the future. However, understanding the relationships between eGFR values from the different equations and how they will affect patient management can help clinicians optimize their use for kidney function evaluation.

A-108

Validation of Plasma Oxalate Measurement on the Roche cobas c501

S. Zilka, G. M. Kroner. *Cleveland Clinic, Cleveland, OH*

Background: Measurement of plasma oxalate is important in patients with renal failure as oxalate may build up due to impaired excretion and lead to organ damage due to precipitation of calcium oxalate crystals. However, the measurement of plasma oxalate is challenging due to the low concentrations present in individuals with healthy kidney function and the pre-analytical steps needed to prevent erroneous oxalate results due to conversion from ascorbate. In order to provide improved turnaround-time for plasma oxalate testing, our objective was to develop and validate a method to measure plasma oxalate on the Roche cobas c501.

Methods: Samples used in the validation study included both fresh collections and residual plasma from specimens collected in lithium heparin, no-gel tubes. Samples from fresh collections were kept on ice prior to centrifugation, and then frozen if not analyzed immediately. Thawed plasma was acidified by addition of 12.1 M HCl to a pH between 2 and 3 and mixed thoroughly by vortexing. One milliliter of the acidified plasma was added to a Centrifree filter (Millipore). The samples were then centrifuged for 90 minutes at 1500xg 20°C in a fixed angle rotor to remove plasma proteins. The resulting filtrate was assayed on the Roche cobas c501. The assay (Trinity Biotech) uses oxalate oxidase to produce an indamine dye through the production of hydrogen peroxide from oxalate in the sample, which is measured spectrophotometrically at 600 nm at a single time point. The analytical measuring range (AMR) was evaluated by performing serial dilutions of a high sample. Interferences from hemolysis, icterus, and lipemia were assessed at both low and high oxalate concentrations. Precision was evaluated using two QC concentrations, and patient samples with low and high concentrations of oxalate. Samples were tested ten times in a single day, or twice per day for ten days to estimate intra- and inter-precision, respectively. Spike recovery studies were used to assess method accuracy. Lastly, reference intervals were verified by assaying samples from twenty healthy volunteers. Total allowable error was ±30% or 2 µmol/L with a maximum 15%CV.

Results: The assay was validated to be linear across an AMR of 2 to 30 µmol/L, with an average recovery of 105% across five concentrations. The total assay imprecision was <15%. Initial spike recovery studies across the AMR showed an average recovery of 99.4% (n=12), with a range of 76.6 to 132.4%. In a mixing study, hemolyzed and lipemic sample recovery was on average 82.1% and 76.1% respectively, which was low, but acceptable. Samples mixed with icteric specimens demonstrated unacceptably low recovery (average of 64.1%). The reference interval of <2 µmol/L was successfully verified as all twenty healthy volunteers had results <2 µmol/L.

Conclusion: Initial validation studies support the clinical utility of the newly developed method. Plasma oxalate methods are documented to have significant inter-method variability and variable recovery, which is hypothesized to be due to variation at the filtration step. This semi-automated assay will require less technologist time and will reduce the possibility for human error affecting the result.

A-109

Application of Lean manufacturing in Electrophoresis Test processing in order to reduce lead time

J. R. Oliveira, I. B. Escalante, I. R. Fernandes, K. F. Carrero, P. G. Costa, R. H. Jacomo, L. F. Abdalla. *Sabin Medicina Diagnostica, Brasilia, Brazil*

Background: There has been an increase on the demand for Protein Electrophoresis and Hemoglobin Electrophoresis exams over the past few years. This growth drew attention and motivated the laboratory's Production Planning and Control (PPC) team to carry out a process flow analysis of the electrophoresis samples coming from the external units, which are responsible for approximately 60% of the total samples analyzed of these two exams. The study aimed to minimize waste throughout the process, optimize the use of resources, standardize continuous flows of activities, and reduce the Turnaround Time (TAT) of the samples in question, disseminating Lean culture and increasing the satisfaction of internal and external customers.

Methods: In order to reduce the TAT of electrophoresis exams from external units, the PPC team carried out on-site visits and revised the entire flow of processes related to these exams and others that could indirectly influence it, from collection and transport until the analyst's signature and release of the result to the patient, mapping the entire flow covered by the samples and identifying possible important bottlenecks inherent to the activities, aiming at possible opportunities for improvement, through interviews, brainstorming, flowcharts and Design Thinking. Data such as the volume of monthly tests, percentage of external samples, TAT at the regional units, TAT at the Immunochemistry sector and total TAT were extracted from the laboratory's automated data system and analyzed by the PPC team. With this, it was possible, through the Pareto analysis, to prioritize the obstacles to be overcome and focus on the main identified bottleneck, the amount of time for signing and releasing the reports, in addition to focusing on the random flow between the receiving and triage area and the processing technical area.

Results: Upon discovering that the greatest impact on processing time was located in the post-analytical phase of signing and releasing of the reports we suggested the capacitation of three extra analysts for the execution of the mapped action, the retraining of the technicians of the sectors responsible for the distribution of samples and supply of the machines and the standardization of a continuous flow between the areas, strengthening the Lean philosophy and the FIFO dynamics (First in - First out). Thus, we obtained a considerable decrease in the TAT of protein and hemoglobin electrophoresis, in which the total TAT of the operation was reduced by approximately 34% and the TAT referring to the time inside the Center of Technical Operations (CTO) by approximately 40%.

Conclusion: With the implementation of the above mentioned processes and the use of the Lean philosophy, the laboratory was able to considerably optimize the TAT of the Hemoglobin and Protein Electrophoresis exams, in addition to training employees to release results and enhance the standardization of receipt processes and distribution of these samples, with this, it was possible to optimize resources, increase the productivity of the sector, reduce the time of patient care and consequently maximize added value to the service.

A-110

Analytical Performance Evaluation of Diazo Bilirubin Assays on the Atellica CH Analyzer

C. Robinson, A. Sinopoli, D. Blagovcanin, J. Rhea McManus, J. Cheek. *Siemens Healthineers, Tarrytown, NY*

Background: Measurements of bilirubin are used to evaluate liver function. The two most common methods to measure bilirubin are the diazo method (based on measuring the color of azobilirubin) and the vanadate method (based on chemical oxidation). Advantages and disadvantages of each method are well-documented in the literature. This study was designed to evaluate the analytical performance of two new diazo-based methods: the Atellica® CH Total Bilirubin (D_TBil)* assay and the Atellica CH Direct Bilirubin (D_DBil)* assay on the Atellica CH Analyzer.

Methods: Precision and method comparison (MC) were used as performance indicators for the diazo-based assays. Precision studies were performed according to CLSI EP05-A3. Human serum samples were spiked with either conjugated or unconjugated bilirubin. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart for ≥20 days. MC studies were performed according to CLSI EP09-A3. For total bilirubin comparisons, human serum samples (n = 100) were analyzed using the Atellica CH D_TBil assay and the commercial Dimension® Total Bilirubin (TBI) assay. Direct bilirubin comparisons were performed by analyzing human serum samples (n = 100) using the Atellica CH D_DBil assay and the commercial ADVIA® Chemistry Direct Bilirubin_2 (DBil_2) assay.

Results: Representative precision and MC results for each assay across the indicated sample ranges are listed in the table. Over the two assays tested, repeatability and within-lab %CVs were <2.0% and <3.5%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1.

Conclusion: Evaluation of the Atellica CH D_TBil and D_DBil assays demonstrated good precision and equivalent performance compared to commercially available assays.

	Precision				Method Comparison	
	Unit	Sample Range	Repeatability %CV Range	Within Laboratory %CV Range	Sample Range	Regression Equation for Comparative Assay
Analyte (Assay)						
Total Bilirubin (D_TBil)	mg/dL	1.02—22.39	0.3—1.5	0.8—3.3	0.14—22.55	y = 1.02x + 0.08 mg/dL
Direct Bilirubin (D_DBil)	mg/dL	0.37—9.16	0.3—1.9	0.7—1.9	0.10—11.10	y = 0.95x – 0.03 mg/dL

*Products under development. Not available for commercial use or sale.

A-111

There is More to Diluting Icterus Interference than Meets the Eye

M. A. Lasho, N. A. Baumann, D. R. Block. *Mayo Clinic, Rochester, MN*

Background: Total protein is measured in serum and body fluids as part of routine evaluations of general nutritional status and differentiating exudative and transudative effusions. Spectral interference thresholds for bilirubin (e.g., icterus) is set by assay manufacturers to prevent inaccurate results from being reported. The Roche Cobas serum total protein assay package insert states no significant interference from bilirubin up to an icterus (I) index of 20. Body fluids tend to have lower total protein concentrations; therefore, thresholds were derived during prior validation studies and set to I=10, a threshold that is exceeded routinely. Laboratories can mitigate some interferences by recollecting (e.g., hemolysis) or ultracentrifuging (e.g., lipemia) specimens. Serial dilutions are another option used; however, it is imperative that the laboratory understands the mechanism of interference and can demonstrate that it is mitigated by sample dilution. The aim of this study was to investigate whether bilirubin interference can be mitigated by sample dilution in pleural fluid, peritoneal fluid, and serum specimens.

Methods: Residual clinically ordered pleural fluid (n=3), peritoneal fluid (n=3), and serum (n=3) samples submitted for protein testing were split into two equal volume aliquots with concentrations ranging 2.7 to 4.3 g/dL in pleural fluids, 3.9 to 5.4 g/dL in peritoneal fluids, and 3.8 to 11.0 g/dL in serum. One aliquot was spiked (<10% by volume) with a bilirubin conjugate solution (~1000 mg/dL) to surpass the icterus index threshold (spiked). The second aliquot was spiked with an equal volume of 0.9% saline (control). Total protein was measured on the Roche Cobas c701 instrument in both samples to calculate difference (Spiked-Control). The mean(SD) difference was calculated for each sample type. Each spiked sample was serially diluted (2-fold, 4-fold and 8-fold) with 0.9% saline. Total protein was measured for each diluted sample. Mean(SD) difference was calculated ($Diluted_X$-Control), where X=2-fold, 4-fold, 8-fold. Linear regression analysis was performed by plotting the measured (x-axis) vs expected (y-axis) total protein concentration. Slope, y-intercept, and R^2 were determined from serially diluted samples where the expected concentrations were derived using the spiked concentration to replicate the practice of diluting an icteric sample.

Results: Bilirubin spiking (average icterus index=33.5) into pleural fluid, peritoneal fluid, and serum, respectively demonstrated mean(SD) bias (g/dL) = -1.0(0.2), -0.9(0.0), and -0.7(0.0). Subsequent 2-fold dilution demonstrated mean(SD) bias (g/dL) = -1.0(0.2), -1.0(0.0), and -0.8(0.1), 4-fold dilution with mean(SD) bias (g/dL) = -1.1(0.2), -1.0(0.0), and -1.0(0.1), and 8-fold dilution with mean(SD) bias (g/dL) = 1.3(0.2), -1.0(0.0), and -1.4(0.1). Diluting pleural, peritoneal, and serum samples, respectively spiked with bilirubin resulted in slope = 0.99, 1.00, and 0.99; y-intercept = 0.04, 0.01, and 0.07; R^2 = 0.99, 0.99, 0.99.

Conclusion: The dilutions exhibited a linear dilution response, however the dilution results never returned to baseline (non-spiked) concentration. Laboratories should exercise caution when conducting serial dilutions to diminish bilirubin interference in samples when measuring total protein as the mechanism of interference does not appear to be reversible by dilution. This behavior was demonstrated in both body fluids and serum.

A-112

A Consensus Reference Range for Ionized Magnesium

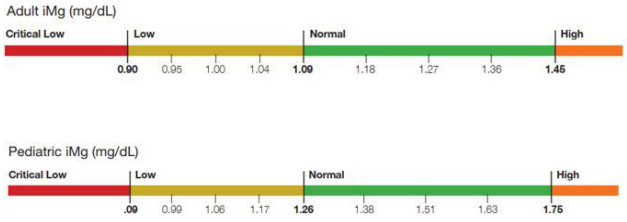
D. Begos. *Nova Biomedical, Waltham, MA*

Objective: Ionized magnesium (iMg) is the physiologically active form of the analyte. Despite this, clinical adoption of iMg is infrequent, and there is only one commercially available analyzer that measures iMg. There are now numerous studies that have examined iMg levels in healthy individuals, and this report will summarize their findings and report a consensus reference range. **Relevance:** Although reference ranges may vary based on population and location, among other factors, a baseline reference range is an important starting point to introducing a new test to any laboratory. **Methods:** A literature search was performed using the search term "ionized magnesium". Papers with reference ranges for healthy individuals measured by ion-selective electrodes were selected and an attempt was made to establish a reference range for adults and children. **Results:** A total of 10 studies were identified, with 422 healthy individuals[1-10]. There were 322 adults and 100 children. Studies looked at venous samples and serum samples. Venous samples were anticoagulated with lithium heparin.Reference ranges for adults and children are shown below:

To convert from mg/dL to mmol/L multiply by 0.41.

To convert from mg/dL to MEq/L multiply by 1.22

Conclusions: Although a large-scale study would be helpful in determining a more precise reference range, there is reasonably good agreement among studies evaluating iMg reference ranges in healthy adults and children. Further work to evaluate differences among different age groups, genders, and ethnic groups would be helpful to clinicians and researchers. There has been a call for updating reference ranges for total magnesium, as there is a wide variation in reported ranges[11].



A-114

Mas™ Omni·Core™ Controls, ready-to-use tube format

N. Adams, M. Sunderraj, N. Krishnaswami, D. Crandall, Y. Zhang, R. Hu. *Thermo Fisher Scientific, Fremont, CA*

Background: The Thermo Fisher Scientific™ MAS™ Controls* are assayed controls to monitor assay performance within clinical laboratory settings. The user can compare observed results of controls with their expected ranges as a means of assuring consistent performance of both reagent and instrument.

The objective of developing MAS™ Ready-to-Use Tube Controls is to provide MAS™ controls in a new automation-friendly plastic tube format, as an alternative to the current glass vials. The MAS™ controls contained in the plastic tubes are intended to be used by core labs to be placed directly onto the analyzers, eliminating the need to aliquot into sample cups or other primary tubes.

In this presentation we summarize the results of MAS™ Omni·CORE™ in the new Ready-to-Use Tube Controls configuration.

Methods: The purpose of these studies is to verify material compatibility and acceptable product performance of MAS™ Omni·CORE™ in the new Ready-to-Use Tube Controls configuration.

Study	Purpose
Torque and Leak	Determine application/removal torque for caps + observe for any liquid leakage
Cap Characterization	Quantify tube/cap ability to maintain Argon gas overlay as well as prevent outgassing
In-Use (CO_2) Study	Determine CO_2 analyte in-use stability to mimic time-on-platform performance, in three scenarios: 1 - Capped 2-8°C: Ortho Automation Platform 2 - Un-Capped 2-8°C: Platforms with onboard storage 3 - Un-Capped 25°C: Platforms without onboard storage
Plastic Interaction	Determine potential interaction of claimed analytes with plastic (PET) material. Performed closed vial testing, up to 14 days in two scenarios: Plastic (PET) vs. current Glass (Fiolax)
Extractables / Leachables	Determine (if any) material leeched from plastic and/or butyl rubber plug. Performed accelerated stress testing at elevated temperatures in three scenarios: 1 - PET tube alone 2 - PET tube with cut up butyl rubber in product 3 - Current Fiolax glass material as baseline comparison
Light Transmission	Determine tube"s ability to prevent photodegradation of light sensitive analytes. Performed 15 days closed vial testing (under constant light exposure (464lx)) in three scenarios: 1 - PET tube alone 2 - PET tube covered with foil 3 - Current Fiolax glass material as baseline comparison

The controls were also assessed for Fit/Form/Function on various platforms from manufacturers such as Siemens, Roche, Abbott, Ortho Clinical Diagnostics, Thermo Fisher Scientific, and Beckman.

Results: The MAS™ Omni·CORE™ Ready-to-Use Control results demonstrated comparable performance and Fit/Form/Function criterion of the specified platforms.

Torque/Leak, Cap Characterization, and Plastic Interaction studies yielded passing results per protocol criterion. The In-Use (CO2) study demonstrated acceptable "time-on-platform" performance of 9 hours in 2-8 °C (4 hours at 25°C). The Extractables

/ Leachables study demonstrated passing results for all claimed analytes, with minor exceptions. All light sensitive analytes yielded passing results during the Light Transmission study.

Conclusion: To conclude, the MAS™ Omni·CORE™ Ready-to-Use Controls will contribute to the increased efficiency of core lab workflow by allowing the analyzer(s) to aspirate controls directly from the tube and allow for control storage in an on-board refrigeration unit after use.

*Availability of product in each country depends on local regulatory marketing authorization status

A-116

Evaluation of Lp(a) Ultra assay for the immunoturbidimetric quantitative determination of lipoprotein (a) in human serum and plasma with Siemens Healthineers Atellica CH 930 Analyzer

A. Cugini, L. De Angelis, R. Lucini, S. Brambilla. *Sentinel CH. SpA, Milano, Italy*

Objective The aim of this study is to evaluate the analytical performances of the Lp(a) Ultra assay for the immunoturbidimetric quantitative determination of lipoprotein (a) in human serum and plasma on Siemens Healthineers Atellica CH 930 Analyzer.

Background Lipoprotein (a) has been found in artery walls and can have atherogenic effects. Because of its structural similarity to plasminogen, it can also inhibit fibrinolysis and hence acts thrombogenically. High Lp(a) concentrations in serum correlate with premature manifestation of atherosclerosis and strokes. In combination with elevated LDL Cholesterol concentrations, the coronary risk increases approximately six-fold.

Methods Lp(a) Ultra is a latex immunoassay manufactured by Sentinel Diagnostics and developed to measure Lp(a) levels. If the antigen-antibody reaction takes place between Lp(a) in the sample and the anti-Lp(a) antibodies, which have been adsorbed to latex particles, agglutination occurs. This agglutination is detected as an absorbance change, with the magnitude of the change being proportional to the quantity of Lp(a) contained in the sample.

Results The Limit of Blank LOB (4 samples x 5 replicates x 3 runs) was ≤ 2.0 mg/dL. The Limit of Detection LOD (4 samples x 5 replicates x 3 runs) was ≤ 3.0 mg/dL. The Limit of Quantitation LOQ (8 dilution levels x 10 replicates x 3 runs) was ≤ 5.0 mg/dL at %CV lower than 20%. The Intra Assay (5 samples x 20 replicates x 3 runs) gave the following %CVs: 2.4% at 12.1 mg/dL, 0.8% at 30.3 mg/dL, 0.6% at 47.8 mg/dL, 0.5% at 58.8 mg/dL, 1.7% at 87.1 mg/dL. Total precision study (5 samples x 2 runs x 2 replicates x 20 runs) gave the following %CVs: 4.0% at 11.5 mg/dL, 3.6% at 21.7 mg/dL, 3.0% at 32.0 mg/dL, 2.8% at 42.2 mg/dL, 2.3% at 50.7 mg/dL. The test was linear up to 130 mg/dL. Calibration stability was up to 30 days. Reagent on board stability was up to 30 days. This application (y) was compared with the same test applied on Beckman Coulter AU680 Analyzer (x) and gave the following results: using Passing Bablok analysis: y = 0.8 + 1.0x; correlation coefficient (r) = 0.997; using Deming analysis: y = 1.0 + 1.0x; correlation coefficient (r) = 0.997; number of samples = 120. There is not Hook effect/antigen excess up to 600 mg/dL.

Conclusions Analytical performances of Lp(a) Ultra assay on Siemens Healthineers Atellica CH 930 Analyzer meet the requirements for its use as immunoturbidimetric quantitative determination of lipoprotein (a) in human serum and plasma and make this assay very suitable for the routine measurement of this analyte.

A-117

Performance evaluation of five Sentinel Diagnostics' assays on Beckman Coulter DxC 500 AU Clinical Chemistry Analyzer

A. Cugini[1], D. O'Meara[2], F. Di Gennaro[1], N. Di Rocco[1], C. Assalini[1], R. Lucini[1], S. Brambilla[1]. *[1]Sentinel CH. SpA, Milano, Italy, [2]Beckman Coulter Ireland Inc., Maryfort, O'Callaghansmills, Co. Clare, Ireland*

Objective The aim of this study is to evaluate the analytical performances of the following Sentinel Diagnostics' assays: ACE Liquid, Lp(a) Ultra, Pancreatic Amylase, Lipase NG, Albumin BCP on the Beckman Coulter DxC 500 AU Clinical Chemistry Analyzer.

Methods Performance evaluation included Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantitation (LoQ), linearity, intra assay precision, inter assay precision, calibration stability, on board reagent stability and instrument correlation, following the current CLSI guidelines protocols. Analytical Measuring Range (AMR) was defined through linearity and sensitivity.

Results Summary of the analytical performances for all the assays tested is reported in table below.

Parameters	ACE Liquid	Lp(a) Ultra	Pancreatic Amylase	Lipase NG	Albumin BCP
Sensitivity - LOB	2.8 U/L	1.0 mg/dL	1 U/L	0.3 U/L	0.4 g/L
Sensitivity - LOD	5.8 U/L	1.5 mg/dL	2 U/L	1.0 U/L	0.7 g/L
Sensitivity - LOQ	7.4 U/L	3.0 mg/dL	3 U/L	3.0 U/L	0.9 g/L
Linearity	up to 120 U/L	up to 130 mg/dL	up to 2000 U/L	up to 300 U/L	up to 70 g/L
Intra assay	%CV ≤ 6.3%	%CV ≤ 3.1%	%CV ≤ 2.5%	%CV ≤ 3.2%	%CV ≤ 1.4%
Inter assay	%CV ≤ 7.0%	%CV ≤ 4.9%	%CV ≤ 4.3%	%CV ≤ 3.2%	%CV ≤ 2.2%
On board reagent stability	up to 30 days	up to 30 days	up to 30 days	up to 30 days	up to 28 days
Calibration stability	up to 30 days	up to 15 days	up to 14 days	up to 30 days	up to 14 days
Correlation vs. AU480	y = 1.097x + 1.98 r = 0.982	y = 1.00x – 0.03 r = 1.000	y = 1.003x – 0.08 r = 1.000	y = 1.00x + 0 r = 1.000	y = 1.04x – 1.50 r = 0.997

Conclusions All assays showed good results for the parameters tested on the Beckman Coulter DxC 500 AU Clinical Chemistry Analyzer making these kits very suitable for a routine measurement of these analytes.

A-118

Evaluation of Mindray Cystatin C Assay

Y. Bao[1], J. Hu[1], M. Jiang[1], J. Wang[1], J. Dai[2]. *[1]Mindray Bio-Medical Electronics Co., Ltd., shenzhen, China, [2]Mindray IVD Innovation Center Minnesota, Oakdale, MN*

Background: Cystatin C is a low molecular weight non-glycosylated alkaline protein, with a molecular weight of 13kDa. It is formed by all nucleated cells in human at a constant rate. The only pathway of Cystatin C in vivo is excretion through glomerular filtration and absorbed by proximal renal tubular epithelial cells, which was decomposed finally. Therefore, the concentration of Cystatin C in serum is almost not affected by external factors, such as weight, age, gender, etc. Compared with creatinine and urea, it is an ideal endogenous marker for monitoring glomerular filtration function. Currently, the most methods on the market for testing Cystatin C are either latex enhanced immunoturbidimetric method, or immunoturbidimetric method. These methods usually use polyclonal antibodies from different animal species, which are sometime susceptible to the interference from rheumatoid factors reported in the literature, resulting in false positive results. The anti-RF interference ability of this reagent was enhanced by selecting the optimal antibody and optimizing reagent formulation. This study evaluated performances of the Cystatin C assay on Mindray BS-2000M system.

Method: Mindray Cystatin C assay is based on latex immunno-agglutination. Latex nanoparticles were coated with anti-Cystatin C antibody. Reagent of this assay contains R1 buffer and R2 with latex particles coupled with anti-Cystatin C antibodies. The assay starts with R1 and sample incubation for 5 min and then R2 is added. After another 5 min of incubation, agglutination occurs, leading to turbidity increasing. The turbidity is measured at 570 nm. Multi-level (5-point) calibration is used. Cystatin C concentrations are obtained from the calibration curve with signals from the assay with patient samples. Following basic assay performances were evaluated: precision, limit of detection, linearity, method comparison, interference and high dose hook.

Results: The precision study followed EP05-03 protocol. The repeatability and within-lab CVs on the BS-2000M system with 2 QC and 3 patient samples were ranged from 0.9% to 5.3%, respectively. Limit of detection was 0.1mg/L and linearity was given up to 8.63mg/L. The assay (y) shows good accuracy when compared with Roche Cystatin C (x) assay on the cobas 701: y= 1.0055x - 0.0268 (r^2 = 0.9994, n=109). The assay showed no significant with bilirubin up to 40mg/dL, with hemoglobin up to 500mg/dL, with lipids up to 500mg/dL, and with rheumatoid factors up to 1200 IU/mL that is about 0.3% out of a total of 55184 samples collected from our customers in China with RF measurements. No prozone was observed with the assay up to the high cystatin C concentration of 800 mg/L tested.

Conclusion: From this evaluation, we confirmed that Mindray Cystatin C assay can measure serum Cystatin C precisely and accurately, and with good performance against rheumatoid factors interference. It is suitable for use in routine clinical laboratories.

A-119

Performance Evaluation of a New Enzymatic Creatinine Assay on Mindray Clinical Chemistry BS-2800M Analyzer with Excellent Accuracy

X. Zheng[1], H. Wang[1], M. Wu[1], Z. Liu[1], J. Wang[1], M. Jiang[1], J. Dai[2]. *[1]Shenzhen Mindray Bio-medical Electronics CO., LTD., Shenzhen, China, [2]Mindray IVD Innovation Center Minnesota, Oakdale, MN*

Background: Serum creatinine is used to estimate glomerular filtration rate (GFR) and monitor the progression of renal diseases. Accuracy and precision for this assay is very important. False estimates of serum creatinine were found in some cases due to the interference by exogenous and endogenous substances in addition to inadequate standardization. Substances, such as lipid, bilirubin, Hemoglobin(hemolysis) and ascorbic acid have been recognized as the cause of interference. Other endogenous substances such as paraprotein may also cause interference that has rarely been systematically studied. This study specifically evaluated such performance for the new enzymatic creatinine assay, in addition to the general assay characteristics. **Method:** This assay is based on the sarcosine oxidase creatinine method with dual reagents. The reagent formulation was optimized. Concentrations are calculated from a calibration curve with a calibrator. Assay precision was evaluated using EP05-A3. The assay linearity was evaluated following EP06-Ed2. On board stabilities were tested with opened reagent for the duration of the testing. Accuracy studies were conducted against in-house ID-LC-MS/MS reference method that is calibrated with SRM 914a and validated by SRM 967a. Interference testing was run in accordance with EP07-Ed3. Paraprotein interference was evaluated by serum samples with high concentration of globulin and serum samples from patents with rheumatoid diseases and hematologic cancers. All these samples were measured by three commercial creatinine methods in parallel. Ten samples were found with discrepant results. Target values for these 10 samples and other 8 normal samples were obtained by the ID-LC-MS/MS reference method. **Results:** The repeatability and within-lab CVs on the BS-2800M system were found ranging from 0.5% to 2.0% and 1.0% to 3.5% respectively, at creatinine concentrations of ~85 µmol/L (0.96 mg/dL) and ~372 µmol/L(4.21mg/dL). The assay linearity was from 10 to 7000 µmol/L. The reagent onboard stability was found to be for 28 days. The assay (Y) showed excellent accuracy when it compared with ID-LC-MS/MS reference method(X): Y= 1.0339X-3.5404 (r=0.99, n=31). There is no significant interference (<±10%), at creatinine concentration of 53µmol/L with bilirubins up to 20 mg/dL, hemolysate hemoglobin up to 200 mg/dL, Intralipid up to 1000 mg/dL, triglycerides up to 2000 mg/dL, and ascorbic acid up to 30 mg/dL. Three hundred patient samples with high globulin and 200 samples from patients with rheumatoid diseases and hematologic cancers were used for paraprotein interference study. With these samples, the average bias vs. Roche enzymatic creatinine method was found less than 2.5%. The recoveries of 18 samples were compared with ID-LC-MS/MS reference method. The mean bias in that comparison was -4.1%. **Conclusion:** The new Mindray creatinine assay demonstrates excellent accuracy and anti-paraprotein interference performance on BS-2800M system. It is suitable for the use in clinical laboratories.

A-120

Performance evaluation of three Sentinel Diagnostics' assays on Roche cobas c 303 analytical unit

M. De Lorenzis, F. Di Gennaro, M. Valdambrini, L. De Angelis, R. Lucini, S. Brambilla. *Sentinel CH. SpA, Milano, Italy*

Objective The aim of this study is to evaluate the analytical performances of the following Sentinel Diagnostics' assays: Total Bile Acid Liquid, Copper, Aldolase, on Roche cobas c 303 analytical unit.

Methods Performance evaluation included Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantitation (LoQ), linearity, intra assay precision, inter assay precision, on board reagent stability, prozone effect, instrument correlation following the current CLSI guidelines protocols. Data were evaluated using Microsoft Excel statistical tool Analyse-it. Analytical Measuring Range (AMR) was defined through linearity and sensitivity.

Results Summary of the analytical performances for all the assays tested is reported in table below.

Parameters	Total Bile Acids	Copper	Aldolase
Sensibility - LOB	≤ 0.2 µmol/L	≤ 3.0 µg/dL	≤ 1.0 U/L
Sensibility - LOD	≤ 0.5 µmol/L	≤ 5.0 µg/dL	≤ 2.0 U/L
Sensibility - LOQ	≤ 1.0 µmol/L	≤ 7.0 µg/dL	≤ 3.0 U/L
Linearity	up to 200 µmol/L	up to 500 µg/dL	up to 30 U/L
Precision - Intra assay	%CV ≤ 0.8%	%CV ≤ 1.7%	%CV ≤ 4.1%
Precision - Inter assay	%CV ≤ 3.2%	%CV ≤ 2.6%	%CV ≤ 4.1%
On board reagent stability	up to 60 days	up to 60 days	up to 30 days
No prozone effect up to	820 µmol/L	1500 µg/dL	90 U/L
Correlation vs. cobas c503 analytical unit	n = 110 slope = 0.99 intercept = 0.07 µmol/L r = 0.994	n = 110 slope = 1.00 intercept = 1.94 µg/dL r = 1.000	n = 110 slope = 1.04 intercept = 0.29 U/L r = 0.998

Conclusion All assays showed good results for all the parameters tested on Roche cobas c 303 analytical unit making these kits very suitable for a routine measurement of these analytes.

A-121

Performance evaluation of two Sentinel Diagnostics' assays on Roche cobas c 503 analytical unit

M. De Lorenzis, F. Di Gennaro, L. De Angelis, R. Lucini, S. Brambilla. *Sentinel CH. SpA, Milano, Italy*

Objective The aim of this study is to evaluate the analytical performances of the following Sentinel Diagnostics' assays: Zinc, Total Bile Acid Liquid on Roche cobas c 503 analytical unit.

Background Zinc is a component of metalloenzymes involved in nucleic acid and protein synthesis, therefore it is a necessary complement for cell replication. Acute zinc deficiency can result in skin lesions, irritability, loss of hair, growth retardation. Also impaired immunological function is associated with zinc insufficiency.Total Bile Acids (TBA) are metabolized in the liver and hence serve as a marker for liver function. Serum TBA are increased in patients with acute hepatitis, chronic hepatitis, liver sclerosis and liver cancer.

Methods Performance evaluation included Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantitation (LoQ), linearity, intra assay precision, inter assay precision, calibration stability, on board reagent stability, and instrument correlation following the current CLSI guidelines protocols. Data were evaluated using Microsoft Excel statistical tool Analyse-it. Analytical Measuring Range (AMR) was defined through linearity and sensitivity.

Results Summary of the analytical performances for both the assays tested is reported in table below.

Parameters	Zinc	Total Bile Acids
Sensibility - LOB	≤ 3.0 µg/dL	≤ 0.2 µmol/L
Sensibility - LOD	≤ 5.0 µg/dL	≤ 0.5 µmol/L
Sensibility - LOQ	≤ 7.0 µg/dL	≤ 1.0 µmol/L
Linearity	up to 2000 µg/dL	up to 200 µmol/L
Precision – Intra assay	%CV ≤ 0.9%	%CV ≤ 1.0%
Precision – Inter assay	%CV ≤ 3.0%	%CV ≤ 1.4%
On board reagent stability	up to 60 days	up to 60 days
Calibration stability	up to 30 days	up to 30 days
Correlation	vs. ICP/MS n = 146 slope = 0.99 intercept = -4.2 µg/dL r = 0.998	vs. Abbott Architect c16000 n = 147 slope = 0.96 intercept = -0.13 µmol/L r = 0.999

Conclusions Both assays showed good results for all the parameters tested on Roche cobas c 503 analytical unit making these kits very suitable for a routine measurement of these analytes.

A-122

Fluid Type (STILL) Matters: An Analysis of Quality Control Material Suitability for Measuring Eleven Body Fluid Analytes in the Clinical Laboratory

R. C. Fyffe-Freil, N. A. Baumann, D. R. Block. *Mayo Clinic, Rochester, MN*

Background: Measuring the concentration of albumin, amylase, bilirubin, cholesterol, creatinine, glucose, lactate dehydrogenase(LDH), lipase, total protein(TP), triglycerides, and urea nitrogen(UN) in body fluids(BFs) provides important information to aid in clinical decision-making. The range of expected values of these analytes in BFs may not match serum, and the distribution by fluid type is not well described. Quality control (QC) materials are designed to reflect clinically relevant concentrations in blood. Hence, the measuring ranges for assays modified for BF testing vary in suitability. Laboratorians and manufacturers would benefit from understanding the overlap in BF and serum analyte concentrations. The aim of this study was to characterize the range of BF analyte concentrations compared to serum and assess the suitability of QC materials used on these assays.

Methods: A retrospective analysis was performed for the following analytes in BFs with reportable ranges(including maximum dilution factor): albumin=0.2-12.0g/dL(x2), amylase=3-140,00U/L(x100), bilirubin=0.1-72.2mg/dL(x2), cholesterol=4-8000mg/dL(x10), creatinine=0.1-92.0mg/dL(x4), glucose=2-3,375mg/dL(x5), LDH=10-9000U/L(x10), lipase=3-300,000U/L(x1000), TP=0.2-36.0g/dL(x3), triglycerides=4-8,850mg/dL(x10), and UN=2-300mg/dL(x3). Chemistry tests were run on a cobas®6000 c501 (Roche Diagnostics,Indiana). Patient analyte concentration and fluid type results reported between 9/16/2019 and 1/27/2022 at all Mayo Clinic locations were extracted from an institutional database of electronic medical record data. Serum results were included for comparison. Descriptive statistical analyses (median and range) were performed with R(version 4.2.2)/R Studio(version 2022.12.0). QC materials for BF testing were purchased from Bio-Rad (Multilevel Qual, Levels 1 and 3; Hercules,CA,USA). Target concentrations are reported.

Results:

Analyte	Serum N	Serum Median	Serum Range	BF N	BF Median	BF Range	QC Target Level 1	QC Target Level 3
Albumin (g/dL)	950461	4.1	<0.3 to 8.5	5021	1.0	<0.2 to 4.1	2.6	4.4
Amylase (U/L)	20213	60.0	<3 to 7002	4389	55.0	<3 to 166160	43.0	257.0
Bilirubin (mg/dL)	883892	0.5	0.1 to 67.8	2025	1.3	0.1 to 76.2	0.6	6.4
Cholesterol (mg/dL)	473888	176.0	12 to 2557	1205	45.0	<4 to 549	109.0	271.0
Creatinine (mg/dL)	2022216	1.0	<0.1 to 38.8	4674	1.6	0.1 to 103	0.8	6.6
Glucose (mg/dL)	1628208	108.0	2 to 1434	7507	128.0	2 to 3136	63.0	362.0
LDH (U/L)	145298	211.0	<10 to >24120	7717	148.0	<11 to 31835	117.0	418.0
Lipase (U/L)	80469	30.0	<3 to 20288	883	33.0	<3 to 560000	18.0	149.0
Total Protein (g/dL)	100232	6.6	2.7 to 16.2	10335	2.6	<0.2 to >8.3	4.0	6.7
Triglycerides (mg/dL)	480998	108.0	<9 to 9170	2713	44.0	1 to 8090	91.0	199.0
Urea Nitrogen (mg/dL)	1698010	18.0	<2 to 265	1881	37.0	2 to 294	15.0	68.0

Conclusion: BF median(range) concentrations are similar or higher for bilirubin, creatinine, glucose, lipase, and UN. Serum QC material is likely appropriate for these assays in BFs. BF median(range) concentrations are lower for albumin, cholesterol, TP, and triglycerides suggesting that serum QC material may *not* be well suited for BFs. The need for extended dilutions may impact BF amylase and lipase.

A-123

Increased Rate of Recurrent Hemolysis is Associated with COVID-19 Infection and Propofol Administration in the Intensive Care Unit (ICU)

R. Fyffe-Freil, A. A. Wockenfus, E. A. Hain, M. A. Nicklas, N. A. Baumann, B. M. Katzman. *Mayo Clinic, Rochester, MN*

Background: Hemolysis is a pre-analytic issue that challenges clinical laboratories. When a specimen with significant hemolysis is obtained in our laboratory, tests where the hemolysis threshold is exceeded are not reported and blood is recollected. Unfortunately, in a subset of patients, the recollected specimen is also hemolyzed (termed "recurrent hemolysis"). In these cases, the second recollection utilizes special collection and handling protocols (i.e. hand-carried to lab, plasma collection, etc.) to mitigate preventable causes of hemolysis. Lipemia is also a known contributor to specimen hemolysis. Mayo Clinic Rochester experienced an increased rate of hemolysis as well as recurrent hemolysis during the height of the COVID-19 pandemic (January 2022). The objective of this study was to investigate the increased rate of recurrent hemolysis and compare to a rate prior to the COVID-19 pandemic (January 2019).

Methods: Retrospective data analysis was performed for January 2019 (negative control) and January 2022. The number of Mayo Clinic Rochester hospital admissions, admitting diagnosis, medications administered, and number of test collections performed were obtained using reporting functionality in our Electronic Health Record (EHR), Epic. The Laboratory Information System, SoftLab, was utilized to obtain recollection rates due to hemolysis. Data were normalized to total patient admissions for the specified period to account for inter-year variability. R/R Studio was utilized to analyze and visualize the data. Pearson's Chi-squared test for independence was performed on categorical variables (significance defined as $p<0.05$).

Results: A 28% decrease in hospital admissions (n=3958:Jan-22 vs. n=5517:Jan-19) and a 23% decrease in total specimens collected on hospitalized patients (n=40,557:Jan-22 vs. n=52,373:Jan-19) was observed between Jan-19 to Jan-22. However, the laboratory observed a 6% increase in hemolyzed specimens (n=256:Jan-22 vs. n=241:Jan-19). Recurrent hemolysis occurred in 24% (n=62/256) of Jan-22 and 17% (n=43/241) of Jan-19 hemolyzed specimens and required special handling protocols. COVID positive patients comprised 48% (n=30/62) of those requiring special handling protocols in Jan-22. Further examination revealed 34% (n=21/62) of Jan-22 and 12% (n=5/43) of Jan-19 cases of recurrent hemolysis were due to lipemia ($p<0.01$). Chart review highlighted a potential source of lipemia from the administration of propofol, a lipophilic sedative emulsion. Of those COVID positive cases with recurrent hemolysis due to lipemia (n=15/30), 93% (n=14/15) were receiving propofol at the time of collection ($p<0.001$).

Conclusion: We observed increased rates of specimen hemolysis during the COVID-19 pandemic. While most hemolysis resolves upon specimen recollection, patients with recurrently hemolyzed specimens often require special specimen collection and handling protocols to mitigate hemolysis. Approximately half of the recurrent hemolysis cases in Jan 2022 were COVID-19 positive patients. Lipemia-induced hemolysis was also more frequent in this patient population presumably due to the administration of propofol, a lipophilic emulsion sedative commonly used for COVID positive patients requiring sedation and ICU-level care. It is critical to understand the variety of factors which may contribute to recurrent hemolysis, as intervening with specialized collection and handling protocols to reduce observed pre-analytical hemolysis is required to provide accurate and timely laboratory results.

A-124

Evaluation of the Analytical Performance of 10 General Chemistry Assays on the Atellica® CI 1900 Analyzer

K. Kolewe, C. Micklitsch, K. Ostolaza-Venegas, T. Pham, C. Tyler, W. Varhue, G. Arrode-Bruses, J. Rhea-McManus, J. Snyder. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput, integrated chemistry and immunoassay analyzer employing both Atellica chemistry and Atellica immuno assays. This study was designed to evaluate the analytical performance of the Atellica CH Alkaline Phosphatase concentrated (ALP_2c), Direct Bilirubin 2 (DBil_2), Total Bilirubin_2 (TBil_2), Albumin (Alb), Albumin BCP (AlbP), Total Protein II (TP), Aspartate Aminotransferase (AST), Aspartate Aminotransferase P5P (ASTPLc), Alanine Aminotransferase (ALT), and Alanine Aminotransferase P5P (ALTPLc) assays* on the Atellica CI 1900 Analyzer.

Methods: The Atellica CI 1900 ALP_2c, DBil_2, TBil_2, Alb, AlbP, TP, AST, ASTPLc, ALT, and ALTPLc assays use the same reagents and calibrators as the Atellica Chemistry assays. Precision and method comparison (MC) were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 using native and contrived human serum samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. MC studies were performed according to CLSI EP09-A3. Individual native and contrived human serum samples were analyzed using the Atellica CH assays on both the Atellica® CH 930 and Atellica CI 1900 Analyzers.

Results: Representative precision and MC results are listed for each assay in the table below. For the nine assays tested, repeatability and within-lab %CVs were <6.0% and <9.0%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1.

Conclusion: Evaluation of the Atellica CH ALP_2c, DBil_2, TBil_2, Alb, AlbP, TP, AST, ASTPLc, ALT, and ALTPLc assays using the Atellica CI 1900 Analyzer demonstrated acceptable precision and equivalent performance compared to the same assays on the Atellica CH 930 Analyzer.

	Precision				Method Comparison	
Analyte (Assay)	Unit	Sample Range	Repeatability %CV range	Within Laboratory %CV range	Sample Range	Regression Equation for Atellica CH 930 Comparative Assay
Alkaline Phosphatase (ALP_2c)	U/L	86–838	0.3–0.6	0.6–1.4	17–988	y = 0.97x + 0 U/L
Direct Bilirubin (DBil_2)	mg/dL	0.4–13.4	0.3–2.5	2.3–5.0	0.1–13.8	y = 1.04x + 0.0 mg/dL
Total Bilirubin (TBil_2)	mg/dL	0.7–24.0	0.4–5.7	1.5–8.6	0.2–33.8	y = 0.97x – 0.0 mg/dL
Albumin (Alb)	g/dL	2.1–5.4	0.9–1.9	1.9–2.8	1.2–5.8	y = 1.02x + 0.0 g/dL
Albumin (AlbP)	g/dL	2.7–7.1	0.6–1.3	1.7–2.6	0.6–7.5	y = 0.98 + 0.0 g/dL
Total Protein (TP)	g/dL	7.0–10.0	0.4–0.8	1.7–2.6	2.2–10.7	y = 0.96x + 0.4 g/dL
Aspartate Aminotransferase (AST)	U/L	35–897	0.2–2.0	1.1–2.6	10–951	y = 1.01x – 1 U/L
Aspartate Aminotransferase (ASTPLc)	U/L	38–888	0.2–1.6	0.5–2.4	13–948	y = 1.02x – 4 U/L
Alanine Aminotransferase (ALT)	U/L	31–818	0.3–2.3	0.9–3.2	7–1019	y = 0.99x + 1 U/L
Alanine Aminotransferase (ALTPLc)	U/L	41–850	0.2–1.5	1.5–2.9	11–941	y = 1.01x – 2 U/L

*The products mentioned are not commercially available in all countries. Their future availability cannot be guaranteed.

A-125

Analytical Performance of Chemistry Assays Comprising the Basic Metabolic Panel on the Atellica CI 1900 Analyzer

A. Bogard, P. Gupta, J. Kellogg, K. Ostolaza-Venegas, S. Singh, C. Tyler, W. Varhue, G. Arrode-Bruses, J. Rhea-McManus, J. Snyder. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput, integrated chemistry and immunoassay analyzer utilizing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica CH Urea Nitrogen (UN_c), Creatinine (CREA_2), Enzymatic Creatinine (ECre_3), A-LYTE™ Multisensor (IMT Na K Cl), Carbon Dioxide (CO2_c), Glucose Oxidase (GluO), Glucose Hexokinase_3 (GluH_3), and Calcium_2 (CA_2) assays* on the Atellica CI 1900 Analyzer*.

Methods: The Atellica CI 1900 CH UN_c, CREA_2, ECre_3, IMT Na K Cl, CO2_c, GluO, GluH_3, and CA_2 assays use the same reagents and calibrators as the Atellica CH assays. Precision and method comparison (MC) were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 using native and contrived human serum samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. MC studies were performed according to CLSI EP09-A3. Individual native and contrived human serum samples were analyzed using the Atellica CH assays on both the Atellica CH and Atellica CI 1900 Analyzers.

Results: Representative precision and MC results are listed for each assay in the table. Over the 10 assays tested, repeatability and within-lab %CVs were <5.0% and <6.0%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1.

Conclusion: Evaluation of the Atellica CH UN_c, CREA_2, ECre_3, IMT Na K Cl, CO2_c, GluO, GluH_3, and CA_2 assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on the Atellica CH Analyzer.

	Precision				Method Comparison	
	Unit	Sample Range	Repeat-ability %CV Range	Within Laboratory %CV Range	Sample Range	Regression Equation for Atellica CH 930 Comparative Assay
Analyte (Assay)						
Blood Urea Nitrogen (UN_c)	mg/dL	16-115	0.4-2.5	2.7-3.8	8-107	y = 1.01x + 0 mg/dL
Creatinine (CREA_2)	mg/dL	0.43-26.43	0.3-2.1	1.1-4.0	0.68-29.18	y = 0.99x - 0.08 mg/dL
Enzymatic Creatinine (ECre_3)	mg/dL	0.40-26.37	0.2-4.5	1.1-5.8	0.21-28.36	y = 0.97x + 0.03 mg/dL
Sodium (A-Lyte Multisensor)	mmol/L	70.1-154	0.3-0.4	1.0-1.2	53.2-192	y = 1.00x - 2.69 mmol/L
Potassium (A-Lyte Multisensor)	mmol/L	2.44-7.16	0.3-0.5	1.1-1.2	1.40-9.85	y = 0.97x + 0.0353 mmol/L
Chloride (A-Lyte Multisensor)	mmol/L	75.1-176	0.3-0.5	1.0-1.7	52.7-196	y = 0.99x + 0.161 mmol/L
Carbon Dioxide (CO2_c)	mEq/L	14-29.5	1.1-1.3	1.9-4.7	10.7-36.4	y = 0.96x + 0.5 mEq/L
Glucose (GluO)	mg/dL	56-514	0.4-1.1	1.4-3.0	16-664	y = 1.04x - 3 mg/dL
Glucose (GluH_3)	mg/dL	57-518	0.4-0.7	1.0-2.2	10-681	y = 1.01x + 1 mg/dL
Calcium (CA_2)	mg/dL	6.0-13.4	0.6-1.0	1.9-2.7	1.8-12.9	y = 1.03x + 0.0 mg/dL

*The products mentioned are not commercially available in all countries. Their future availability cannot be guaranteed.

A-126

Evaluation of NMR-based GFR estimation in dosing of high-dose methotrexate

A. N. Schwäble Santamaría[1], S. Einhell[2], A. N. Robertson[1], M. Grassi[1], T. Pukrop[2], E. Schiffer[1]. *[1]Numares AG, Regensburg, Germany, [2]Universitätsklinikum Regensburg, Regensburg, Germany*

Background The cytotoxic agent methotrexate (MTX) is mainly used in oncology and rheumatology. MTX is 80-90% excreted unchanged in the urine, thus, impaired renal excretion because of kidney disease leads to accumulation and prolonged exposure, with a consequent increased risk of myelosuppressive and other toxic adverse effects. Direct kidney damage from MTX crystal precipitation and tubular injury may also occur. Therefore, precise and accurate measurement of renal function prior to MTX administration is of paramount importance to determine accurate dosing and to minimize such adverse risks. Commonly used estimated glomerular filtration rate (eGFR) equations perform poorly in patients with cachexia and at later stages of life - the typical oncology patient. Here we evaluate the feasibility of applying the recently introduced nuclear magnetic resonance (NMR)-based GFR equation (GFR_{NMR}) using creatinine, cystatin C, myo-inositol, and valine, in oncology patients receiving high-dose MTX.

Methods A series of residual sera from patients scheduled to receive high-dose MTX infusion for oncological indications were collected from two sites: n=52 sera from the University Hospital Regensburg (Regensburg, Germany), at four time points (t0h, t24h, t42h, and t48h), and n=10 cross-sectionally from the Mayo Clinic (MN). Standard drug-monitoring trough levels were used as a reference. Serum was prepared and NMR-measured in five replicates for all samples. GFR_{NMR} was compared with the current standard GFR equations: i) CKD-EPI_{2021}Cr (creatinine) and ii) CKD-EPI_{2021}CrCys (creatinine and cystatin-C). Coefficient of determination for linear regression, kappa coefficient for categorical regression and bootstrapped confidence intervals were calculated.

Results MTX was shown not to increase the GFR_{NMR} failure rate or affect the intra-assay precision of GFR_{NMR}. When monitoring eGFR during high-dose MTX treatment, MTX affected GFR according to the RIFLE criteria for AKI in 13 cycles in 9 patients; 1/13 were classified as 'risk' (creatinine increased 1.5-fold) and 1/13 were classified as 'injury' (creatinine increased 2.0-fold). Using MTX clearance as a surrogate for measured GFR, GFR_{NMR} was shown to reflect the MTX plasma clearance constant k more accurately (using a fitting function $c=c_0*e^{-kt}$) with r=0.758 (95% CI 0.36-0.92, p=0.004) compared to CKD-EPI_{2021}Cr (r=0.401, 95%CI -0.19-0.78, p=0.176) and CKD-EPI_{2021}CrCyc (r=0.632, 95% CI 0.12-0.88).

Conclusion The robustness of GFR_{NMR} test results is not affected by HD-MTX treatment. Compared to standard eGFR equations, GFR_{NMR} is more closely associated with MTX renal clearance rates and may therefore be a novel method to improve the accuracy of MTX dosing in the future.

A-127

Bias and precision of a NMR-based GFR estimating equation in kidney transplant recipients

A. N. Schwäble Santamaría[1], J. Meeusen[2], J. Lieske[2], R. J. Scott[2], A. N. Robertson[1], M. Grassi[1], E. Schiffer[1]. *[1]Numares AG, Regensburg, Germany, [2]Mayo Clinic, Rochester, MN*

Background An accurate and reliable measurement of glomerular filtration rate (GFR) is essential to monitor for rejection and disease progression post-kidney transplantation. Measured GFR (mGFR) is the current gold standard. However, it has scarce availability at many centers and is labor intensive. Newer estimated GFR (eGFR) methods containing cystatin-C and/or other biomarkers have not been clinically validated in a post-transplant cohort. In this study, we evaluated a new NMR-based GFR (GFR_{NMR}) equation that contains serum creatinine, cystatin C, myoinositol, and valine in post-kidney transplant recipients in a clinical routine setting.

Methods Venipuncture for serum collection was performed immediately prior to mGFR measurement with urinary iothalamate clearance in 67 post-kidney transplant recipients. Serum was stored at 4°C and measured by NMR no later than four days after collection. eGFR was assessed using the following equations: i) GFR_{NMR}, ii) $CKD\text{-}EPI_{2021}Cr$ (creatinine) and iii) $CKD\text{-}EPI_{2021}Cr,Cys$ (creatinine and cystatin-C). Bias was calculated as 'eGFR - mGFR' for all equations. The Bootstrap method was employed to assess pairwise significance levels between bias distributions interquartile ranges (IQR), and p-value correction was determined with the Benjamini & Hochberg method. Precision, which was defined as percentage of samples within ±30% and ±15% from the mGFR value (P_{30} and P_{15}, respectively), was calculated for each equation. Pairwise comparisons were made using McNemar's Chi-squared and Benjamini & Hochberg correction. Precision was defined as the proportion of eGFR values concordant with mGFR values by CKD clinical stage.

Results P_{30} for GFR_{NMR} was significantly higher than $CKD\text{-}EPI_{2021}$ Cr,Cys (97% vs. 84%, p<0.01) and higher than $CKD\text{-}EPI_{2021}$ Cr (88%, p=0.08). Similarly, P_{15} for GFR_{NMR} was 63%; higher than for $CKD\text{-}EPI_{2021}$ Cr (54%, p=0.35) and $CKD\text{-}EPI_{2021}$ Cr,Cys (51%, p=0.19). Bias IQR for GFR_{NMR} was 9.5 mL/min/1.73m^2 (median bias 2.0 mL/min/1.73m^2); significantly smaller than for CKD-EPI2021 Cr (Bias IQR 15.55 mL/min/1.73m^2, p<0.05; median bias 1.47 mL/min/1.73m^2), and smaller than $CKD\text{-}EPI_{2021}$ Cr,Cys (Bias IQR 14.4 mL/min/1.73m^2, p=0.09; median bias 2.3 mL/min/1.73m^2). A broad range of medications such as immune modulators, and comorbidities such as hypertension, thyroid dysfunction, and coronary artery disease, did not interfere with GFR_{NMR} performance.

Conclusion NMR-based eGFR was less biased, more precise, and more accurate compared to creatinine and cystatin-C CKD-EPI eGFR equations in a post-kidney transplant setting. These findings suggest GFR_{NMR} may more closely resemble mGFR in post-transplant patients and warrants further study on its potential use to improve clinical management in this patient group.

A-128

Analytical Performance Evaluation of Assays Used for Iron Studies on the Atellica® CI 1900 Analyzer

J. Freeman, K. Kolewe, S. Singh, B. Valdivia, W. Varhue, G. Arrode-Bruses, J. Rhea-McManus, J. Snyder, H. Leipold. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput, integrated chemistry and immunoassay analyzer employing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica CH Iron_2 (Iron_2), Total Iron Binding Capacity (TIBC), and Transferrin (Trf) assays* and the Atellica IM Ferritin (Fer) assay on the Atellica CI 1900 Analyzer. **Methods:** The Atellica CI 1900 Iron_2, TIBC, and Trf assays use the same reagents and calibrators as the comparable Atellica chemistry assays. The Atellica CI 1900 Fer assay uses the same reagents and calibrators as the Atellica immunoassay. Precision and method comparison (MC) were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 using native and contrived human serum samples. MC studies were performed according to CLSI EP09-A3. Native and contrived human samples were analyzed using the Atellica CH Iron_2, TIBC, and Trf assays on both the Atellica® CH 930 and Atellica CI 1900 Analyzers. For Fer, native and contrived human samples were analyzed using the Atellica IM Fer assay on both the Atellica® IM 1600 and Atellica CI 1900 Analyzers. **Results:** Representative precision and MC results for each assay are listed in the table. For the four assays tested, repeatability and within-lab %CVs were <3.5% and <4.5%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1. **Conclusion:** Evaluation of the Atellica CH Iron_2, TIBC, and Trf and Atellica IM Fer assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on their respective Atellica CH 930 and Atellica IM 1600 Analyzers.

	Precision				Method Comparison	
Analyte (Assay)	Unit	Sample Range	Repeatability %CV range	Within Laboratory %CV range	Sample Range	Regression Equation for Comparative Assay
Iron (Iron_2)	µg/dL	63–821	0.2–1.6	1.2–3.0	10–939	y = 0.99x + 2 µg/dL
Total Iron Binding Capacity (TIBC)	µg/dL	221–614	0.3–0.9	1.1–2.0	61–609	y = 0.98x + 5 µg/dL
Transferrin (Trf)	mg/dL	154–344	0.7–0.9	1.8–2.4	4–417	y = 0.98x – 3 mg/dL
Ferritin (Fer)	ng/mL	4.0–1459.3	1.3–3.2	2.1–4.3	2.0–1478.0	y = 1.03x + 3.7 ng/mL

*The products mentioned are not commercially available in all countries. Their future availability cannot be guaranteed.

A-129

Analytical Performance Evaluation of Vitamin D, Intact Parathyroid Hormone, and Inorganic Phosphorus on the Atellica CI 1900 Analyzer

J. Freeman, R. Aleo, C. Tyler, B. Valdivia, J. Rhea-McManus, H. Leipold, J. Snyder. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput, integrated chemistry and immunoassay analyzer utilizing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica IM Vitamin D (VitD) and Intact Parathyroid Hormone (PTH) and Atellica CH Inorganic Phosphorus (IP) assays* on the Atellica CI 1900 Analyzer*. **Methods:** The Atellica CI 1900 VitD, PTH, and IP assays use the same reagents and calibrators as the Atellica IM VitD and PTH and CH IP assays. Precision and method comparison (MC) were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 using native and contrived human serum (VitD and IP) or plasma (PTH) samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. MC studies were performed according to CLSI EP09-A3. Native and contrived human samples were analyzed using the Atellica IM VitD and PTH assays on both the Atellica IM and Atellica CI 1900 Analyzers. For IP, native and contrived human samples were analyzed using the Atellica CH IP assay on both the Atellica CH and Atellica CI 1900 Analyzers. **Results:** Representative precision and MC results for each assay across indicated sample ranges are listed in the table. For the three assays tested, repeatability and within-lab %CVs were <4.5% and <10%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1. **Conclusion:** Evaluation of the Atellica IM VitD and PTH and Atellica CH IP assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on their respective Atellica IM and Atellica CH Analyzers.

	Precision				Method Comparison	
	Unit	Sample Range	Repeatability %CV range	Within Laboratory %CV range	Sample Range	Regression Equation for Comparative Assay
Analyte (Assay)						
Vitamin D (VitD)	ng/mL	6.17–129.16	1.0–4.4	2.6–9.7	4.37–137.38	y = 1.03x + 0.83 ng/mL
Intact Parathyroid Hormone (PTH)	pg/mL	8.3–1540.9	1.7–2.7	2.6–4.5	7.3–1813.5	y = 1.02x – 1.0 pg/mL
Inorganic Phosphorus (IP)	mg/dL	3.0–12.1	0.4–1.3	1.4–2.3	0.3–19.2	y = 0.95x + 0.2 mg/dL

*The products/features mentioned are not commercially available in all countries. Their future availability cannot be guaranteed.

A-130

Analytical Performance Evaluation of Assays Measuring Acetaminophen and Ethanol on the Atellica CI 1900 Analyzer

J. Freeman, N. Morjana, S. Singh, G. Arrode-Bruses, J. Rhea-McManus, J. Snyder. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput, integrated chemistry and immunoassay analyzer employing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica CH Ethyl Alcohol (ETOH) and Acetaminophen (Acet) assays on the Atellica CI 1900 Analyzer. **Methods:** The Atellica CI 1900 ETOH and Acet assays use the same reagents and calibrators as the comparable Atellica CH assays. Precision and method comparison (MC) were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3. Native and contrived human serum samples were used. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. MC studies were performed according to CLSI EP09-A3. Individual human serum samples were analyzed using the Atellica CH assays on both the Atellica CH and Atellica CI 1900 Analyzers. Precision and MC were evaluated with three reagent lots on one system. **Results:** Representative precision and MC results observed for each assay are listed in the table. Over the two assays tested, repeatability and within-lab %CVs were <2.5% and <4.0%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1 for the ETOH and Acet assays. **Conclusion:** Evaluation of the Atellica CH ETOH and Acet assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on the Atellica CH Analyzer.

	Precision				Method Comparison	
Analyte (Assay)	Unit	Sample Range	Repeatability %CV range	Within Laboratory %CV range	Sample Range	Regression Equation for Atellica CH 930 Comparative Assay
Ethanol (ETOH)	mg/dL	53.1–259.6	0.8–2.1	1.6–3.9	7.5–293.8	y = 0.98x + 1.0 mg/dL
Acetaminophen (Acet)	mg/dL	4.6–13.6	0.5–0.9	1.5–2.2	0.3–19.2	y = 1.02x – 0.2 mg/dL

*The products/features mentioned here are not commercially available in all countries. Their future availability cannot be guaranteed.

Hematology and Coagulation

A-134

Correlation study of haematology analyser Sysmex XS 1000 vs Beckman DXH 520

j. teng. *national healthcare group diagnostics, singapore, Singapore*

Background:

An evaluation was conducted on 2 different haematology analysers to determine its result correlation. Both Sysmex XS1000i and Beckman DXH 520 are 5 part differential count analysers.

Methods:

The evaluation was conducted on Sysmex XS 1000 and 2 units of Beckman DXH 520 at NHGD YIS lab in 2020.All instruments underwent precision study, carryover test and linearity to ascertain its performance prior to the concordance study.63 samples were used in the correlation all reportable parameters and WBC differential count and its flagging.

Results:

The 2 units of DXH 520 demonstrated acceptable performance in precision, carryover and Linearity. The correlation of the parameters were within RCPA allowable error. However, we noted a low concordance of 39% in the WBC differential count flagging . To validate the flagging, manual differential count was conducted.

Conclusion:

During the correlation, Sysmex XS appeared to be more sensitive to Atypical lymphocytes where manual differential count read < 5%. This inevitably resulted in more manual slide review. In the 63 samples, 4 cases with high atypical lymphocytes (>5%) seen in manual differential count and they were flagged as lymphocytes in sysmex XS1000i and as monocytes in DxH 520. To counter the potential missed out in the reporting of Atypical lymphocytes in DXH, a lower threshold for monocytes in slide review may be needed.

A-135

Improving cell count turnaround times with the GloCyte® Automated Cell Counter for CSF: a retrospective study at MercyOne Des Moines Medical Center

A. Haynes[1], J. MacKenzie[2]. *[1]MercyOne, Des Moines, IA, [2]Advanced Instruments, LLC, Norwood, MA*

Background: Accurate, STAT cerebrospinal fluid (CSF) cell counts are critical in the diagnosis of meningitis, malignancy, demyelinating disease, and hemorrhage. Due to historical limitations with the linearity of automated analyzers, CSF cell counts have traditionally been performed manually with a hemocytometer and the aid of a brightfield optical microscope. Manual cell counting is time-consuming, subjective due to reliance on human interpretation, and requires a high degree of skill. This poses significant challenges for clinical laboratories of all sizes facing unprecedented med tech shortages. The purpose of this study was to evaluate the impact of implementing the GloCyte Automated Cell Counter for CSF, an FDA-cleared device with linearity down to 0 cells/µL, on result turnaround times. **Methods:** Result turnaround time data for CSF specimens processed in the hematology lab at MercyOne Des Moines Medical Center, a 600-bed hospital in Des Moines, Iowa, was analyzed in a retrospective study of two cell counting methods- (1) manual cell counting using a Neubauer-Improved hemocytometer and (2) GloCyte, a benchtop cell counting device that uses fluorescence technology and highly specific reagents to automate RBC and TNC cell counts in less than 5 minutes. Turnaround times for 227 CSF specimens counted manually during an approximately six-month period prior to the implementation of GloCyte (January 2, 2017 to June 29, 2017) were compared to those for 133 specimens analyzed on GloCyte over a similar duration (January 4, 2021 to June 30, 2021). In this study, turnaround times were defined as the time between specimen arrival in the lab and result verification. Correlation between the methods was previously established in the MercyOne Des Moines Medical Center laboratory. **Results:** The data demonstrated a significant, 16-30-minute improvement in turnaround times with GloCyte compared to the manual hemocytometer method. Median turnaround times, analyzed to adjust for add-ons that resulted in longer turnaround times pre- and post-GloCyte implementation, were 1:38h for specimens counted manually versus 1:08h for specimens analyzed on GloCyte. The average turnaround time for the 227 CSF specimens counted manually was 2:03h compared to 1:46h for the 133 samples analyzed on GloCyte. The MercyOne Des Moines Medical Center laboratory processed 456 CSF cell counts in 2022, equating to a reduction in turnaround time of 122-228 hours annually with the implementation of GloCyte. **Conclusion:** This study demonstrates that GloCyte can be implemented to significantly improve turnaround times for CSF cell counts. The MercyOne Des Moines Medical Center laboratory realized several benefits from the reduction in turnaround times- GloCyte has (1) freed up significant tech time for other tasks, (2) standardized CSF cell counting to mitigate tech to tech variability, (3) increased lab staff satisfaction and decreased stress levels, and (3) increased physician satisfaction. For MercyOne Des Moines Medical Center's sister site, MercyOne West Des Moines Medical Center, a 146-bed hospital that infrequently performs CSF cell counts, GloCyte has provided peace of mind in performing cell counts accurately with techs of all skill levels.

A-136

Usefulness of presepsin and thrombomodulin measurements for predicting COVID-19 mortality

A. Yamazaki[1], Y. Nukui[2], T. Kameda[1], R. Saito[3], Y. Koda[4], N. Ichimura[4], S. Tohda[4], R. Ohkawa[1]. *[1]Analytical Laboratory Chemistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), Tokyo, Japan, [2]Department of Infection Control and Prevention, Tokyo Medical and Dental University (TMDU) Hospital, Tokyo, Japan, [3]Department of Molecular Microbiology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), Tokyo, Japan, [4]Clinical Laboratory, Tokyo Medical and Dental University (TMDU) Hospital, Tokyo, Japan*

Background: Coronavirus disease (COVID-19) is a novel infectious disease developed into a pandemic since early 2020, resulting in extensive morbidity and mortality globally. Therefore, it is important to identify biomarkers for predicting the severity and mortality of COVID-19. We focused on presepsin (P-SEP) and thrombomodulin (TM), which are biomarkers of sepsis and endothelial dysfunction, respectively. Some reports have already suggested that these biomarkers can be associated with COVID-19, however, most of them assessed blood P-SEP and TM levels using only a single day measurement. In addition, since both P-SEP and TM are excreted by the kidney, renal dysfunction should be considered when evaluating these levels. In this

study, to evaluate the usefulness of P-SEP and TM for the prognosis and prediction of COVID-19 severity, we investigated the changes in these levels after admission with or without adjustment for creatinine (CRE) level in COVID-19 patients.

Methods: Samples were residual sera obtained from COVID-19 patients (n = 674) admitted to the TMDU Hospital. Serum P-SEP and TM levels were measured using the STACIA clinical assay system. Disease severity was classified as mild, moderate, or severe based on hemoglobin oxygen saturation and the history of intensive care unit transfer or use of ventilation at admission. Patients in the severe group were further classified into survivors and non-survivors. This study was approved by the institutional research ethics committee (No. M2020-110).

Results: Since patients who received recombinant human TM treatment had abnormally high TM levels (> 1000 U/mL), data after administration were excluded from the analysis. P-SEP and TM levels during hospitalization were significantly correlated with CRE levels. In the correlation between P-SEP and CRE, the slope of the severe group was different from that of the non-severe group. When compared the data extracted within 5 days after admission (n = 183) among the three severity groups, CRE levels showed no significant difference among them. On the other hand, P-SEP and TM levels at admission were significantly higher in the severe group than those in the mild group, even after adjusting for CRE levels. In addition, there was no significant difference in the P-SEP levels between survivors and non-survivors, whereas TM levels were significantly higher in non-survivors than in survivors. Changes in the P-SEP levels at two time points (interval: 4.1 ± 2.2 days) were significantly different between survivors and non-survivors. The second measurement of TM levels were elevated in both groups from the first measurement at admission.

Conclusion: This study suggested that the elevation of P-SEP and TM levels in severe COVID-19 patients might not only fluctuate with renal dysfunction, but might reflect severity. TM and continuous P-SEP measurements are available for predicting mortality in patients with COVID-19. Moreover, P-SEP and TM values after CRE adjustment would be independent predictive markers, apart from renal function.

A-137

Performance Evaluation of an Automatic, Rapid and Non-Westergren based Method for Erythrocyte Sedimentation Rate Measurement

H. Hsiang-Ling, W. Fang-Yu, L. Hao-Ru. *Taipei Veterans General Hospital, Taipei, Taiwan*

The erythrocyte sedimentation rate (ESR) is widely used for the assessment of acute or chronic inflammation, especially for rheumatoid diseases. The gold standard for determining ESR is the Westergren method, which measures the distance in millimeters that red blood cells fall in one hour. However, this method needs to be performed manually, which increases the biohazard risk to laboratory personnel, and the results are often influenced by many preanalytical and analytical factors. Several automated ESR analyzers with different methodologies are now available to provide faster results directly from whole blood anticoagulated with K3EDTA. In this study, we evaluated the performance of an automatic ESR analyzer, Alifax TEST1, and compared it with that of another automatic analyzer, Diesse Cube 30 touch, and also the Westergren method. TEST1 extrapolates the ESR values by measuring the kinetics of red cells aggregation in 20 seconds, while Cube 30 is a modified Westergren method that could obtain the results in 20 minutes. A total of 75 clinical samples were analyzed for ESR using the three methods. The hematocrit (Hct) and C-reactive protein (CRP) values were also analyzed. The Spearman correlation coefficient between TEST1 and Westergren methods was 0.90 (P<0.0001) with a mean difference of -5.45; the correlation between Cube 30 and Westergren methods was 0.80 (P<0.0001) with a mean difference of 10.74; the correlation between TEST1 and Cube 30 was 0.69 (P<0.0001) with a mean difference of -15.9. The precision values (CV %) of TEST1 in low-, medium- and high-level controls were 2.71%, 1.21% and 1.05% for inter-assay, and 3.70%, 1.66% and 0.86% for intra-assay. The normal range of TEST1 was also established by analyzing ESR values of 125 healthy individuals where it is 0-15 mm/hr for men ≤ 50 years old, 0-20 mm/hr for men > 50 years old and for women ≤ 50 years old, and 0-30 mm/hr for women > 50 years old. The ESR values obtained from the three methods all significantly correlated with plasma CRP levels. However, the ESR values from Cube 30 and Westergren methods negatively correlated with Hct values, while that from TEST1 did not.The present study demonstrates that 1) TEST1 has a better correlation and a lower difference to the Westergren method compared to Cube 30, suggesting that TEST1, the automatic, rapid and non-Westergren based method is an effective assay for clinical practice; 2) the Westergren based methods are more easily influenced by Hct; 3) the large mean difference between TEST1 and Cube 30 highlights a need for harmonization of the ESR values obtained from different automatic analyzers, especially when it is applied to monitor the disease activity score (DAS28) in rheumatoid arthritis patients.

A-139

Investigating the Utility of Reticulocyte Hemoglobin Equivalent for Diagnosis of Iron Deficiency Anemia

H. Lee[1], F. Wang[1], H. Ho[2]. *[1]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [2]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; Department of Biotechnology and Laboratory Science in Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan*

Background:

Iron deficiency anemia (IDA) is one of the most common leading global health problems, which could cause extreme fatigue, chest pain, headache, and even neurodevelopment deterioration in children. Traditionally, assessing serum ferritin, transferrin, and iron are the tools to diagnose IDA. However, a high false-positive rate of these conventional biomarkers might be observed due to inflammation, infection, or malignancy. Reticulocyte hemoglobin equivalent (RET-He) is a new parameter that reflects the recent functional availability of iron for erythropoiesis, with the advantage of real-time results as part of automated reticulocyte analysis. The present study, therefore, aimed to investigate the utility of RET-He for the diagnosis of patients with IDA.

Methods:

This retrospective study collected laboratory data from patients with both serum ferritin and reticulocyte results from January 2022 through December 2022, and excluded the high white blood cell count (WBC > 9,380 cells/μL) and high C-reaction protein (CRP > 0.5 mg/dL). RET-He was measured by Sysmex XN-10 or XN-20 automated hematology analyzer (Sysmex, Kobe, Japan). Serum ferritin was measured by Roche Cobas e801 (Roche Diagnostics, Mannheim, Germany). Iron and total iron binding capacity (TIBC) were measured by Roche Cobas c702 (Roche Diagnostics, Mannheim, Germany). Anemia was defined as hemoglobin concentration < 13 g/dL for men and < 12 g/dL for women. Iron deficiency was defined as serum ferritin < 15 μg/L. Student T-tests and Pearson correlation were performed, and *p*-values less than 0.05 were considered statistically significant. Statistical analyses were performed with SPSS software (Version 22.0).

Results:

A total of 2,208 patient data were enrolled in this study and were classified into the IDA, iron deficiency, anemia, and control groups according to ferritin level and hemoglobin concentration. The RET-He were 20.6, 27.7, 30.4, and 32.3 pg in the IDA, iron deficiency, anemia, and control groups, respectively. The IDA group showed significantly lower RET-He levels than the control group (p<0.001). By Pearson correlation analysis, the RET-He showed a weak positive correlation with ferritin and hemoglobin (r=0.236, 0.274), moderate positive correlation with iron (r=0.354), and moderate negative correlation with TIBC (r=-0.369). In addition, the average turn-around time (received-reported) of RET-He was 16.4 minutes, which was shorter than other biochemical markers such as ferritin, iron, and TIBC (57.7, 34.2, 34.4 minutes).

Conclusion:

The RET-He could be an assessment of the iron actually used for the biosynthesis of hemoglobin and as a useful and rapid tool for diagnosing and monitoring iron deficiency anemia.

A-140

Evaluating the Performance of Various Lupus Anticoagulants Tests among Different Coagulation Platforms

F. Wang[1], H. Lee[1], Y. Chen[1], H. Ho[2]. *[1]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [2]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; Department of Biotechnology and Laboratory Science in Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan*

Background:

Lupus Anticoagulants (LAs), one of the antiphospholipid antibodies, are risk factors for arterial or venous thrombosis with complications such as stroke, acquired thrombophilia, and pregnancy loss. Detection of LAs is challenging for laboratories as the heterogeneity of antibodies and epitopes on protein in the phospholipids. Thus, no single test is sensitive for all LAs, and the International Society on Thrombosis and Haemostasis Scientific and Standardization Committee (ISTH) recommended laboratory should provide two test systems based on different principles, which dilute Russell's viper venom time (dRVVT) should be as the major test, and partnered with LA-sensitive activated partial thromboplastin time (aPTT). There are many different

mechanisms of tests proposed by various manufacturers, however, the comparison of different platforms by different manufacturers on LAs remains unknown. In this study, we aimed to evaluate the performance of various LA tests among different platforms.

Methods:

A total of 76 specimens from September to December 2022 were collected. dRVVT ($dRVVT_{IL}$) and silica clotting time (SCT_{IL}) were performed by ACL TOP 500 analyzer (Instrumentation Laboratory, US). In addition, ellagic acid-based aPTT (Siemens Dade Actin FSL reagent, $aPTT_{AFSL}$), silica-based aPTT (Siemens Pathromtin SL reagent, $aPTT_{PSL}$), and dRVVT ($dRVVT_{sysmex}$) were performed by Sysmex CN-6000 analyzer (Sysmex Corporation, Japan). Analytical performances, agreements, and McNemar's test were calculated.

Results:

All 76 specimens were originally tested by $dRVVT_{IL}$ and SCT_{IL}, and showed that 30 specimens were positive for both assays, 6 were only positive for dRVVT, 5 were only positive for SCT, and 35 specimens were negative for both assays. In addition, all samples were further tested by $dRVVT_{sysmex}$. Compared with the integrated use of $dRVVT_{IL}$ and SCT_{IL}, $dRVVT_{sysmex}$ showed 100.0% (41/41) positive agreement and 91.4% (32/35) negative agreement. 3 specimens could only be tested positive on the $dRVVT_{sysmex}$ platform, of which one patient was diagnosed with systemic lupus erythematosus (SLE), another patient was diagnosed with infertility, and the other patient was diagnosed with antiphospholipid syndrome (APS). To further evaluate the performance of aPTT on LA screening, 44 specimens with either $dRVVT_{IL}$, SCT_{IL} and $dRVVT_{sysmex}$ positive were considered LA-positive and further tested by different aPTT assays. The positive agreement was 65.9% (29/44; 95% CI: 50.1-79.5%) and 77.3% (34/44; 95% CI: 62.2-88.5%) on $aPTT_{AFSL}$ and $aPTT_{PSL}$, respectively, however, there was no significant difference between the positive agreement of two assays (p=0.13).

Conclusion:

Compared with the integrated use of $dRVVT_{IL}$ and SCT_{IL}, $dRVVT_{sysmex}$ could achieve the same detection rate on LA. Furthermore, $aPTT_{AFSL}$ and $aPTT_{PSL}$ showed no significant difference between the positive agreements for LA-positive specimens.

A-141

Evaluation of the Novel Blood Coagulation Analyzer S400CF Comparison on Basic Performance and Turnaround Time with the CP 3000)

Y. Abe[1], K. Uchida[2], S. Munekata[2], Y. Kanoh[3]. *[1]Kitasato University Hospital, sagamihara-shi,kanagawa, Japan, [2]Kitasato University Hospital Clinical Laboratory Department, kanagawa-ken, Japan, [3]Kitasato University School of Medicine Clinical Laboratory Diagnosis, kanagawa-ken, Japan*

Background: Coagulation and fibrinolytic tests are widely used as screening of abnormalities on hemostatic mechanisms, and for thrombosis. Our laboratory routinely receives large number of orders for coagulation and fibrinolytic tests. The monthly average of order is 9700, and these orders are reported as emergency tests. To address the demands, fully automated blood coagulation analyzers that offer a variety of measuring principles such as coagulation time method and photometric method. We have operated three units of the automated blood coagulation CP3000 for eight years. In this eight years, the number of samples in our hospital has been increased. On the other hand, our preparation time before daily set-up has been extended because of the stricter management on quality control to comply with ISO 15189. As a result, we need to do the same procedure on three units and our turnaround time (TAT) has been extended. In November 2021, the blood coagulation analyzer S400CF that offers high throughput and performance was launched. We evaluated basic performance of S400CF and TAT and compared with the CP3000.

Methods: In the evaluation of basic performance, S400CF (Sekisui Medical) and was used. Correlation was compared with CP3000 (Sekisui Medical). In the comparison of TAT, three units of CP3000 and one unit of S400CF were used and the orders corresponding for the 243 samples that were requested by 7:45 AM - 10:08 AM on July 11, 2022 were demonstrated on both analyzers.

Results: Repeatability:C.V.% 2% or less for all items. Between-day precision: C.V. 8% or less for all items. Correlations were PT-% (N = 135): Y = 1.0477 X -6.40, PT-INR (N = 135): Y = 0.9612 X + 0.046, APTT (N = 124): Y = 1.0150 X + 0.42, Fbg (N = 112): Y = 1.0284 X + 2.4, AT (N = 52): Y = 0.9700 X, FDP (N = 49): Y = 0.9326 X + 0.06, DD (N = 80): Y = 1.0322 X + 0.279, SF (N = 50): Y = 1.0423 X -1.73. TAT of CP3000 and S400CF were MIN 1 minutes(min.) 41 seconds(sec.), MAX 14 min. 45 sec. and MIN 1 min.20 sec. , MAX 12 min. 27 sec., respectively.

Conclusion: It was indicated that one S400CF has three times or more productivity of CP3000. Therefore, the number of blood coagulation analyzers can be reduced from the current three units. And the total time for quality related process such as calibration and control can be saved.Results were equivalent to current CP3000 or better. It indicates that the quality of our current test can be maintained. S400CF has a capability to connect laboratory automation system that saves the time for placing specimen and improves the working efficiency of laboratory technicians. We concluded S400CF can solve the problems in our current operation in blood coagulation test and can contribute to faster report of high-quality test results.

A-142

Comparison of Alinity c Hemoglobin A1c Immunoassay with Premier Hb9210 Automated HPLC Assay: Very Few Patients May be Outside Reportable Range Using Alinity c.

A. Walton. *The University of Kansas Health System, Kansas City, KS*

Background: Hemoglobin A1c (HbA1c; glycosylated hemoglobin) is used for diagnosis and monitoring of patients with diabetes. In the clinical laboratory both immunoassays and high-performance liquid chromatography (HPLC) based tests are available for such measurement. We use Premier Hb9210 analyzer (HPLC method; Trinity Biotech, Jamestown, NY) for measuring HBA1c in whole blood. As our laboratory is transitioning to Abbott chemistry systems, we compared HbA1c values obtained by the Alinity c and Premier Hb9210. **Methods:** The Premier Hb9210 analyzer is based on boronate affinity high performance liquid chromatography with output in 66 sec. The analytical measurement range is 3.8 to 18.5% although values as low as 2% can be reported by the analyzer but result may not be accurate. The Alinity c Hemoglobin A1c assay measures both total hemoglobin and HbA1c (enzymatic assay) in whole blood and then calculates %HbA1c. The analytical measurement range of this assay is 4 to 14% of HbA1c. Specimens cannot be diluted for Alinity c HbA1c measurement. **Results:** Alinity c HbA1c has excellent total precision (0.4% at HbA1c of 5.1% and 0.25% at Hb A1c of 9.4%). In contrast, Premier HB9210 has total precision of 1.89% at HbA1c of 5.5% and 1.81% at HbA1c of 8.4%. However, analytical measurement range of Premier HB9219 is slightly wider compared Alinity c assay. Plotting HbA1c results obtained by the Premier Hb9210 analyzer in the x-axis and the corresponding values obtained by the Alinity c assay, we observed the following regression equation: y = 0.9499 x+ 0.1108 (n=50, 0.99). The 95% confidence interval of the slope was 0.9241 to 0.9757. Rarely, we get specimen where HbA1c is between 3.8 and 4.0% (42 specimens out of 52,803 between 2/7/22 to 2/7/23; 0.08%) or above 14% (174 specimens out of 52,802; same period; 0.33%) and these specimens would not produce any result with Alinity c assay. We recently observed HbA1c result of 2.4 % in a sickle cell patient (reported as <3.8% per our lab policy). **Discussion:** Our result indicates that HbA1c immunoassay on the Alinity c analyzer showed values comparable to HPLC method. However, for very few patients HbA1c may exceed 14% due to uncontrollable diabetes. Because specimen cannot be diluted, real value could not be measured using Alinity c but can be measured by the HPLC method. **Conclusions:** We conclude that HbA1c assay on the Alinity c analyzer is a viable alternative to HPLC for measuring HbA1c in clinical laboratory. However, for very few patients (sickle cell trait, uncontrolled diabetes), HbA1c may be outside the range of Alinity c, requiring HPLC analysis.

A-143

Imaging Flow Cytometry: a novel method using ADVIA 2120i System Reagents and Microscopic Image Analysis

M. Azhar[1], I. Chakraborty[1], A. Joshi[2], F. Stelling[3]. *[1]Center for Innovation in Diagnostics (CID), Siemens Healthineers, Bangalore, India, [2]Siemens Technology and Services Pvt. Ltd, Bangalore, India, [3]Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

Background: Flow cytometry based ADVIA 2120i Hematology System reagents are used to modify blood cells. Laser-scattering flow cytometry replaced with digital microscopic image analysis significantly simplifies the complex system to a compact, low-cost microscope. A novel method for imaging flow cytometry is proposed here that simultaneously generates complete blood count (CBC) and high-resolution images of blood cells.

Methods: Image processing algorithms are implemented to process the pixels containing blood cells. RBCs are made isovolumetrically spherical using the RBC reagent (Figure 1a) and imaged. RBC volumes are easily determined from the diameter of the isovolumetrically spherical RBCs. By illuminating the spherical RBCs with ~400 nm light, the increasing grayscale values of the pixels corresponds to increasing haemoglobin concentration due to absorption.

WBC nuclei are stained using the Perox reagent (Figure 1b). The grayscale values of the nuclei pixels can be used to determine the peroxidase stain component, thereby obtaining cell size-to-peroxidase activity scatter plots similar to those on the ADVIA

2120i system. Using the Baso reagent, the cytoplasm of all WBCs are stripped off except for basophils, as shown in Figure 1c. All the microscopic images have good contrast and high resolution. Image processing techniques can thus provide a 5-part differential using the nuclei/lobular density of WBCs.

Results: Images were obtained under a 20x Nikon microscope with a Thorlabs DC-C1545M monochrome camera. ADVIA reagents were mixed with venous blood and <5 µL of reagent treated sample was spotted between a microscopic slide and cover-slip. Image analysis was performed using ImageJ particle analysis tools.

Conclusion: Analysis of digital microscopic images of blood cells acted upon by ADVIA 2120i system reagents can offer a novel, simplified, cost-effective method of hematology analysis. Simultaneous CBC scatter plots and high-resolution images of the blood cells can be obtained using this new approach.

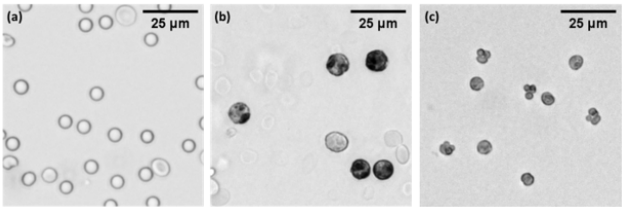


Figure 1. (a) Isovolumetrically spherical RBCs, (b) peroxidase stain of WBC nuclei, and (c) cytoplasm of WBCs stripped off (except for basophils). Images taken under a 20x Nikon microscope.

*Under development and not available for commercial sale.

A-144

Three-wavelength Polarization Imaging Method for the Analysis of Individual Human Blood Cells With Dye-Enhanced Dispersion of Dilution Buffer

R. Seliger[1], M. Kraus[2], T. Engel[3]. *[1]Center for Innovation in Diagnostics, Siemens Healthcare GmbH, Erlangen, Germany, [2]Technology Excellence, Siemens Healthcare GmbH, Erlangen, Germany, [3]Siemens AG, Munich, Germany*

Background: Performing basic hematology analysis in a near-patient setting enables more rapid clinical decision making in the process of diagnosis. Traditional blood analyzers in central labs use a complex combination of sensing principles and chemical modification of the sample to derive the necessary parameters for a complete blood count (CBC). To reduce cost and maintenance, varying approaches have been employed to determine a CBC with a single-step measurement technique. One common option is the use of optical microscopy to deliver highly reliable results, examples are fluorescence microscopy, digital holographic microscopy, or spatial light interference microscopy. However, most of these technologies lack a proper determination of cellular-level RBC parameters. Therefore, a non-interferometric, quantitative-phase measurement technique was developed to derive relevant CBC parameters from a single measurement, comparable to a standard lab analyzer. **Methods:** The optical setup employed by this method enables the parallel acquisition of brightfield and differential interference contrast (DIC) images by polarization-sensitive cameras. By using three cameras with individual optical filters, quantitative phase and absorption information can be calculated, which enables the determination of mean cellular volume (MCV), mean cellular hemoglobin (MCH), and mean cellular hemoglobin concentration (MCHC) for red blood cells (RBCs). The whole-blood sample is diluted in a buffer containing a non-cell-penetrating dye to enhance optical refraction contrast and therefore precision in the dependent calculated quantities. The sample is then introduced to a glass flow cell, and the cells are allowed to sediment to the bottom for imaging. Machine learning is applied to the derived images to classify unstained native white blood cells (WBCs) to yield a 5-part differential, which distinguishes between neutrophil, monocyte, lymphocyte, eosinophil and basophil. The algorithm is trained by imaging FACS-validated, purified WBC subgroups. All blood samples are measured on an ADVIA® 2120 Hematology System for control. **Results:** A one-step, optical CBC hematology system based on quantitative phase and absorption image analysis was developed. The correlations for MCV and MCHC compared to a standard lab CBC analyzer were 0.77 and 0.84. The accuracy for the 5-part differential WBC classification was approximately 0.8. To enable a visual inspection of the classification results, a pseudo-colored image of each cell was generated and uploaded in a review tool. **Conclusion:** With a more-refined, less-expensive optical setup, a high-quality CBC analyzer suitable for use at the point of care can be derived with the presented method. A possible simplification could be the use of low-cost but high-resolution Fourier ptychography microscopy (FPM) technology. Imaging of blood samples on a single-cell level offers the potential for the analysis of even more morphological parameters, for example, disease-related changes in the cell membrane. Also, the adhesion behavior of the WBCs in the glass flow cell could indicate cell activation status and therefore give information about infection processes. *Trademark disclaimer: "ADVIA, and all associated marks are trademarks of Siemens Healthcare Diagnostics, Inc. or its affiliates. All other trademarks and brands are the property of their respective owners."

A-145

Development of a Novel Algorithm Using Red Blood Cell Indices for Screening of Alpha Globin Gene Deletions

X. Zhang[1], J. Oliveira[1], B. Parikh[2]. *[1]Mayo Clinic, Rochester, MN, [2]Washington University School of Medicine, St. Louis, MO*

Background: Distinguishing between different causes of microcytic anemia is essential for management of anemic patients. More than 20 different RBC indices have been used to screen thalassemia trait with inconsistent results. Differentiation between iron deficiency anemia (IDA) and alpha-thalassemia is challenging based on RBC indices alone. Moreover, thalassemia and IDA may coexist. A highly sensitive screening algorithm is needed to select clinical cases for alpha globin gene analysis to reduce unnecessary testing and improve pre-test probabilities. **Methods:** A retrospective study was performed to evaluate the reliability of 22 RBC indices (RBC, MCV, RDW, MCH, Mentzer Index, RDW Index, etc) and formulae in differentiating cases with 1-2 alpha globin gene deletions from negative cases, in a cohort of 90 individuals with confirmatory alpha globin gene analysis results. **Results:** The number of patients with each alpha globin gene deletion pattern were: 37 patients with alpha-thalassemia trait, 21 silent carriers (one gene deletion), 31 patients with negative result and 1 patient with triplicated alpha globin gene. The mean RBC count in each group were 4.79 +/- 0.65 (trait), 4.45 +/- 0.73 (carrier), and 4.21 +/- 0.84 (negative) (million/mm). The mean MCV in each group were 71.1 +/- 4.8 (trait), 76.1 +/- 5.9 (carrier), and 74.2 +/- 9.3 (negative) (fL). The mean RDW in each group were 16.5 +/- 3.0 (trait), 17.2 +/- 3.4 (carrier), and 18.3 +/- 4.9 (negative) (%). The mean MCH in each group were 22.0 +/- 1.8 (trait), 23.8 +/- 2.3 (carrier), and 23.5 +/- 3.7 (negative) (pg). None of the RBC indices showed significant difference between carrier and negative groups, while 20 RBC indices showed significant difference ($p<0.05$) between trait and negative groups. The sensitivities of the RBC indices in detecting 1-2 alpha globin gene deletions based on published cut-offs ranged between 3.4% and 81.0%, while the specificities ranged between 41.9% and 100%. A novel scoring algorithm was evaluated based on the combination of 12 selected RBC indices and formulae, with each index or formula contributing to a score of 0 or 1. The average value of the negative cases was used as the score cut-off value for each index or formula. The scoring algorithm gave a score distribution between 0 and 12, with lower scores indicating alpha globin gene deletions. It achieved a sensitivity of 96.6% in detecting 1-2 alpha globin gene deletions, with a negative likelihood ratio of 0.15. The negative likelihood ratio of the scoring algorithm outperformed each of the individual RBC indices at similar levels of sensitivities (>95%). **Conclusion:** The novel algorithm is a highly sensitive tool for screening of alpha globin gene deletions, including one gene deletion. It is more effective at identifying negative cases compared to individual RBC indices. Implementation of the algorithm could efficiently reduce 10.0% (9/90) of the test volume of alpha globin gene analysis. A more stringent version of the algorithm that incorporates standard deviation in the cut-off values could potentially achieve nearly 100% sensitivity and reduce 5.6% (5/90) of the alpha globin gene analysis test volume.

A-146

A Review of RDW-CV and RDW-SD Measurements in Patients with Iron Deficiency Anemia in an Acute Care Hospital in Singapore

Z. Chen, F. Ilagan, P. Heng, V. Prasad. *Khoo Teck Puat Hospital, Singapore, Singapore*

Background: Red Cell Distribution Width (RDW) is a routinely available parameter on automated haematology analysers. This parameter is often used to help in the diagnosis of iron deficiency anaemia. RDW quantitatively measures the degree of anisocytosis of the red blood cells. The more common way of reporting RDW is RDW-CV, which is based on the coefficient of variation of the red blood cell distribution volume and is calculated using mean red cell volume. RDW can also be presented as RDW-SD, which is a direct measurement of the width of the red blood cell distribution curve and is not influenced by mean red cell volume. Conventionally, laboratories report RDW-CV as part of the red blood cell indices in full blood count (FBC) reports. However, it has been proposed that RDW-SD is a better indicator of iron deficiency

anaemia compared to RDW-CV. The authors seek to review and assess the clinical utility of both RDW measurements in patients with iron deficiency anaemia in an acute care hospital in Singapore.

Methods: RDW data of samples from 271 unique individuals from Sysmex XN-9000 (Sysmex, Japan) automated haematology analysers over a period of two months were reviewed. These individuals were selected as they had plasma iron measured in the same blood collection. The individuals were sorted into two groups based on the plasma iron measurement: with iron deficiency (80%) and without iron deficiency (20%). The correlation of RDW-CV and RDW-SD with the plasma iron concentration of the individuals was studied. The RDW-CV reference range used were established for the local population by an in-house reference range validation study while that of the RDW-SD is from a recent Participant Summary Report for a College of American Pathologists (CAP) Hematology Automated Differential survey (FH9-B 2022). The sensitivity, specificity, predictive values, and overall efficiency of RDW-CV and RDW-SD were calculated and compared using the data collected.

Results: The sensitivity and specificity of RDW-CV in detecting iron deficiency anaemia are 45.2% and 68.5%. The positive and negative predictive values for RDW-CV are 85.2% and 23.7% respectively. The sensitivity and specificity of RDW-SD in detecting iron deficiency are 33.2% and 64.8%. The positive and negative predictive value of RDW-SD are 79.1% and 19.4% respectively. The overall efficiency of RDW-CV is 49.8%, significantly higher than 39.4% of RDW-SD.

Conclusion: This retrospective study suggested that RDW-CV is still a better blood cell index than RDW-SD in the differential diagnosis of iron deficiency anaemia in a predominantly Asian population. However, the use of RDW-SD reference range that is appropriate for the local population may improve the clinical utility of the RDW-SD.

A-148

Thromboelastography utilization in a community hospital setting

J. L. Allen, M. Christian, A. Zuretti, M. H. Bluth. *Maimonides Medical Center, Brooklyn, NY*

Background: Thromboelastography (TEG) applications have been gaining momentum in various clinical settings and service lines. However, the utilization of the technology varies among clinical establishments and requires additional assessment for TEG optimization among its user groups

Methods: TEG (Haemonetics TEG6s) was applied in a community hospital based setting in SICU, OR and ED service lines to identify instrument prompted blood product support over a two month time period.

Results: Forty one measurement were obtained from twenty patients. SICU represented the majority of TEG use (78%) followed by OR (15%). Global TEG run assessments were completed on 27%, platelet mapping on 46%, and heparin assessment on 56% of samples. Global TEG recommendations included factor deficiency with suggested FFC or PCC use (12%) or no recommendations (10%). Platelet assessments recommended no product (19.5%), or ADP inhibition with platelet consideration if bleeding (14.6%). Heparin assessment indicated no treatment (44%) and above normal CK/R time with FFP or PCC if bleeding (10%).

Conclusion: TEG applications in various services suggested no need for blood product or factor application in the majority of platelet mapping and heparin assessments. Other assessments yielded about a half dozen suggestions from the instrument's 150 suggestion options. TEG application appears to be easily implementable and suggests no treatment or a few repetitive treatment options for the majority of patients assessed thereby reducing onboarding educational and improving management requirements for select user groups.

A-149

Performance characteristics of VerifyNow PRU and ARU tests during a clinical method verification exercise

S. Ibrahim[1], M. Pandiri[1], J. Bailey[1], E. Housman[2], V. Johari[1], M. A. Barbhuiya[3]. *[1]Department of Pathology, UMass Chan Medical School-Baystate, Springfield, MA, [2]Department of Pharmacy Services, Baystate Health, Springfield, MA, [3]Department of Pathology; Healthcare Delivery and Population Sciences, UMass Chan Medical School-Baystate, Springfield, MA*

Background: Dual antiplatelet therapy with aspirin and a platelet P2Y12 receptor antagonist is indicated for the prevention of atherothrombotic events in patients with acute coronary syndromes and for those undergoing percutaneous coronary intervention procedure. Two different assays are available in the VerifyNow Test device for monitoring the anti-platelet therapy response. The aspirin assay (ARU Test) utilizes arachidonic acid as an agonist and is sensitive to acyl salicylic acid. The P2Y12 assay (PRU test) is sensitive to thienopyridines (such as clopidogrel, prasugrel, ticagrelor) and uses adenosine diphosphate (ADP) as an agonist. The PRU test is a non-waived test and ARU test is a waived test. A comprehensive method performance verification is required for the PRU test. We evaluated performance characteristics of the VerifyNow PRU Test and ARU Test for clinical use.

Methods: The standard precision and accuracy study using two levels (low and high) of quality control materials were performed (N=10) for both the assays. Alternative qualitative method comparison was carried out (N=49) using the VerifyNow PRU Test in a reference lab and in-house. The in-house inter-instrument comparisons were carried out (N=42). The blood samples were obtained from patients taking P2Y12 inhibitors and from normal donors not on any drugs. The results of the PRU Test are expressed in P2YI2 reaction units (PRU). The VerifyNow-Aspirin assay results are expressed as aspirin reaction units (ARU). The statistical analysis to evaluate performance characteristics was carried out using the EP Evaluator.

Results: The VerifyNow device 1561 precision characteristics for low QC (Obs Mean= 1.5 PRU (N=10), SD = 0.2; 95% CI for Obs Mean 1.0 to 2.0 PRU) and high QC (Obs Mean= 258.3 PRU (N=10), SD = 8.2; 95% CI for Obs Mean 252.4 to 264.2 PRU) were within acceptable performance limits. The CV for high QC was 3.2%. The ARU test also demonstrated high precision for two levels (Low QC Obs Mean= 350ARU (N=10), SD= 0.0); High QC Obs Mean= 583.3 (N=10), SD= 7.2; 95% CI for Obs Mean= 578.1- 588.5) with a CV of 1.2%. The PRU Test Method PRU 1561 In House vs Ref Method Verify Now PRU Test (N=49) demonstrated overall agreement of 93.9% with positive agreement (intended use population on drugs, N= 27) of 92% and negative agreement (normal non-drug group, N=22) of 95.8% (Cohen's Kappa= 87.8%; Kappa > 75% indicates "high" agreement). The McNemar Test for Symmetry passed (p >0.999, Fisher's Exact test) in this qualitative method comparison. A value of $p<0.05$ would suggest the methods are significantly different. The other in-house instrument 1562 also demonstrated comparable or better performance characteristics.

Conclusion: It is important to confirm the medical decision limit for the intended use population and determine acceptance criteria while considering biologic variability. However, the reference range verifications with drug responders and non-responders are not always feasible and cost-effective. Based on the qualitative method comparison in this study, we have transferred the medical decision limits from the peer group. The in-house multi-instrument comparison should be repeated every six months to monitor the performance of the instruments.

A-150

Abo subgroup incompatibility leading to red cell engraftment failure and fatal outcome: a rare but preventable complication in related allogeneic hsct

F. M. Conti[1], A. Valveson[2], R. Pereira[2], F. Nascimento[2], M. Hori[3], M. C. Mello[4], M. C. Castelo[5], A. C. Lopes[6]. *[1]Dasa, Rio de Janeiro, Brazil, [2]Hemocentro São Lucas, Rio de Janeiro, Brazil, [3]Américas Oncologia, Rio de Janeiro, Brazil, [4]Dasa, São Paulo, Brazil, [5]Dasa, Fortaleza, Brazil, [6]Dasa, Florianópolis, Brazil*

Background Pre-allogeneic hematopoietic stem cell transplant (HCST) testing includes, among others, ABO and HLA typing and testing for anti-HLA antibodies. ABO subgroup typing is not routinely performed, as A_2 subgroup representes 1% of the total group A population and only 0,4% of A_2 individuals have anti-A_1 in their serum. ABO antigens are also found on the platelet surface. We describe a case of alloHSCT performed between siblings, the donor A_1 and the recipient A_2, with a fatal outcome caused by red cell and platelet engraftment failure due to anti-A_1 antibodies, and emphasize the need to routinely investigate subgroup incompatibility in the pre-alloHSCT workup **Methods** Patient data was collected from hospital and laboratory electronic records. Blood typing and immunohematological investigation was performed at the hospital blood bank using gel cards for ABO typing, DAT and antibody screening panels (Grifols, Barcelona). Tube method was used for A subgroup typing (Fresenius, Germany). Bone marrow analysis and other blood tests were performed at local hospital laboratory. HLA typing and antibody screening (Luminex, USA) were sent to a specialized laboratory **Results** A 51 year-old male with Non-Hodgkin lymphoma underwent a full-match ABO compatible allogeneic HSCT, both brother/donor and recipient group A. Pre-transplant anti-HLA or ABO antibodies were negative. WBC recovery was achieved on D+22. The patient had a catheter infection on D+15 (Permcath removed), CMV infection on D+24 (treated with gancyclovir) and received several blood transfusions. On D+22, he had a hemolytic transfusion reaction with fever (38,5°C), chills and a decline in hemoglobin (7.3 g/dL to 6.6 g/dL in the following day), low haptoglobin and increased indirect bilirrubin levels. Immunohematological investigation revealed a positive DAT with an anti-A antibody

in the eluate. Subgroup incompatibility was suspected and the patient was found to be subgroup A_2, whereas the donor typed A_1. Three days later the DAT became negative and anti-A_1 was no longer detected. Other RBC antibodies were also excluded. Only group O RBCs and A / AB platelets were transfused thereafter to prevent further hemolysis. On D+35 the patient was diagnosed with GI-GVHD, which responded to corticosteroids. He never achieved transfusion independence and received RBC and platelet transfusions on a regular basis. Failure of RBC and platelet engraftment was confirmed by a bone marrow aspirate on D+45, showing only 20% erythroid lineage and absence of megakaryocytes. Marrow chimerism revealed 83% of donor origin. Sepsis by *S. aureus* ensued on D+57 and three days later he had fever (38,2°C) and dyspnea after a platelet transfusion. Despite entubation and mechanical ventilation, he evolved to RCP and died on D+60. **Conclusion** ABO subgroup typing is a simple and low-cost technique routinely available in the blood bank. Detection of A subgroup incompatibility impacts on key decisions, such as donor choice (if more than one candidate) and the need for erythrocyte depletion of donor stem cells and recipient plasmapheresis if the anti-A_1 titer is high. Since graft failure due to ABO subgroup incompatibility can be hazardous, we recommend routinely ABO subgroup typing in the pre-alloHSCT workup to increase donor safety.

A-151

Evaluation of Monocyte Distribution Width as an Early Marker for Diagnosis of Sepsis

J. Park[1], J. Kang[1], Y. Choi[1], H. Choi[2], M. Shin[1]. *[1]Chonnam National University Hwasun Hospital, Hwasun, Korea, Republic of, [2]Chonnam National University Hospital, Gwangju, Korea, Republic of*

Background: Since sepsis has a high mortality rate, early diagnosis and treatment can increase the survival rate and improve prognosis. However, it is difficult to diagnose sepsis accurately in actual clinical practice. Determination of Procalcitonin or C-reactive protein (CRP) levels, which are conventionally used for diagnosing sepsis, are time-consuming. Recent studies have shown that monocyte distribution width (MDW), which is observed simultaneously during the complete blood count (CBC)/diff test in Beckman Coulter DxH900, is a potential marker for the early detection of patients with or developing sepsis. This study evaluated MDW for early detection of sepsis in patients visiting the emergency department. **Methods**: This study enrolled a total of 1,167 patients (>18 and ≤80 years of age) who visited the emergency department of a single institution from August 4, 2020 to March 31, 2021, whose initial evaluation included CBC with differential tests and other blood tests (CRP and Procalcitonin). Samples for CBC and MDW were collected in K3-EDTA tubes and analyzed in a UniCel DxH 900 analyzer (Beckman Coulter, Inc., USA). Sepsis was diagnosed according to the sepsis-3 definition through a medical record review. All patients were grouped into no sepsis, sepsis, and septic shock. The diagnostic performance of MDW and other biomarkers for the detection of sepsis was evaluated through statistical analysis. The subgroup analysis for diagnostic performance was performed in subjects with malignancy or without malignancy. All statistical analyses were carried out using Analyze-it, and $P<0.05$ was considered statistically significant. **Results**: Of the 1167 patients enrolled, 156 patients (13.4%) were diagnosed with sepsis (135 (11.6%) sepsis and 21 (1.8%) septic shock). Among sepsis patients, those with positive culture results were 46.8%, and the rest either had negative culture results (43.6%) or did not proceed with culture testing (9.6%). The MDWs of the no sepsis group, sepsis group, and septic shock group were 19.70, 27.20, and 31.10, respectively, CRPs were 1.82 mg/dL, 8.84 mg/dL, and 20.44 mg/dL, and Procalcitonins were 0.19 ng/mL, 1.72 ng/mL, and 18.20 ng/mL (median, $P<0.0001$). All biomarkers showed a higher value in the sepsis group compared to the no sepsis group. Median MDW showed an increasing trend in patients with malignancy and without malignancy. The AUC of MDW, WBC, CRP, and Procalcitonin for the prediction of sepsis were 0.869, 0.604, 0.773, and 0.868, respectively ($P<0.0001$), thus MDW and Procalcitonin showed similar diagnostic performance. At the cutoff of MDW (21.5), the sensitivity was 91.0% and the negative predictive value (NPV) was 98%. The diagnostic performance of MDW showed similar results in cancer patients and cancer-negative patients (AUC 0.873 vs. 0.869). **Conclusions**: MDW, which is automatically measured in Beckman Coulter DxH900 hematology analyzer without the requirement of additional reagents during CBC/diff test, has high accuracy in detecting patients with sepsis and could be a reliable tool for early detection of patients with sepsis in the emergency department compared to results of culture or other biomarkers which take longer time to get the result. Furthermore, high NPV may help clinicians rule out sepsis.

A-154

Cost-effectiveness of on-site vs. send-out measurement of ADAMTS13 activity in the management of acquired thrombotic thrombocytopenic purpura

P. Toulon[1], A. Sudria[1], I. Harzallah[2]. *[1]Pasteur University Hospital, Nice, France, [2]Emile Müller Regional Hospital, Mulhouse, France*

Background: Thrombotic thrombocytopenic purpura (TTP) is a rare thrombotic microangiopathy (TMA) caused by congenital or acquired severe deficiency of disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13), an enzyme cleaving von Willebrand factor (VWF). ADAMTS-13 deficiency leads to the accumulation of ultra-large VWF multimers, and subsequent platelet aggregation and microthrombi formation. Aside clinical symptoms, the diagnosis of TTP is based thrombocytopenia, hemolytic anemia with the presence of schistocytes, and severely reduced activity of ADAMTS-13 (<10%). As TTP is a life-threatening condition, it is of great importance to shorten the time to the treatment, and according to the French recommendations, the triplet therapy including plasma exchange (PEX), rituximab, caplacizumab, plus corticosteroids, must be administered to patients with suspected TTP until the results of ADAMTS-13 was obtained, and be pursued only in the case of deficiency. Therefore, a rapid ADAMTS-13 testing is critical for early diagnosis and optimal management of acute TTP.

Methods: We compared the cost-effectiveness of a strategy based on on-site measurement of ADAMTS-13 activity using a rapid fully automated chemiluminescent assay (HemosIL AcuStar ADAMTS-13) vs. its centralized measurement in a reference laboratory, as it is currently the case at our institution. For that purpose, we created two scenarios based on either its local measurement with a maximum time-to-result of 16 hours (assay performed during daytime, 7/7) or its centralized measurement with a median time-to-result of 4 days (range: 2-8, as demonstrated from the 2018-2021 estimates). The triplet therapy was hypothesized to be started on admission and stopped if ADAMTS-13 activity was >10%. In case of ADAMTS-13 activity <10%, an inhibitor screening would be performed at the reference laboratory, as this assay was not expected to be locally implemented. For the calculation, we considered the costs of reagent, shipping/handling of samples to the reference laboratory, laboratory staff workload, reimbursement rates, and treatments administered in patients with suspected TTP until availability of the ADAMTS-13 result.

Results: Based on 2018 to 2021 estimates, there was a mean of 60 prescriptions of ADAMTS-13 activity per year. Most were for the follow-up of TTP patients and for patients with non-TTP TMA. Only seven were for the diagnosis of acute TTP, as suggested by the French score, which was confirmed in four cases (mean values). The local measurement of ADAMTS-13 activity was found to be cost-effective with a 16%-reduction of the costs compared to its centralized measurement in those seven patients with suspected TTP during the first 4 days.

Conclusion: Together with its short turnaround time (33 min) and its full automation, our results suggest that the HemosIL AcuStar ADAMTS-13 activity assay could be an assay of choice to rapidly measure ADAMTS-13 activity in the plasma and thus to establish the diagnosis of acute TTP in emergency settings. Considering the local costs, using the strategy based on the local evaluation of ADAMTS-13 activity led to a 16%-reduction of the overall costs during the first 4 days after admission of the seven patients with suspected acute TTP.

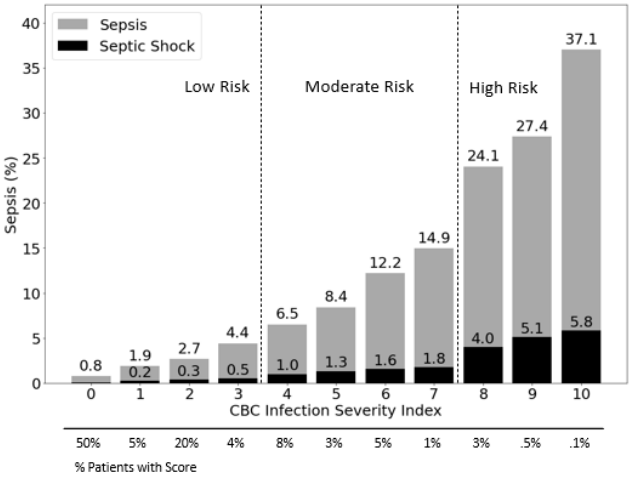
A-155

Unlocking the complete blood count: Derivation of a single-panel laboratory test that includes monocyte distribution width to create a universal sepsis screening tool

J. S. Hinson[1], N. Sarani[2], A. Smith[3], A. Debraine[3], O. S. Badaki-Makun[1], A. Malinovska[1], S. Levin[3]. *[1]Johns Hopkins University School of Medicine, Baltimore, MD, [2]Kansas University Medical Center, Kansas City, MO, [3]Beckman Coulter, Brea, CA*

Background: Sepsis is the leading cause of in-hospital death. Outcome improvement requires rapid detection and early antimicrobial therapy. The complete blood count (CBC) is ordered for more than two-thirds of all patients in the emergency department (ED). Individual CBC components have limited utility for sepsis screening due to low specificity and sensitivity. We sought to create a reliable sepsis screening tool by combining components of the CBC differential. **Methods**: In this single-center retrospective cohort study, all adult patients who had a CBC with differential performed within 6 hours of ED arrival during a 20-month period were eligible for inclusion. Repeat ED visits by the same patient were excluded. Our primary outcome was manifestation of sepsis within 72 hours of ED arrival, detected from timestamped electronic health records using a previously validated electronic phenotype based on Sepsis-3

consensus criteria. Multivariable logistic regression was performed to identify individual components of the CBC differential that were independently associated with our outcome and to identify optimal cutoffs for discretization. Odds ratios were used to assign weights to each component and to derive a 10-point system for scoring likelihood of sepsis. **Results**: 34,864 patients were included; 1306 (3.7%) met sepsis outcome criteria within 72 hours of ED arrival and 181 (0.5%) had septic shock. White blood cell (WBC) count, monocyte distribution width (MDW), and the neutrophil to lymphocyte count ratio were found to predict sepsis. As shown in the figure, there was a linear relationship between our CBC infection severity index and likelihood of developing sepsis and septic shock. Patients with a high risk score (8 or above) had a greater than one in four chance of developing sepsis within 72 hours. **Conclusion**: A simple ten-point index derived from the most commonly used laboratory panel in medicine reliably estimated likelihood of developing sepsis.



A-156

White Blood Cell Measurands, Flagging and Scatter Plot Patterns in Chronic Lymphocytic Leukemia

S. Silva[1], Y. Li[2], Y. Chen[1], M. Ismail[1], S. Musolino[3], I. Talarico[3], D. Cossedu[3], B. Montaruli[3], A. Insana[3], C. Rongey[1]. *[1]Abbott Diagnostic Division, Santa Clara, CA, [2]Abbott Laboratories, Lake Forest, IL, [3]Azienda Ospedaliera Ordine Mauriziano di Torino, Torino, Italy*

Background: In this study, we use Alinity hq hematology analyzer to investigate the differences between white blood cell measurands of a healthy control population versus Chronic Lymphocytic Leukemia (CLL). A repeatability study was also conducted to determine the precision of the white blood cell parameter results on the Alinity hq. In addition, we used Alinity hq to identify unique flagging and patterns in the optical channels for CLL.

Methods: One hundred and fifteen peripheral blood samples of CLL were compared to a population of four hundred and fourteen healthy controls on the Alinity hq analyzer. Statistical differences in Alinity hq's white blood cell measurands compared between the control population and CLL. For the repeatability study, five CLL samples (with sufficient volume) will be tested in 10 replicates for WBC measurands. These samples can be part of the 115 samples above or can be different samples. Comparison of scatterplots were also analyzed between CLL and control samples. Lastly, a peripheral smear was performed on all CLL samples.

Results: Statistical analysis of the means of the white and red cell parameters showed that in comparison to the means of the control population there was a significant statistical ($p < 0.05$) and clinical difference with CLL for the following parameters: absolute white blood cells (WBC) X 103/µL (43.6 CLL vs 7.41 control), percent neutrophils (%N) (18.25 CLL vs 53.71 control), percent lymphocytes (%L) (77.72 CLL vs 33.47 control), and percent monocytes (%M) (2.71 CLL vs 8.75 control). The WBC repeatability study results showed good concordance on the Alinity hq CBC+Diff+Retic test mode with the following percent coefficient of variance (%CV) results: absolute WBC X 103/µL (0.64, 5.31), percent neutrophils (%N) (1.32, 5.57), percent lymphocytes (%L) (0.20, 2.49), and percent monocytes (%M) (3.99, 26.39). As expected, the %CV range for monocytes is wide due to lymphocyte/variant lymphocyte population extending into the monocyte cluster. Evaluation of flagging showed 67 of the 115 (58.26%) CLL samples had a blast flag and 88 out of the 115 (76.52%) CLL samples had a variant lymphocyte flag. Review of the raw data from the optical channels shows a dominant and elongated lymphocyte cluster on the IAS, FL1 and PSS channels versus ALL for the CLL sample group compared to the control population. In the IAS and PSS versus ALL scatter plots in the CLL samples the lymphocyte cluster extends into the monocyte population triggering the blast flag. Peripheral smear review confirmed the presence of CLL (i.e., numerous smudge cells, mature-looking lymphocytes with characteristic condensed "soccer ball" pattern chromatin and scant cytoplasm) in the experimental group.

Conclusion: CLL is a cancer of the blood and bone marrow that predominately affects the quantity, and morphology of lymphocytes. Patients present with an abnormally high relative and variant lymphocyte populations. As such, there are a number of signs found in WBC measurands, flagging, and scatter plots on the Alinity hq that can be used as an early screening tool for CLL.

A-157

Evaluation of Cellular Stability in Bone Marrow for Flow Cytometry Analysis

A. Correa Wengerkievicz Lopes[1], I. Sousa[2], C. Muniz Ribeiro Franzon[2], A. Oliveira Martin Wagner[2]. *[1]Laboratório Médico Santa Luzia DASA, Florianópolis, Brazil, [2]Laboratório Médico Santa Luzia DASA, Florianópolis, Brazil*

BACKGROUND Plasma cells are sensitive and can rapidly deteriorate after sample collection or due to roughhandling. Delayed sample processing remains a common challenge due to logistical limitations.Specialized tests performed in central pathology laboratories are frequently located aconsiderable distance from healthcare providers. According to the Clinical Flow CytometricAnalysis of Neoplastic Hematolymphoid Cells; Approved Guideline-Second Edition, BM samplescollected in EDTA anticoagulant should be analyzed within 12 to 24 hours. The objective of thisstudy was to define the stability of a bone marrow (BM) sample within 48 hours through theevaluation of the main cell populations with a focus on plasma cells. **METHODS** Nineteen bone marrow samples (without the use of preservative medium) were evaluated at 0,24h and 48h, collected in EDTA anticoagulant and stored at 4 °C before processing. To definethe stability of the samples, the MFI (mean fluorescence intensity) was evaluated. The samplepreparation protocol was performed based on the monoclonal gammopathy screening panel attime 0 (CD38 FITC, CD138V500; CD45V450; CD19PECY7, CD56PE, CD27PERCP and KAPPA APCand LAMBDAAPCH7), and at times 24h and 48h it was performed a panel with anchor markers(CD45V450, CD19PEcy7, CD38FITC, CD138V500, CD56PE), acquisition in FACS CANTO II and FACSLyric equipment and data analysis performed with Infinicyt software. To assess the impact ofsample storage and the MFIs of individual markers, positive reference populations wereanalyzed using the antigen CD38 for plasma cells, CD19 for B lymphocytes, CD56 for NK cells andCD45 for other populations. Statistical analysis was performed using the Paired t-test,considering statistically significant results those with $p < 0.05$. **RESULTS** In the present study, the evaluation at 24 and 48 hours showed that the populations ofgranulocytes, B lymphocytes and plasma cells did not present a significant difference betweenthe evaluated times. The monocyte population showed a decrease of $p < 0.05$ in the time 48hours an in the first 24 hours it remains stable. In NK cells, despite $p < 0.05$ between 0-24h and0-48h, it can be observed that between 24h-48h the population remained stable. **CONCLUSION** Evaluating these results, based on the cell type of interest, different delays are acceptable.Despite the greater sensitivity of plasma cells, it was concluded that the evaluation of CD38 asan anchor marker for plasma cells can be used in up to 48 hours without significant change inthe MFI evaluation for analysis of this cell with this marker. The differences found in NK lymphoidcell may be due to methodological problems, considering that the analyzed panel is not ideal forevaluating this population. Therefore, it is necessary to carry out more detailed studies for othertypes of cell populations.

A-158

Evaluation of nucleated red blood cells NRBC) count on Mindray BC 6800Plus

A. Correa Wengerkievicz Lopes[1], A. Siqueira Castanhel[2], C. Muniz Ribeiro Franzon[2], J. Valim Franzen[3]. *[1]Laboratório Médico Santa Luzia DASA, Flórianópolis, Brazil, [2]Laboratório Médico Santa Luzia DASA, Florianópolis, Brazil, [3]Vyttra Diagnosticos, São Paulo, Brazil*

BACKGROUND The detection of NRBCs in the peripheral blood is a marker of many pathological conditions andhas significant prognostic value. Microscopy is the traditional method for NRBC count, but it´stime consuming and has low precision

and efficacy and requires experienced technicians. TheMindray BC-6800 Plus is a hematological analyzer that brings SF Cube technology, whichquantifies NRBCs in the WNB channel. This channel differentiates basophils and NRBCs, andcounts white blood cells. The present study aims to evaluate if the automated method is preciseand comparable to the manual microscopy. **METHODS** Blood samples from 40 patients with NRBC flag from the laboratory routine were evaluated bymicroscopy. Blood films were prepared automatically with a Mindray SC-120 slidemaker/stainer.NRBC were manually counted and reported as number of NRBC per 100 white cells, which wasconsidered the reference method. The results of microscopic counts were compared with thoseof the automated method and statistically analyzed using the F-test, T-test and Pearson'scorrelation coefficient. **RESULTS** The results show good correlation between the methods: F-test of 1.36 (below 1.69); t test(0,2791) below the critical T(2,0210) and Pearson's correlation coefficient was 0.9, which isconsidered an adequate result. Samples used in this evaluation comprised mainly low results andfew samples with higher counts. The maximum observed value was 41%, which was confirmedby microscopy. It is important that samples with larger counts be evaluated to be sure of thelinearity value of the test. **CONCLUSION** The results demonstrated that the NRBC counts obtained by Mindray BC-6800 analyzer are inagreement with data from manual counts. However, it is necessary that the accuracy of sampleswith very high counts be investigated and compared with the reference method. Thisimplementation of the automatic release of NRBC can allow the laboratory to reduce the time forreleasing tests and optimize the productivity of microscopists.

A-159

Role of TCRB1 antibody in the evaluation panel of T lymphoproliferative diseases.

A. Correa Wengerkievicz Lopes[1], C. Muniz Ribeiro Franzon[2], A. Oliveira Martin Wagner[2]. *[1]Laboratório Médico Santa Luzia DASA, Flórianópolis, Brazil, [2]Laboratório Médico Santa Luzia DASA, Florianópolis, Brazil*

Background: The diagnosis of T lymphoproliferative diseases is usually a challenge due to the difficulty indifferentiating pathological T cells from reaction cells and also due to the limitation of clonalitytests currently available. TCRB1 is a monoclonal antibody specific for region 1 of the TCR T cellreceptor Beta chain constant, detecting clonal populations of TCRab positive T cells in caseswhere its expression is monotypic. Its application is similar to immunoglobulin light chainrestriction in the detection of B cell lymphoproliferations and in normal T CD3+ TCRab+ cells, theexpression of this marker is polytypic. The objective of the present work is to report theexperience of including this antibody in the lymphoproliferative T panel in a private laboratoryin southern Brazil. **Methods:** Samples received with indication of lymphoproliferative disease and lymphocytosis wereevaluated using the tube Lymphocyte Screnning Tube - LST (Euroflow) and were acquired usingFACS CANTO or FACS LYRIC cytometers. Analyzes were performed using Infinicyt software. After,all, samples that showed alteration in the CD4/CD8 ratio, or antigen alteration in the expressionof pan T markers were evaluated for the complete investigation panel according to Euroflowprotocol. This panel had the incorporation of the tube for the evaluation of clonality that hasthe TCRB1 antibody in the PE channel associated with the anchor markers CD45V500,CD3PerCpcy5, CD4V450 and CD8APCH7. The channels that were not used (FITC, APC, Pecy7) areused when necessary, to evaluate antigens that can help select the desired aberrant population,such as CD5, CD2, CD7 antibodies or to assess maturation, like CD45RA and CD27. Data werecollected from the beginning of the use of this antibody from February 2021 until the presentmoment. **Results:** During this period, 2870 samples for immunophenotyping analysis were received, of which 17cases were T chronic lymphoproliferative diseases. Of the 17 cases found, 11 (65%) were caseswith the pathological population TCRab positive and the expression of TCRB1 could beevaluated. In these cases, the expression of the patologic cells was monotypic for the TCRB1antigen. The cases were: 4 large granular lymphocytic (LGL) leukemia, 4 Adult T-cellleukemia/lymphoma /Peripheral T-cell lymphoma, unspecified , 1 Sezary syndrome, 1 T-celllymphoblastic lymphoma in cortical/common thymocyte stage and 1 T-cell prolymphocyticleukemia (*T*-PLL). Recent studies have demonstrated the high specificity of this test for clonality,as long as it is associated with an expanded and standardized lymphoproliferative panel. Thepresent cases demonstrated good results for peripheral blood samples, bone marrow and lymphnode biopsy. **Conclusion** T-lymphoproliferative disorders remain a diagnostic challenge, due to the difficulty of a simpletest to assess clonality. The inclusion of the TCRB1 antibody in the lymphoproliferative panelincreased the specificity of the test for diagnosis, being a method of rapid execution and lowcost. Another advantage of using this marker is the possibility of evaluating small cell clonesthat may not be detected in molecular biology techniques due to the sensitivity of the test.

A-160

Factor VIII Chromogenic Low Activity Sample Analysis in a Low Calibration Curve Model

R. F. de Paula[1], G. R. Ramos[2], M. Yanquen[2], C. Borella[3], C. M. Perez[4]. *[1]Siemens Healthineers, São Paulo, Brazil, [2]Hemostasia and Hematology H&H Lab, Bogotá, Colombia, [3]Siemens Healthineers.com, São Paulo, Brazil, [4]Siemens Healthineers, Bogotá, Colombia*

Background: Adequate laboratorial diagnostics of hemophilia is essential and could be done by chromogenic and one-stage assays based on APTT methodologies. Adverse effects of medications became less severe recently, but it remains important to be aware of to ensure that treatment is effective and ensure the best possible conditions for affected individuals. Classification of hemophilia according to FVIII activity: mild 25% - 5%; moderate, 5% - 1% and severe < 1%. For new therapies such as emicizumab and some drugs with an extended half-life, the World Federation of Hemophilia recommends the use of a chromogenic assay of FVIII containing bovine FX for monitoring factor FVIII activity. Objetive: evaluate the applicability of a low calibration curve (LCC) in the chromogenic factor VIII (FVIII-Chr) assay by standardizing the dilution of the standard (calibrator) and thus ensuring the accuracy of the analyses in the lower values like severe hemophilia range. **Methods**: The samples were obtained from H&H LAB SAS, which guarantees all requirements. Sysmex® CS 2100i Siemens Healthineers. Calibration was performed with Standard Human Plasma (SHP), together with FVIII-Chr. Quality Control (QC); the accuracy of the LCC was checked. **Results**: Preparation of the LCC: SHP reconstitution, followed by 1:10 dilution between the SHP and the plasma deficient in FVIII. The curve was named FVIII-Chr Low. QC was performed through pathological control (PC) with 2 dilutions: 1) identical to the formation of the low curve, i.e., dilution 1:10 (2.3 – 3.9%). 2) dilution 1:30 (0.2 - 2%) to accommodate the range of activity comprising severe hemophilia. The values found confirms the curve's quality. **Discussion:** This study sought to develop a way to detect FVIII activities < 1.5% in a reproductive manner, even with a reduced "N" number of samples for severe state. The chromogenic test presents a limit of detection for the optical density of D.O. 0.0080 reporting < 0,3% FVIII-Chr activity, which is very close to the most diluted point of the LCC. **Conclusion:** QC performance with PC 1/10 and 1/30 showed excellent reproducibility. With the protocol FVIII Low and LCC (SHP+PDF) it is possible to obtain results of FVIII-Chr < 1.0% compatible patients with severe hemophilia clinical condition.

A-161

Analytical Performance of Reagents for Determination of Activated Partial Thromboplastin Time: A Sigma Metric Perspective

R. F. de Paula[1], L. Cicarelli[2], R. Fock[2], C. Borella[1], I. Rodrigues[3], C. M. Perez[4]. *[1]Siemens Healthineers, São Paulo, Brazil, [2]Clin. Lab. Div. of the Universitary Hosp. of the Un. of S. Paulo (DLC-HU-USP), São Paulo, Brazil, [3]Siemens Healthineers.com, São Paulo, Brazil, [4]Siemens Healthineers.com, Bogotá, Colombia*

Background: Sigma Metric (SM) assesses the degree of deviation of lab. tests from established specifications, combining Total Acceptable Error (ETa), imprecision and analytical bias. Quality criteria, defined by models of desirable specification, are used to determine the analytical performance of lab. tests. The concepts of biological variation are used as the preferred approach for establishing specifications. Reagents (RE) for the determination of activated partial thromboplastin time (APTT) use thromboplastin obtained from different tissue extracts and activation factors, being standardized to reduce the variability of the tests, and ensure equivalence of clinical diagnosis. This work evaluated three RE for APTT and the fulfillment of the desirable specifications of analytical quality, using the SM as an analysis tool. **Material & Methods:** APTT determinations were performed on the Sysmex®CS-2500 (Siemens Healthineers) with Actin®FS (AFS), Actin®FSL (FSL), and Pathromtin®SL (PSL) RE and bilevel Control Plasma N (CN) and Ci-Trol®2 Control (CI). Two different lots were used for each RE and control (CT), and the results were analyzed in RE-CT combination. We determined (a)analytical bias (Bias), from intra-assay imprecision determined by 30 repetitions in a single analytical run and by comparison with manufacturer's data, and (b)analytical coefficient of variation (CVa), from inter-assay imprecision, by triplicate in 3 periods each 3 hs, for the 2 CT levels. Analytical quality specifications for APTT were selected in the literature, adopting desirable ETa values of 4.5% and minimum of 6.7%, obtained from a database for biological variation and acceptable limit of 15%, based on CLIA-CAP. SM was calculated with formula: Sigma=ETa–Bias/CVa, with graphic representation adapted from the Westgard's model. **Results:** For CVa, AFS and FSL results with CN and CI were <1.0%. PSL presented results above 1.0% for CN and CI. All RE presented results lower than those recommended

by the manufacturer, respectively 4.0% for AFS; 3.0% for FSL; and 2.8% for PSL. For bias, a greater variation was observed for the combinations of RE and CT, ranging from 1.16% to 4.07%, being related to the differences between the averages obtained and the values defined in the leaflets. For the desirable specification of ETa (4.5%), 3 combinations of RE and CT level AFS-CN, FSL-CN and PSL-CI presented Sigma values > 3 (respectively, 3.61, 3.87 and 3.33). When using the minimum specification (6.7%), the PSL-CN combination presented a Sigma=2.06. With the CLIA-CAP specification, all RE and CT combinations had Sigma>6. **Discussion:** All RE showed similar analytical performance when compared by SM, demonstrating that different commercial formulations for APTT determination do not show differences in analytical Bias and CVa determinations. The graphical representation by SM allows you to visually compare analytical imprecision and inaccuracy values. **Conclusion.** All RE showed no differences in analytical performance, with SM being a visual tool for analyzing and comparing RE performance.

Laboratory Management and Patient Safety

A-163

Abnormal HbA1c Results Lead to an Unexpected Chronic Myeloid Leukemia Diagnosis

N. J. Mathewson, L. N. Pearson, K. Doyle. *University of Utah / ARUP Laboratories, Salt Lake City, UT*

Background: Hemoglobin A1c (HbA1c) is used to assess and monitor glycemic control, as it reflects average levels of blood glucose over the previous two to three months. HbA1c can be measured by capillary electrophoresis (CE) which calculates percent HbA1c from the HbA1c and HbA peaks in the electropherogram. CE is a prominent method in reference labs as it provides complete automation with fast separation and less interference than what can be observed with other assay formats. The high-resolution capability of CE allows the quantification of HbA1c, even in the presence of labile HbA1c, carbamylated and acetylated hemoglobins, and major hemoglobin variants. These variants are commonly recognized as causes of interference in HbA1c measurements.

Case presentation: A 70-year-old male with a history of type 2 diabetes mellitus presented to an outpatient laboratory for routine venipuncture to evaluate glycemic management and cardiovascular disease risk. HbA1c analysis by CE resulted with a flag for an atypical profile in the electropherogram and no HbA1c value was reported. HbA1c measurements throughout the past year demonstrated HbA1c values ≥ 6.5% yet trending downward, consistent with his diagnosis and treatment of diabetes mellitus. HbA1c was repeated with a different instrument and fresh specimen collected at a different phlebotomy location and showed similar inconclusive results. Unusual shifts in hemoglobin peak migration times were observed upon close review of the electropherogram, suggesting a potential analytical interference of the HbA1c measurement.

Results: Because atypical peak migration times due to elevated leukocyte counts or increased viscosity are documented by the manufacturer, a follow up complete blood count (CBC) was recommended for this patient. CBC results revealed a highly elevated leukocyte count of 175.80 k/µL (reference interval: 4.30-11.30 k/µL), suggesting the abnormal HbA1c migration profile was possibly due to leukocytosis. The elevated leukocyte count led the patient to visit an oncology clinic where leukemia/lymphoma phenotyping, ancillary studies, and cytogenic testing, confirmed a diagnosis of chronic myeloid leukemia. The diagnosis was completely unexpected, as the patient's only complaint was feeling fatigued over the last few weeks. The patient began chemotherapy treatment immediately.

Conclusion: The present case is an example of how leukocytosis and hyperviscosity can interfere with HbA1c measurement by CE. Although this specific clinical situation is uncommon, clinicians and laboratorians should be aware of these potential interferences, as they have not been discussed in detail in the literature. Further, these interferences warrant recognition in the cancer patient population, as this population may be directly impacted by elevated leukocyte count and hyperviscosity in their specimens, which may impact glycemic control monitoring during treatment. The incidence of diabetes in cancer patients is six times greater than in the general population, making glycemic control monitoring key for cancer patients. Many diabetic patients undergoing chemotherapy will prioritize their cancer treatment over management of their diabetes, which increases their risk for poor glycemic control. These patients should be carefully monitored to ensure HbA1c results are accurate as poor glycemic control can lead to increased risk for infections and hospitalizations, which may decrease survivorship.

A-164

Key Performance Indicators (KPIs) can be polluted by add-on tests

T. Ellison[1], D. Fraser[1], P. Cane[1], D. James[2]. *[1]Synnovis, London, United Kingdom, [2]Synlab, London, United Kingdom*

Background:

Key Performance Indicators (KPIs) are a measurement of service performance which are measured frequently and can demonstrate the clinical effectiveness of services. We undertook a review of a subset of KPIs following feedback from clinicians in the emergency department that was at odds to our reported KPIs. Our reported KPIs suggested that we were not meeting the turnaround times (TATs) required (1 hour from receipt in the laboratory to result availability), however the view of the clinicians was that we were achieving the KPIs needed to manage their patients. We undertook an exercise to quantify the impact of add-on tests to our published KPIs as our laboratory information systems are not able to discriminate tests that are added on at a later time from those that go though as a first pass.

Methods:

We reviewed all data (February 2022) for 4 common tests requested in the Emergency Department at King's College NHS Foundation Hospital: amylase, CRP, paracetamol and high sensitivity troponin. Through a laborious process, we reviewed all records where a test had been added on to compare the turnaround time (TAT) for tests that pass through the first time and those that are added on so that we could quantify the impact of add-ons.

Results:

In the month of February 2022, we processed 754 amylase, 4014 CRP, 121 paracetamol and 1414 high sensitivity troponin tests. The highest proportion of add-on tests was experienced with troponin (9.7%), amylase (9.4%) and paracetamol (8.3%). The lowest percentage of add-on tests was CRP (1.9%). Figures to be presented will demonstrate the impact of add-on tests as compared to those that go through as a first pass yield in whisker box plot format, non-truncated.

Conclusion:

Add-on tests were polluting the KPIs that we were reporting. The service was delivering a TAT that the clinicians required, however our legacy internal reporting systems were not able to discern the date / time of add-on from that of the receipt of the tube into CSR or strip out the add-ons from the KPI reports and hence the negative impact that they have on reporting.

A-165

Test harmonisation is a key enabler of pathology transformation

T. Ellison[1], P. Cane[1], C. MacFarlane[1], D. James[2]. *[1]Synnovis, London, United Kingdom, [2]Synlab, London, United Kingdom*

Background: In April 2021 a new joint venture was formed to deliver and transform services across South East London. Two large NHS Foundation Trusts (Guy's and St Thomas', and King's College hospitals) partnered with SYNLAB who are specialist pathology providers, bringing extensive global experience and expertise in setting up, transforming and running diagnostic services. The Trusts had been working in partnership for many years but were operating in numerous areas as two separate entities. There was duplication of services and tests, with different analytical platforms and very different ways of working, which led to variations in resulting and reporting. Our aim was to establish a single consolidated service, with a harmonised test catalogue, facilitating the provision of consistent results regardless of the origin of sample, and to have a single governance framework to drive improved accountability and optimisation of resources. Our view was that test harmonisation was essential to enable the deployment of a common core Laboratory Information Management System (LIMS) from the 24 legacy systems, and that the harmonisation process should be clinically led. **Method:** We established forums (through a series of workshops) for comprehensive engagement and dialogue to allow key clinical leads from all services to meet and agree the path forward. After extensive collaborative engagement, a new model was created with a single 'Strategic Clinical Lead' with pan-site responsibility appointed for each service from the existing senior clinical staff. The Strategic Clinical Lead works with their colleagues to drive consensus, seeking wide stakeholder engagement from end service users as required. **Results:** From an initial repertoire of circa 7,100 tests, test harmonisation has resulted in a 25% reduction, and in some services such as Virology, we identified 31% duplication in tests. The establishment of the new clinical model has resulted in improved efficiency and better use of resources including staffing as well as a reduction of variation of services. Importantly, test harmonisation is essential to move samples seamlessly in a hub and spoke model. The implementation of a single common core LIMS this year will significantly improve business continu-

ity by facilitating seamless transfer of work between sites. **Conclusion:** Extensive clinical engagement is key to transforming pathology services. The benefits of consolidation include an overall reduction in process variation leading to improved standardisation and therefore enhanced patient safety as a result of consistency in result reporting. We established a framework and new clinical model which has resulted in improved links between staff across all sites who are working collaboratively on the new target operating model (hub and spokes) which will be live in April 2024.

A-166

Severity of Harm Category Designation Survey Results

L. Peltier[1], S. Van Aelst[2], B. Peeters[2], J. Raimbourg[3], J. Yundt-Pacheco[4]. *[1]Hopital Pontchaillou, Rennes, France, [2]Heilig Hart ziekenhuis, Lier, Belgium, [3]Bio-Rad Laboratories, Marnes-la-Coquette, France, [4]Bio-Rad Laboratories, Irvine, CA*

Background: ISO document 14971-Application of Risk Management to Medical Devices and CLSI document EP23-Laboratory Quality Control Based on Risk Management use a Severity of Harm model to designate maximum acceptable probability of patient harm (risk) based on the severity of the consequence to the patient.

When designing a risk managed quality control program for the clinical diagnostic laboratory, the starting point is to select Severity of Harm categories for each analyte being tested. As each Severity of Harm Category (Negligible,Minor,Serious,Critical,Catastrophic) can be mapped to a corresponding maximum acceptable probability of patient harm from erroneous results (0.01,0.001,0.0001,0.00001,0.000001). The laboratory can then design quality control strategies that have a predicted probability of patient harm below this maximum for a risk managed quality control program.

Unfortunately, there is little guidance on setting Severity of Harm designations, and they are by nature, subjective. To remedy this, we conducted a survey of laboratory professionals, asking them to rate the Severity of Harm for 20 analytes.

Method: An international Severity of Harm survey was conducted to solicit Severity of Harm designations for analytes in the clinical diagnostic laboratory. 261 respondents from 42 countries answered the question: "How would you rate the severity of patient harm if a clinician would interpret incorrect reported results from these analytes in the most unfavorable situation?".

The available ratings were described as:

Negligible: Inconvenience or temporary discomfort

Minor: Temporary injury or impairment not requiring professional medical intervention

Serious: Injury or impairment requiring professional medical intervention

Critical: Permanent impairment or life-threatening injury

Catastrophic: Patient death.

Results:

Analyte Type	Analyte	Severity of Harm Designation
Clinical Chemistry	Sodium	Serious
Clinical Chemistry	Potassium	Catastrophic
Clinical Chemistry	Troponin	Catastrophic
Clinical Chemistry	Glucose	Critical
Clinical Chemistry	Creatinine	Serious
Clinical Chemistry	Phosphorus	Serious
Clinical Chemistry	ALT	Serious
Clinical Chemistry	Lipase	Serious
Clinical Chemistry	Total Protein	Serious
Clinical Chemistry	Iron	Minor
Clinical Chemistry	Albumin	Minor
Clinical Chemistry	TSH	Serious
Clinical Chemistry	CA 19-9	Serious
Clinical Chemistry	hCG	Serious
Pharmacology	Digoxin	Critical
Coagulation	D-dimer	Critical
Coagulation	Fibrinogen	Serious
Coagulation	Prothrombin Time	Critical
Hematology	Hemoglobin	Critical
Hematology	Platelet	Critical

Conclusion: While Severity of Harm designations are specific to the local clinical system, guidance can be provided by collecting the opinion of a wide variety of clinical laboratory professionals. This provides support for the clinical diagnostic community in building risk managed quality control programs.

A-167

Repeatedly Positive Serum and Urine Beta-HCG Results in Non-Pregnant Patients - Application of Clinical Decision Support

M. R. Ianosi-Irimie[1], D. Nikolic[2], T. Bocker Edmonston[2]. *[1]Cooper University Health Care, Camden, NJ, [2]Cooper University HealthCare, Camden, NJ*

Background: In January of 2020, a 48-year-old woman, status post bilateral tubal ligation was postponed colonoscopy due to a positive urine and serum pregnancy point of care (POC) test. The quantitative serum HCG levels were normal at 4.9 mIU/mL (reference range 0-8 mIU/mL). Follow-up showed that over the prior year there were other 6 similar incidents when nonpregnant patients showed positive qualitative pregnancy results with quantitative HCG levels within "nonpregnancy" normal range being brought to the attention of the laboratory.

Methods: We assessed the possibility of a detection/measurement error including possible interfering antibodies. The likelihood of interfering substances/antibodies that would interact with 2 different types of POC detection systems (urine qualitative - Clinitest HCG Pregnancy test from Siemens Medical Solutions USA, serum qualitative - Pregnancy HCG Rapid Test from Cardinal Health) and serum quantitative - is very low, especially given that both serum and urine specimens were affected, and interfering antibodies are not normally excreted into urine. Testing of the diluted sample showed results like the neat specimen which further excludes interferences in the quantitative reaction as well as a possible hook effect. Based on this we concluded that the results were accurate.Chart review showed that 6 months prior, she had another quantitative HCG test performed using the same instrumentation obtaining a value of 6.6 mIU/mL. At this time a transvaginal ultrasound excluded the presence of any masses or abnormal findings. FSH (47.8 mIU/ml) and LH (49.8 mIU/ml) were found to be within the perimenopausal range. Qualitative serum and urine test were found to be repeatedly positive, while the quantitative tests were within nonpregnancy range, over several months. Retrospective chart review of the other 6 cases revealed similar pattern. Pregnancy**,** certain tumors (gestational trophoblastic neoplasia, ovarian germ cell tumor), kidney failure and menopause will manifest with increased HCG production. In (peri)menopausal women the pituitary produces HCG up to 14 mIU/mL in association with increased levels of FSH. The qualitative tests were repeatedly positive over several months while the quantitative results remained unchanged, excluding the diagnosis of pregnancy or tumors, and supporting the conclusion that these results are menopause related.

Results: To avoid similar future incidents we added the following comment to the POC test results: "If the result does not fit the clinical picture, please repeat testing on a new sample collected 48 - 72 hours later or order serum HCG quantitative" We also added for the quantitative HCG result, the following comment: "Peri- and postmenopausal patients may have increased HCG of pituitary origin up to 14 mIU/mL. If the result does not fit the clinical picture, FSH measurement can be used for additional information." During the 12 months preceding the implementation of the comments we had 6 incidents of positive qualitative HCG results in non-pregnant (peri)menopausal patients brought for investigation, and none during the 24 months after that.

Conclusion: The addition of the interpretative comment as clinical decision support increased the efficiency of the medical service, staff engagement and most importantly, patient satisfaction and safety.

A-169

Addressing Opportunities and Misconceptions of ASCP BOC International Credentials in the Philippines

J. B. Baker[1], A. Spiczka[2], C. Gebhart[3]. *[1]American Society for Clinical Pathology, Chicago, IL, [2]American Society for Clinical Pathology, Board of Certification, Chicago, IL, [3]LifeLink Transplantation Immunology Lab, Tampa, FL*

Background: The objective of this study is to identify the positive viewpoints while clarifying and mitigating misconceptions Filipino applicants have about the BOC's international credential (ASCP^i) and the credential maintenance program (CMP). With a total of 13,678 Medical Laboratory Scientist MLS(ASCP^i) credential holders, the Philippines is the BOC's highest volume MLS(ASCP^i) credential market. However, despite the Philippines being the BOC's largest international credential market, the BOC only certifies about a third of eligible Filipino graduates each year. Therefore, to encourage additional interest in achieving BOC credentials and to eliminate misunderstanding surrounding CMP, the BOC International Credentialing Committee (ICC) created and deployed a survey in December 2022. This survey was intended to reach ASCP^i credential holders worldwide. One outcome of the survey was to gain insights about the MLS credential market in the Philippines, as well as determine what professional development opportunities Filipino's were afforded after they earned their credential. The resulting report (based on analysis of the survey results) showed there are several opportunities and issues affecting Filipino participation in the BOC's credential and CMP offerings. **Methods:** 1,460 individuals from 74 countries of education participated in the ICC-developed survey. This study is a focused summary of results from the 556 Filipino respondents (only). **Results:** This study confirmed a number of assertions the BOC had about the Filipino credential market. The most important of these assertions is MLS(ASCP^i) certification is appealing to Philippine-educated individuals due to its portability (i.e., work eligibility, emigration, etc.). The study also found that: --35% of respondents found employment outside of the Philippines --51% of those who found employment outside of the Philippines indicated their BOC credential was the reason they were hired --96% of respondents indicated BOC credentials are popular in the Philippines --30% of respondents indicated they do not participate in CMP --64% of respondents indicated when they attend a conference in-person it is primarily to earn additional CE --55% of respondents indicated the BOC Facebook page is what convinced them to apply for a BOC credential --518 out of 556 respondents use social media—91% use Facebook, 66% use Instagram, 60% use YouTube, 36% use LinkedIn, 20% use WhatsApp, 31% use Twitter, and 30% use TikTok **Conclusion:** The BOC should continue leaning into the portability of MLS(ASCP^i) certification. However, the BOC needs to further promote other credentials for which Filipinos may be eligible. The BOC should also place greater emphasis on educating Filipinos regarding the importance of CMP and on what can be used for continuing education (CE) towards CMP. For example, our research provided insights that the BOC needs to create a social media outreach campaign that helps Filipino credential holders understand CE earned for their Professional Regulation Commission requirements, and other professional or skill-development courses, may also be used for CMP. Finally, considering the popularity of BOC credentials in the Philippines, the BOC should develop and optimize content that focuses on promoting the usefulness of pursing an advanced credential, the portability of credentials, and the overall demystification of CMP.

A-170

Investigation of High Rate of Low Positive Quantiferon-TB Gold Plus Results

A. Mehreen[1], K. Isidan[1], D. B. Patel[1], K. Jones[2], M. Laugesen[2], H. Lee[1]. *[1]NorthShore University HealthSystem, Evanston, IL, [2]ACL Laboratories, Rosemont, IL*

Background: Tuberculosis (TB) is a communicable disease, transmitted almost exclusively by cough aerosols carrying pathogens of the M. tuberculosis complex. TB continues to be a major public health threat, causing an estimated 10.4 million new cases and 1.3 million deaths from TB globally in 2016. Many more people may have latent tuberculosis infection (LTBI), which may go undetected without further laboratory investigation. The Quantiferon-TB Gold Plus (QFT-Plus) is an Interferon Gamma Release Assay (IGRA) for the identification of LTBI. It detects the cellular immune response to antigens of M. tuberculosis and represents an indirect marker of M. tuberculosis exposure. In January of 2021, the NorthShore University HealthSystem Core Laboratory transitioned from a reference laboratory ELISA-based QFT-Plus test to an in-house DiaSorin LIAISON® QuantiFERON®-TB Gold Plus test. After implementation, we noticed a higher rate of low positive QFT-Plus results (1.3-5.4%) in our patient population that we regard as a mixed risk population. The expected percent of low positive results in a mixed risk population is 2-3%.

Objective: To determine the potential causes of a higher rate of low positive Quantiferon-TB Gold Plus Results.

Methods: Forty specimens with Antigen-Nil results between 0.35-1.00 IU/mL using LIAISON® QuantiFERON®-TB Gold Plus reagent from 8/2022-10/2022 were collected for this study. All forty specimens were re-tested using ELISA-based methodology. Patient demographics and medical history were reviewed for any factor that may be responsible for the low positive results.

Results: Results from the two different methodologies were compared: 28/40 (70%) specimens showed positive results on the DiaSorin LIAISON®-XL platform, but negative results on the ELISA platform. Review of medical history showed that 92% of the sample population had received more than 2 doses of COVID-19 vaccine and 12.5 % had previous COVID-19 infection. 30% had an underlying autoimmune disease, with psoriasis and rheumatoid arthritis being the most common. However, no correlation was found between medical or vaccination history and the QFT-Plus results.

Conclusion: LIAISON® QuantiFERON®-TB Gold Plus is a Chemiluminescence immunoassay which is more sensitive than ELISA, resulting in an increased number of low positive QFT-Plus results. Physicians need to correlate clinical findings and TB exposure history to the QFT-Plus results when making clinical decisions.

A-174

More Timely Patient Care Amidst Healthcare Staffing Shortages, Reducing Blood Specimen Tube Barcode Errors for Continuous Flow in an Automated Laboratory System

J. R. copeland, J. Guerriero, B. C. Cook. *Henry Ford Health, Detroit, MI*

Background: The Core Laboratory at Henry Ford Hospital utilizes total laboratory automation to support chemistry, hematology and coagulation analyzers, processing ~10,000 specimens daily. Testing and reporting delays can occur when the system encounters a specimen tube barcode error. Specimens will stop moving and causes approximately a 2-minute per error production pause while the error is resolved. This induces congestion for all other specimens in line behind the error. The seemingly trivial minutes of congestion leads to a domino effect, resulting in reduced line productivity, a temporary limit of testing capacity and prolonged TATs. The laboratory receives specimen tubes with labels generated from over 600 printers. The system encounters 200-250 errors daily associated with barcode read failures of various types. Errors account for an estimated loss of ~320 hours of staff productivity per month.

Methods: Automation line error files, manually recorded data from lab staff, observations of automation line errors occurring in real time, data from a printer maintenance blitz and barcode printer service requests were used in root cause analysis. Three progressive Kaizens events were organized in 2022 to induce rapid process change through focused chartering of events and actions. A probing study designed to induce controlled defects and stoppages helped recognize where congestion on the line was occurring. Simulation of barcode application errors allowed an understanding of the impact of label application. Staff and line productivity loss analysis predicted the potential opportunity of error reduction.

Results: Fifty-five percent of the barcode errors were caused by improper label application and 45% were poor print quality of the barcode on the label. Of the label application defects, 55% were caused by the label being placed upside-down, of which

34% were caused by the label format used by our Emergency Department. Print quality issues such as faded or pitted printing labels accounted for 45% of errors. The printer preventive maintenance blitz successfully reduced print quality errors by 30%. Converting to a more robust printer in two high-volume areas resulted in zero print quality errors over three months. Barcode label format changes were 100% effective in eliminating upside-down as a root cause.

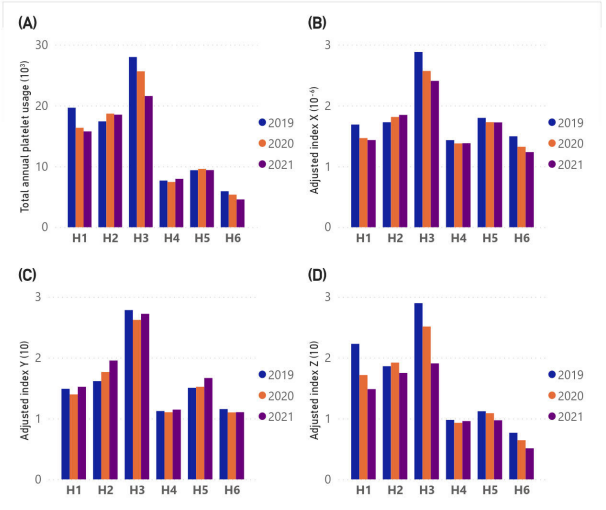
Conclusion: Elimination of barcode line errors can recover 2.0 FTEs in three divisions per month, while increasing automation line productivity and a cost ~$150,000 per year in work delays and unnecessary rework. The Kaizen approach to problem-solving allowed for rapid brainstorming amongst multiple teams. Altered label format allowed built-in engineering control by mistake-proofing the process and making label positioning irrelevant. Identification of areas where high volumes of labels were being printed and replacing those printers with more robust printers reduced service calls and eliminated printer causal factors since these printers require less hands-on maintenance. Finally, instituting preventative label printer maintenance and total quality management reduces or prevents errors associated with print quality. Sustaining mechanisms include continued real-time data documentation and communication of defects, collaboration with colleagues in Nursing for further root cause analysis of barcode application defects.

A-175

Objective indexes for comparing platelet usage among peer hospitals during the COVID-19 pandemic

Y. TU[1], Y. Hsueh[2], Y. Cheng[1], T. Lin[2], T. Chiueh[2]. *[1]Department of Laboratory Medicine, New Taipei Municipal Tu Cheng Hospital (Built and Operated by Chang Gung Medical Foundation), New Taipei City, Taiwan, [2]Department of Laboratory Medicine, Lin-Kou Chang Gung Memorial Hospital, Taoyuan, Taiwan*

Background: Besides hospital size, clinical diagnosis and severity of patients' cases determine the total platelet transfusions of each hospital. Therefore, the appropriateness of platelet usage could not be simply compared with the total units of platelet usage among hospitals of different sizes. This study aimed to establish objective indexes that can appropriately reflect the differences in platelet usage in various hospitals. **Methods:** We used three objective indexes X, Y, and Z to compare the appropriateness of platelet transfusions among different hospitals. Instead of considering various factors such as hospital size, patient population, and disease diagnosis individually, three indexes were generated by dividing the annual total units of platelet usage by total annual reimbursement, the total number of admissions, and the average total reimbursement per admission for each hospital, respectively. **Results:** Total units of platelet usage were only directly related to the sizes of hospitals. New indexes X and Y alleviated the hospital size-dependent difference. Index Y was considered a better option than index X, as its value fluctuated less during the COVID-19 pandemic. Index Z was adjusted with the average total reimbursement per admission, and its results showed that more patients of higher disease complexity did not result in more platelet usage during the COVID-19 pandemic. **Conclusion:** These three objective indexes are suitable for peer comparison and monitoring the platelet usage of hospitals irrespective of their size.



A-176

Barriers and Enablers to Laboratory Stewardship Across a 16 Hospital Regional System

C. R. McCudden[1], J. Brehaut[2], N. McCleary[2]. *[1]University of Ottawa, Ottawa, ON, Canada, [2]Ottawa Hospital Research Institute, Ottawa, ON, Canada*

Background: Evidence indicates substantial inappropriate and inefficient use of resources in laboratory medicine. Commonly-used approaches that can be effective in improving practice include education, changes to processes or systems, and audit & feedback. The overall aims of this project were to survey the experiences of individuals implementing laboratory stewardship activities across a regional lab system, focusing on identifying key enablers and barrier to success.

Methods: We used a mixed methods approach beginning with a questionnaire to gather brief details about implementation efforts (what was done, how interventions were implemented, when, and by whom). These preliminary findings served as a precursor to conducting focus groups with implementation teams at each of the sites. We probed the implementation process, drivers of success, and barriers faced. Focus group transcripts were analyzed using thematic analysis.

Results: Forty individuals took part in the surveys and focus groups. Results revealed a range of progress with lab stewardship initiatives across sites, with a variety of initiatives having been implemented. A wide range of factors impacting progress with laboratory stewardship, both negatively and positively, were discussed. These were grouped into four broad topics: 1) Roles, responsibilities, and relationships - the 'Who'; 2) Generating interest in stewardship - the 'Why'; 3) Implementation strategies and processes - the 'What'; 4) Project management and resources - the 'How'. Key barriers identified included: lack of a clear vision which reduces relative priority, lack of resources, emphasis on financial outcomes, and that over-use problems have complex causes. Key enablers identified included active participation of hospital clinical and administrative champions, ability to implement system level changes (e.g. changing electronic orders and processes), focusing on patient safety outcomes, using established change methodologies which support systematic identification of root causes to help select appropriate change strategies as well as formal evaluation.

Conclusion: While some sites progressed with stewardship initiatives with relative ease, most faced challenges along the way. Surfacing these challenges supports the development of strategies to help address them such that lab stewardship activities can have maximal impact in improving quality of care. Structured and systematic approaches to laboratory stewardship are more likely to be successful than those without designed root cause analysis with targeted interventions and clear outcome metrics.

A-178

Reduced Hemolysis with a Novel Capillary Collection System as Compared with Conventional Capillary Collection Devices

M. Parikh, J. Berube. *BD, Franklin Lakes, NJ*

Background. Capillary blood collection is often cumbersome and lacks consistency in the applied pressure during squeezing of the finger. This may result in poor sample quality (i.e., hemolysis and dilution of the sample with interstitial fluid). Standardizing the capillary collection process and thereby making it less technique dependent can enable diagnostic blood testing to be performed in non-traditional settings (e.g., retail pharmacy). A novel capillary blood collection system has been developed to help standardize the process and may enable blood sampling by non-traditional healthcare workers (e.g., pharmacists, pharmacy technicians). Multiple studies were performed to evaluate the performance of this capillary blood collection system on sample quality in comparison with currently marketed capillary and venous blood collection devices. Sample quality was assessed by visual observation of hemolysis and by quantitative assessment of Plasma Free Hemoglobin (PFH). **Methods.** Participants were enrolled at three sites representative of the intended use environment (i.e., retail pharmacy site or patient service center). Phlebotomists performed venipuncture using a currently marketed venous comparator and conventional capillary collection using a capillary comparator. Capillary collection was also performed using the novel capillary device in the same subjects by targeted trained healthcare workers (e.g., retail pharmacists, pharmacy technicians). Blood was collected using each method (venous and two capillary–conventional and novel capillary devices) into serum separator tubes from the same subject within the same timeframe. Data from the studies were pooled in a meta-analysis to assess device performance across multiple studies. Visual assessment of hemolysis was performed post centrifugation using a rating scale (0=None, 1=Trace, 2=Moderate, 3=Gross). For PFH measurement, a cut-off of >50 mg/dL was selected, as hemolysis interference may be observed at these levels in highly sensitive analytes. **Results.** When compared with the venous comparator, the

incidences of hemolysis, as assessed by visual hemolysis and PFH measurements, were higher with capillary collections. However, the incidences were significantly lower with the novel capillary collection system than with the capillary comparator. The overall incidence of visual hemolysis was significantly lower for the new capillary device when compared to the capillary comparator ("None" rating of 94% vs 64.5%). The frequency of trace, moderate and gross hemolysis was also less in the new capillary device when compared to the capillary comparator (4.4% vs 23.2%, 1.2% vs 9.8%, 0.4% vs 2.5%, respectively). For the quantitative measurement of PFH as an indicator of hemolysis, a similar trend was observed. The mean PFH value in the new capillary device was significantly lower than in the capillary comparator (29.87 vs 55.18), and the frequency of PFH >50 mg/dL was also significantly lower in the new capillary device than in the capillary comparator (11.7% vs 42.0%). **Conclusions.** Improved sample quality was demonstrated with a novel capillary blood collection system, as the incidences of hemolysis, as measured visually and quantitatively as PFH measurement, were significantly lower than in conventional capillary blood collection devices. Consequently, this may result in fewer rejected samples in the lab than conventional capillary methods and help meet the demands for more convenient, patient-centric healthcare services.

A-179

Implementation of an Electronic Competency Evaluation System for Point of Care Testing

N. Sanchez[1], V. Coughlin[1], A. Okulewicz[1], C. Urena[1], M. Mauriello[1], D. Chen[2]. *[1]NYU Langone Health, New York, NY, [2]Dept. of Pathology; NYU School of Medicine, New York, NY*

Background Management of training and competency evaluation for point of care testing personnel is essential to hospital operations. Typically, documentation for training and competency of point of care operators is a paper based manual process and managed by the POCT team. The responsibilities include maintaining these records for the duration of employment and thereafter and retrieving records for regulatory inspections. It is a challenge for any POCT team in a hospital system with a high volume of operators. In an effort to streamline this process, an electronic training and competency format for the operators of point of care glucose meter was developed and implemented in our hospital system. **Method** Initial training and competency requires in person delivery which must capture specific elements of competency and must be traceable to the trainer and trainee. Leveraging a survey based online training platform (Qualtrics) as the repository of the training record, the paper based training checklist was converted to an online format accessed via QR (Quick Response) code. Delivery of the training is performed by a certified trainer or preceptor under observation. The training checklist and competency quiz are documented directly in Qualtrics capturing all of the data required for traceability. The evaluation record of each new trainee is available to the POCT Team in real time. **Results** The online format of initial training and competency was validated by comparing the paper based version to the electronic version. Functionality of the electronic version was confirmed and accuracy of the checklist answers were validated. This electronic format was implemented for initial operator training and evaluation in January 2023. Over 150 trainees have successfully completed the electronic training checklist and competency quiz. **Discussion and Conclusion** The implementation of an electronic evaluation tool for operators of point of care blood glucose testing has streamlined the process of documentation management in a secure, reliable and transparent way. It also helps to standardize the training process and minimize errors related to a manual process. These documents are readily available and can be retrieved upon request. The implementation of the electronic training evaluation system through Qualtrics coupled with our existing electronic re-certification process will allow us to transition to a fully paperless evaluation/documentation workflow.

A-180

A Retrospective Review of Syphilis Serology Interpretation Accuracy

M. Forsythe[1], H. K. Lee[2], R. C. Benirschke[2]. *[1]University of Chicago, Chicago, IL, [2]NorthShore University HealthSystem, Evanston, IL*

Background:

Syphilis is a sexually transmitted disease caused by the spirochaete bacterium *Treponema pallidum.* It is acquired through direct contact, is highly infectious and can survive in an untreated host for years. Multiple tests are available for diagnosis and broadly classified as treponemal and nontreponemal. Treponemal tests are used to detect *T. pallidum* particles and antibodies and the nontreponemal tests include the rapid plasma regain (RPR). Due to this complexity, interpreting syphilis serology tests are a common cause of questions to clinical consultants and have the potential for incorrect diagnosis. Because of this we initiated a retrospective review of past positive serology results to assess provider accuracy in interpreting these tests.

Methods:

The study cohort was composed of 108 patients from July - December 2021 that had at least one positive serology test. The assays used were the Syphilis Total Antibodies and RPR assays and both were performed in-house. The clinical history of each patient was reviewed to obtain relevant data pertaining to the diagnosis. This information included the age, gender, additional send out confirmatory testing, previous history of syphilis, provider type and specialty who ordered the tests. The interpretation by the clinicians was also determined and whether they prescribed antibiotics. Statistical analysis was performed to assess what patient and provider characteristics were associated with a correct or incorrect interpretation of the syphilis test results. This study was approved by the institutional IRB.

Results:

The study cohort showed that 12/108 (11.1%) positive results were interpreted incorrectly. The comparison of correct vs. incorrect interpretation was insignificant across most of the variables, including age, gender, diagnosis and provider type. However, in relation to provider specialty the comparison was significant with a p-value of 0.003. This was due to a high percentage of incorrect interpretations obtained from family medicine providers, reaching roughly 50% of total incorrect interpretations. Empirically, questions to the Pathology laboratory concerning syphilis results interpretation have also been observed more often among the family medicine providers. Additionally, providers from infectious disease, internal medicine and OB/GYN composed 54.3% of the providers in our study and only had one interpretation error indicating very high accuracy.

Conclusion:

Overall, the study demonstrated a significant number of incorrectly interpreted syphilis results, with the majority occurring among the family medicine providers. Assistance with interpretation of syphilis results is one of the most frequently asked questions received at the Pathology laboratory. Appropriate education, in particular for the family medicine providers, will assist in addressing this issue, as well as implementing additional resources to the electronic medical record that can be linked to the reported syphilis results. The distinction of a true positive diagnosis from a false one is important for proper patient care, preventing the treatment of patients who don't need it and allowing for the timely treatment of those who do.

A-181

Comparison of POCT accreditation between TJC and CAP: Which way to go?

J. Lee[1], J. Palmer[1], S. Stoeger[1], A. Quinn[1], M. Rudolph[1], K. Brohmer[1], D. Yang[2], K. Galior[2]. *[1]UWHealth, Madison, WI, [2]UW Madison, Madison, WI*

Background: Point-of-care testing (POCT) at UW Health University Hospital is accredited by the Joint Commission (TJC) while the main laboratory is accredited by the College of American Pathologists (CAP). Consolidating to a single accrediting agency would eliminate the burden of maintaining two separate accreditation programs, and reduce related expenses. In consideration of consolidation, we compared the accreditation requirements between the two agencies for POCT. **Methods:** CAP standards in the All Common (n=84), POC (n=62), and Lab General checklists (n=98) were evaluated for non-waived testing requirements in accordance to the POC test menu. The POC test menu includes the following non-waived tests: activated clotting time, blood gases, urine specific gravity by refractometry, and Tzanck smear; and provider performed microscopy (PPM): wet mount (skin or hair), wet prep, and fern test. A gap analysis was conducted to distinguish differences between CAP and TJC standards and further investigation was initiated when needed. **Results:** The majority of CAP checklist standards aligned with the current processes under TJC. From the CAP All Common checklist, 5 out of 84 standards flagged as a potential gap. One notable difference was found for CAP **COM.04250** *Comparability of Instruments and Methods - Nonwaived testing* where comparison studies are required only for instruments and methods under the same CAP number. In contrast, the TJC requires instrument and method comparisons across the entire organization, regardless of separate CLIA certificates or sites. Shifting POC nonwaived tests to CAP would eliminate the need to perform comparisons across different sites, which in our hands has been labor intensive and of little clinical value. From CAP POC checklist, 10 out of 62 standards flagged as potential gaps. Specifically, CAP **POC.03800** *Problem Resolution* where the ability to provide prompt resolution for POC issues for all shifts in which POC testing is performed is demonstrated. From the CAP Lab General checklist, 4 out of 98 standards flagged as a potential gap. Specifically, CAP **GEN.40470** *Specimen Collection Training* where additional discussion with nursing education would

be needed to assess collection training records. The other key difference was with CAP **GEN.23584** *Interim Self-Inspection* where self-inspection is required during the interim year. **Conclusion:** Overall, our assessment is that moving non-waived POC testing to CAP will align the requirements for this set of largely hospital based POC tests, where the considerable effort saved from eliminating organization wide instrument/method comparisons could be more effectively applied to expanding our existing CAP mandated interim self-inspections to include nonwaived POCT. Because the vast majority of the waived testing performed at our organization occurs at ambulatory sites under CMS oversight, leaving waived POC tests under TJC oversight was felt to align better. Overall, the POCT requirements under CAP checklists were similar to TJC with minor, but potentially impactful, differences. In our situation, moving nonwaived POC testing to CAP accreditation and keeping waived POC testing under TJC accreditation provided the best balance between quality/patient safety and regulatory administrative effort.

A-182

The journey from conceptualizing, designing, and implementing our Integrated Smart Core Lab at Kokilaben Dhirubhai Ambani hospital (KDAH) & Medical Research Institute, Mumbai, India

V. Vadera[1], S. Singhal[2], A. Manjure[3], B. Das[2]. *[1]Kokilaben Dhirubhai Ambani Hospital (KDAH) & Medical Research Institute, Mumbai, India, [2]Kokilaben Dhirubhai Ambani Hospital (KDAH) & Medical Research Institute, MUMBAI, India, [3]Roche Diagnostics India Ltd, Mumbai, India*

Background

KDAH established in 2009, is a 550 bedded tertiary care, multispecialty hospital in Mumbai, India. Annually the hospital manages about 300,000 patients, the clinical lab delivers >1,750,000 results with Compound Annual Growth Rate of 18 %

In the last 14 years, the clinical lab has expanded; increasing the test services, adding equipment, recruiting staff, and requesting for more space

There is increasing pressure to improve speed of service to impact early patient outcomes keeping an awareness on cost, non-availability of space for expansion. There is also demand to bring in new clinical tests and increase outreach beyond the hospital

In October 2018, the laboratory and hospital management decided to apply principles of Lean and design thinking to laboratory processes

The Smart Core Laboratory conceptualized was an outcome of this work and is focused on delivering results in the most efficient way, in terms of quality, cost, and speed

Methods

The team of experts from various lab disciplines and lean consultants, started evaluating the end-to-end process from sample received at the central station to results review, reporting and sample disposal

1. Process Map: The process steps are classified and evaluated by the core team as value added (VA) and non-value added (NVA) & business-value added or necessary waste

Process Maps charts were generated for the following sample flow

a. Central Sample Receipt to Section. Verification, Registration & Distribution Process.

b. In Lab preanalytical, analytical, post analytical processes:

Clinical Chemistry, Immunoassay, Infectious Serology. (Serum, Plasma Fluoride / Heparin)

Hematology (EDTA Whole Blood)Hemostasis (Citrate Plasma)

1. Value Stream Map

A detailed Value Stream Map of all the actions for the above processes was generated to determine VA and NVA process steps

3. Spaghetti Mapping: Motion Study

Observation and analysis of the Spaghetti mapping diagrams exposed inefficient layouts and identified areas with a lot of unnecessary movement. This helped to decide optimization of the flow and re-organization of the entire lab section

Results & Discussion

Based on the analysis of the data generated from the lean tools and identifying process waste and non-value added steps the following immediate improvements were implemented

a. Combining steps extra-processing at centrifugation at each lab section

b. Decreasing sample vacutainers by reducing number of sample draws

c. Eliminating Overproduction by removing extra barcode printing and registers

d. Using Staff Creatively: Reorganize IQC, Calibration and maintenance to off peak hours

e. Space Optimization

Conclusion This consolidation approach has enabled us to free up at least 341 sq ft prime lab space (≈14 % reduction), decrease staff motion & sample transportation. Approximately 50% simplification in overall processes is achieved. It also allowed reduction in additional patient sample draws by at least 40 %.

The team could use this lean analysis to conceptualize and design a multidisciplinary Automation solution that would consolidate 5 sections namely Clinical Chemistry, Immunoassay, Infectious Serology, Hematology and Hemostasis in the Smart Core Laboratory.

A-183

Improving Turnaround Time of Critical Value Reporting by Reducing Redundant Workflow

F. Chuang. *Chiayi Chang Gung Memorial Hospital, Puzi City, Chiayi County, Taiwan*

Background:

Most laboratories and hospital patient safety committee define the critical value parameter together, and laboratory should notice the doctor when critical value reporting. “Critical value” as a result refers to the patient may be life-threatening or in imminent danger and require immediate treatment by a doctor. Especially, Troponin-I result is greatly affect door to balloon time. As soon as the critical value reporting, the patient will get therapy earlier. For patient safety, we implemented some methods to reduce the turnaround time of critical value reporting.

Methods:

(1) Shorten the distance between specimen receiver and testing operator from 2 meter to 0 meter. Specimens are analyzed sooner due to reduced unnecessary walk. (2) Assess the difference of results between first and second exam, there are no significant difference. Reducing unnecessary repeat test to report critical value as soon as possible. (3) Reading barcode by both clockwise and counterclockwise way to improve barcode reading performance of analyzer, and reducing unnecessary re-label or manual operate.

Results:

Emergency patient critical value report in 30 minutes completion rate increased from 80.5% to 93.3%. Inpatient critical value report in 60 minutes completion rate increased from 94.1% to 96.6%. Troponin-I report in 30 minutes completion rate increased from 89.3% to 97.0%.

Conclusion: Doctors treat according to result from laboratory, and delayed report is an impediment to patient care. Particularly critical value reports, that causes delays in urgent patient treatment. We reduced unnecessary work to short turnaround time for critical value reporting, patient will get better therapy faster, and further improve patient safety.

A-184

Racing Against the Clock: Process Improvement Project to Reduce Cardiac Troponin Turnaround Time Through Autoverification of Results

T. M. Tauferner, A. J. Haga, B. R. Kelley, A. M. Wockenfus, L. J. Donato, B. S. Karon, B. M. Katzman. *Mayo Clinic, Rochester, MN*

Background: Turnaround time (TAT), defined as time of collection to time of result verification, is monitored monthly for cardiac Troponin (cTnT). Within our STAT laboratory, our TAT goal is 85% of cTnT results verified within 45 minutes. In October 2021, our lab began observing an increase in TAT. A quality improvement project was launched to identify a root cause and possible solutions.

Methods: cTnT TAT data from the Hospital Clinical Laboratory (STAT lab) at Mayo Clinic, Rochester, MN was extracted from our Laboratory Information System (LIS) using Tableau (Salesforce Inc., Seattle, WA). The data were categorized into three components: collection time, in lab processing and testing time, and time to result verification. Goal metrics for each component are: collection <10 minutes, in lab processing and testing < 25 minutes, and result verification to LIS < 5 minutes. The average time for each component and percentage not meeting TAT goals were calculated in Microsoft Excel. TAT while performing manual verification of results (January 2022) and after autoverification was implemented (December 2022) were compared.

Results: Prior to implementation of cTnT autoverification, total orders for January 2022 were 1,408 with 82.0% meeting TAT of 45 minutes. Of the 18.0% not meeting TAT, 207 (14.7%) orders exceeded goals for one or more components (percentages do not total 100%): collection delays 131 (63.0%), in lab processing and testing delay 175 (85.0%), and delay in result verification to LIS 80 (39.0%). For those orders not meeting the 45 minute TAT goal, mean times were 17.6, 40.3, and 8 minutes for collection time, in lab processing and testing, and result verification to LIS, respectively. Post implementation of autoverification, total orders for December 2022 were 1,444 with 88.6% meeting TAT of 45 minutes. Of the 11.4% not meeting TAT, 93 (6.4%) orders were outside goals for multiple components: collection delays 51 (55.0%), in lab processing and testing delay 67 (72.0%), and result verification to LIS 0 (0.0%) orders. For those orders not meeting the 45 minute TAT goal, mean times 16.6, 39.7, and 0 minutes for collection time, in lab processing and testing, and result verification to LIS, respectively. **Conclusion**: Achieving TAT goals agreed upon by the laboratory and clinical practice is critical to quality patient care. An investigation into our delayed TAT for cTnT highlighted several opportunities for intervention within the control of the lab. We chose to implement autoverification of results to easily and consistently eliminate the time required for manual result verification. TAT was improved by 6.6% (82.0% to 88.6%) through autoverification of results, allowing technologists to focus on other clinical work.

A-186

Enabling laboratories to do more with less: Assessment of workflow efficiencies through instrument consolidation in an Immunology lab

J. Murphy[1], C. Miller[1], E. Yu[2]. *[1]Thermo Fisher Scientific, Portage, MI, [2]Geisinger Medical Center, Danville, PA*

Background As today's laboratories adjust to the ever-changing landscape of growing demands while continuing to provide quality diagnostic testing, the need to evaluate and improve workflow, productivity and efficiency has never been more important. One way to ensure current processes and technology are fully optimized is to perform a Workflow Analysis Study. Geisinger Medical Center (GMC) recognized an opportunity to consolidate their immunology laboratory for allergy and autoimmune testing with annual volume of approximately 138,000. A consolidation solution can result in many improvements for the laboratory, such as: improved turnaround times, decreased manual labor, reduced reagent waste, increased employee morale, enhanced space utilization. Maximizing operational efficiencies is and will always be a good business model. We aimed to assess pre- and post- workflow changes to determine impact to laboratory resources. **Methods** To measure the impact of the instrument consolidation and integration of Phadia250™ systems, operational data was collected through direct workflow observations, time and motion studies, and targeted interviews of testing personnel at GMC's Immunology Laboratory. The initial/baseline study was conducted in May 2022 and the follow-up/post study occurred in October 2022. The baseline study encompassed seven separate platforms that GMC utilized for autoimmune and allergy testing. The following systems were included: two DYNEX DSX®, two Werfen BIO-FLASH®, two Siemens IMMULITE® 2000, one ZEUS IFA™.**Results** The workflow assessment resulted in GMC consolidating down to three platforms for autoimmune and allergy testing: two Phadia250, one ZEUS IFA™. The major benefit of the change was the reduction in technology from three types of technology to one type of technology, resulting in standardization of practices. The decrease in number of testing systems simplified overall test management (reduced LIS interface, process steps, instrument maintenance, reagent management, contract management) and reduced system footprints. A total of 361 square feet of laboratory space was saved equating to a 57% improvement over the baseline metric. After consolidating most of the testing to the two Phadia250 systems, the total daily manual time went from 4.2 hours to just over 2.5 hours. The combined workflow assessment resulted in saving a total of one full time employee (FTE). **Conclusion** To keep up with growing testing demands, laboratories must continue to produce high-quality results in an efficient manner. Test assay quality and utilization should also be evaluated when determining test consolidation options. Instrument consolidation is a viable strategy to save on technologist time, space and costs.This can result in not only economic savings to the laboratory, but also allows medical technologist to be funneled to other more needed areas of the laboratory, while saved space can be used for test expansion. In the climate of major medical technologist shortage nationwide, efficient workflows and productivity are important considerations to ensure the continued success of any laboratory.

A-187

The Effect of Ambient Temperature on Potassium During Hot Months: The Seasonal Pseudohypokalemia Delima!

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

Background: Laboratory tests are vital tools for the clinicians and it is becoming more and more the most the decision maker for the management of patients. Blood samples are drawn from patients and transported to the laboratory for analysis; samples are exposed to various temperatures during transportation which could have an effect on test results. We studied the effect of seasonal change on the potassium results using lithium heparin plasma tubes. **Method:** We compared the results collected from one day/month each from January 2022 to August 2022 from patients residing in Long-Term Care Facilities and were transported to the laboratory during which the samples were exposed to ambient temperature. A total of 16,086 specimens were collected using Lithium heparin plasma tubes up to 8/8/2022, sample on 8/15/22 were collected using serum gel separation tubes. Statistical analysis was done using Analyse-it. **Results:** our results showed an increase in hypokalemia with the increase in ambient temperature, the highest incidence of hypokalemia was during the month of June to August which corresponds with the higher ambient temperature. There was an increase in hypoglycemia at the same time of the increase in hypokalemia. The percentage of samples with hypokalemia was normalized when serum (SST) tubes were used.

	Total samples	% critical samples	% below normal	Tube type
January 2022	1665	0.2%	2.6%	Lithium heparin
February 2022	1944	0.1%	2.3%	Lithium heparin
March 2022	1899	0.6%	5.1%	Lithium heparin
April 2022	1862	0.4%	6.1%	Lithium heparin
May 2022	1792	0.8%	8.7%	Lithium heparin
June 2022	1687	1.2%	10.6%	Lithium heparin
July 2022	1790	1.0%	9.4%	Lithium heparin
8/1/2022	1757	0.9%	10.3%	Lithium heparin
8/15/2022	1690	0.4%	3.2%	SST

Conclusion: Seasonal pseudohypokalemia is phenomena that is seen when the samples are transported to the laboratory from different collection sites during the hot temperature. The increase in hypokalemia could be due to an increase in glucose metabolism and the cellular uptake of potassium or could be mediated by sodium-potassium-exchange-ATPase. Serum samples were less affected by the temperature change than lithium heparin plasma. It is very important to emphasis on the temperature during transporting samples to the laboratory to avoid seasonal pseudohypokalemia in the hot temperature season and hyperkalemia during the cold temperature season.

A-188

Managing a fully-automated paperless laboratory during a cyber-attack

S. K. Datta[1], P. Chopra[2], T. Sehgal[2], R. Yadav[2]. *[1]All India Institute Of Medical Sciences, New Delhi, New Delhi, India, [2]All India Institute of Medical Sciences, New Delhi, India*

Background: On 23rd Nov, 2022 a tertiary care apex medical institute in India was subject to cyberattack which disrupted the Hospital Information system (HIS) and the Laboratory Information system (LIS) servers. The services from the SmartLab, an end-to-end integrated total-lab-automation system, designed to run on paperless mode got disrupted. This is the main service laboratory with an average daily sample load of approx. 5000 including hematology, coagulation, clinical chemistry, immunoassays and serology services.

Methods: All lab operations were immediately shifted to manual mode. Standard operating protocols (SOPs) were designed for manual processing and circulated to OPD and IPD staff through WhatsApp. Instructions were issued to use previously used paper test-request-forms (TRFs) for sending samples. Previous stock of TRFs were retrieved from Stores and distributed to the IPD and OPDs. Staff from other areas of the hospital were mobilized.In OPD sample collection area, patients' registrations were done by writing Patient-Id, Name, Age, Sex, Sample-Id, Treating department and Test requests in registers. A small sticker was introduced and pasted on the blood collection tubes capturing minimal relevant patient details, treating department codes and

sample Id. Duplicates were pasted on OPD cards of respective patients. Traceability of patient Ids and sample Ids were maintained through data entry in registers. In the lab, the LIS were shut down on a precautionary note. Test requests corresponding to each sample Id were programmed directly on the analyzers. When done, print-outs of test results were obtained from the analyzers and hand-written results were entered by data entry operators on the TRFs. Reports were then segregated as per the treating department codes and dispatched to respective places. This continued till partial restoration of HIS services on and from 12th December, 2023.

Results: Despite best efforts, turn-around-time (TAT) for reports got compromised. IPD reports were prioritized and about 60% of the same could be dispatched by 9PM same day, rest being delivered by 9AM, next morning. TAT for OPD reports stretched up to 36 hours. Inspite of introducing separate numbering systems with codes for different OPDs and IPDs, about 40% reports could not be traced to the respective OPDs. These reports were collected by the patients' attendants from the lab itself. Manual report retrieval counters were set up outside the lab. At least 20% reports of the approx. 3000 reports generated per day remained unclaimed even after 30 days. The utilization of lab services also came down by 20-25% during this period. About 5% reports had to be repeated with fresh samples due to inability to retrieve previous reports. The data generated during this 20-days period could not be stored electronically and hence are not available in our database now.

Conclusion: Cyberattacks may happen to any institution. Having a strict cyber-security protocol is a must for all institutions. However, having a back-up plan for handling such a crisis should be also in place in case of any eventuality. Sharing our experience is an endeavour to create awareness regarding the prerequisites of such a plan.

A-189

Volume Matters: Continuous Improvement to Reduce Blood Collection Volumes and Collection Tube Utilization

E. A. Hain, B. K. Hendrix, A. J. Stelpflug, E. A. Elliott, S. A. Boedecker, T. L. Faber, D. R. Block, N. A. Baumann. *Mayo Clinic, Rochester, MN*

Background: Clinical laboratories are constantly changing with automated analyzers and total lab automation (TLA) implemented and replaced over time. Some benefits of newer technologies include decreased specimen volume requirements, less need for repeat testing, and greater throughput. Recent implementation of new analyzers and replacement of TLA in the Central Clinical Laboratory (CCL) at Mayo Clinic, Rochester, MN, prompted the laboratory to reassess blood collection volumes for commonly ordered tests. **Objectives:** The aim of this study was to evaluate whether updating the blood collection volumes for clinical chemistry and immunoassay tests performed in the CCL could reduce the volume of blood collected from patients and the number of blood collection tubes utilized. **Methods:** Nearly 100 different serum assays from the CCL test menu were reviewed including the basic and comprehensive metabolic, hepatic function, renal function, electrolyte, and lipid panels as well as thyroid function tests, enzymes, hormones, electrolytes, and iron status tests. Cortisol, ferritin, folate, and Vitamin B12 testing were performed on the Beckman DxI800; all other testing was on Roche Diagnostics Cobas 8000 chemistry (ISE, c701, c502) and immunoassay (e801) analyzers. Updated blood volumes for each assay were calculated utilizing manufacturer's analytical volumes and "dead volume" requirements. Baseline data retrieved from the laboratory information system (LIS) included blood collection volumes and number of tubes collected for 128 patients with physician-orders for laboratory testing on 5/2/21. The collection logic was then updated in the test LIS environment using the new blood volumes requirements and applied to the same 128 orders. Blood volumes and number of tubes collected per order were compared before and after the volume updates. **Results:** Baseline data from 128 unique orders showed that 414 total tubes were collected with a median(range) of 3(2-6) tubes per order and 5(1-18) tests per tube. The total volume of blood requested at baseline was 2,065 mL. Post-intervention, 197 tubes were needed with a median(range) of 2(1-3) tubes per order and 13(1-30) tests per tube. The total volume of blood requested after updating volumes was 851 mL. **Conclusions:** The practice of reviewing and updating specimen volume requirements when replacing or implementing new analyzers or TLA can greatly reduce the volume of blood collected from patients and the number of blood collection tubes per order. In this study of 128 physician orders for common laboratory tests, updating blood collection information in the LIS reduced the requested blood collection volumes by 59% and decreased the number of blood collection tubes required by 52%. This not only benefits patients because less blood is collected but also conserves blood collection tubes and reduces supply chain demands. Additionally, fewer tubes on the TLA system has the potential to improve throughput and test turnaround time.

A-190

The effect of lipemia on the laboratory assessment of blood gases

M. Nelson, P. Patel, E. Olgaard, B. Osa-Andrews. *University of Florida, Gainesville, FL*

Background: Unlike the electrolyte exclusion phenomenon, little is known about the effects of lipemia on the laboratory assessment of total carbon dioxide (tCO_2) and bicarbonate (HCO_3^-). Depending on the method used, lipemia-induced turbidity of a specimen can impact the outcome of the results. The photometric-enzymatic, rather than the indirect ion selective method is more easily interfered with by a turbid sample. Consequently, HCO_3^- and tCO_2 results from a severely lipemic sample may be falsely diminished. A recent case report suggests an association between high lipids and HCO_3^-. A larger study to investigate this trend is imperative to guide laboratory and medical practice, since preanalytical component accounts for approximately 68% of all clinical laboratory errors. This study aims to investigate the analytical relationship between lipemia and blood gases analyses. **Methodology**: Three months' worth of patient test results totaling 660 data points were retrospectively extracted from the electronic health record and filtered using an excel spreadsheet. Data collected included patient results of triglycerides (TGs), total cholesterol (tChol), HDL-Cholesterol as well as tCO_2 (using enzymatic method), HCO_3^-, pH and pCO_2 from both ambulatory and in-patient setting. The data was analyzed using excel spreadsheet and GraphPad prism. **Results:** 399 patients had TGs results, 372 had tCO_2, and 162 had TGs and tCO_2 simultaneously resulted. Of the 372 tCO_2 measurements, 79 had lower than the reference interval and 87 were simultaneously performed with pH and TGs, out of which 22 had low tCO_2, unextreme TGs(<1000mg/dL) and low pH; only 2 had low tCO_2, unextreme TGs and normal pH; 1 patient had low tCO_2, high TGs (>1000mg/dL) and low pH and 4 patients had low tCO_2, high TGs and normal pH. All low HDL-C measurements also had low tCO_2 and high TGs. Of the 31 patients who had TGs above 1000mg/dL, 20 had normal tCO_2 while 11 recorded low tCO_2 and none had high tCO_2; 3 had low tCO_2 and low pH and, no patient recorded normal tCO_2 and low pH, 4 had low tCO_2 and normal pH of which 3 had TGs>4000mg/dL. In all 5 instances of high initial TGs with low tCO_2 measurements, tCO_2 continually increased as the high TGs were being serially resolved. **Discussion and Conclusion:** Overall, our data shows an inverse correlation between extremely high TGs >4000mg/dL and tCO_2 which in the presence of normal pH, rules out metabolic disturbance. Since tCO_2 chiefly represents HCO_3^-, low tCO_2 in the setting of low pH and unextreme TG, indicates metabolic acidosis. The serial increase in tCO2 as the extremely high TGs are resolved corroborates this converse correlation. The present data demonstrates that resolution of the lipemic sample is essential not only for the accuracy of electrolytes results, but also to improve the interpretation of blood-gas results. The outcome of this study is beneficial for minimization of preanalytical interferences, quality improvement of laboratory process and the improvement of the management of patients suspected with metabolic abnormalities. In the future, an expanded data set and interventional studies will be explored to further authenticate our present understanding.

A-192

Cause and Departmental Origin of Errors - An Investigation of Questionable Patient Test Results

A. L. Brady, M. Hayden, M. B. Elkins, M. R. Nasr, A. Reyes, Z. T. Cao. *SUNY Upstate Medical University, Syracuse, NY*

Introduction: Reliable patient test results are crucial for quality patient care, involving pre-, post- and analytical phases. Questionable Patient test Results (QPRs), either not matching patient clinical presentation or apparent outliers for the patient population, are often encountered. We aimed to investigate the source and nature of possible errors that cause QPRs.

Methods: 960 QPRs were identified during July-December 2022 at our institution. We examined the nature of the QPRs, the corresponding patient clinical records, the procedures involved in pre-, post- and analytical phases, and quality control systems.

Results: The QPRs were found to be originated from 31 departments and medical specialty units. Of those, 5 departments/units had > 50 QPRs (accounting for 64% of total QPRs) and are listed in the table provided. The table also lists the information on 7 types of errors identified by our analysis. Of all errors, 78% occurred at pre-analytical phase, with the majority of errors occurring during sample collection.

Discussion: The most common types of errors were either diluted or contaminated samples accounting for 38% and 34%, respectively. Such errors often occur during sample drawing from an existing I.V. access port if not adequately flushed after saline solution infusion (causing sample dilution) or dextrose solution (causing glucose contamination). Many errors were due to samples drawn in the wrong collection tube

type. Sample integrity issues included hemolysis, lack of sufficient sample volume, or clotting. For those QPRs that we did not find apparent errors, they were either due to random errors or the results fit the patient's true medical condition.

Conclusions: Our findings highlight the importance of pre-analytical quality control measures and the need for regular staff education/training. This study pinpointed the errors that require targeted education materials, as well as the hospital units that would benefit most from this focus on error prevention.

	Department (All In-Patient)								
	General Medicine	Medical ICU	Adult ED	Neurology	Surgical ICU	Cardiopulmonary ICU	Coronary Care ICU	Pediatric ICU	Total (%)
Causes									
Diluted sample	38	31	47	43	27	21	15	11	233 (38%)
Contaminated-Line infusion	43	30	8	19	27	27	23	32	209 (34%)
No apparent error fund - Unknown	11	17	19	6	3	9	9	6	80 (13%)
Sample collected in wrong tube	6	3	9	2	0	0	0	0	20 (3%)
Sample integrity issue	1	6	13	5	0	2	1	0	28 (5%)
True results - no lab causes	6	7	2	2	4	2	2	2	27 (4%)
Instrumentation issue	5	3	2	5	2	2	2	0	21 (3%)
Total (%)	110 (18%)	97 (16%)	100 (16%)	82 (13%)	63 (10%)	63 (10%)	52 (8%)	51 (8%)	618 (100%)

A-193

Effects of Delayed Centrifugation and Delayed Testing on the Stability of Comprehensive Metabolic Profile Analytes

C. W. Chan, A. Gant Kanegusuku, K. J. Yeo. *The University of Chicago, Chicago, IL*

Background: The centralization of clinical laboratory testing has become increasingly common in recent years. While centralization allows for consolidation of resources, expanded test menus, procedural standardization, and improved efficiencies, central laboratories can face significant logistical challenges related to sample transport. In particular, problems may arise when samples are received from distant sites or if the courier services used to transport samples are infrequent or unreliable. Policies aimed to mitigate pre-analytical issues such as the time-to-centrifugation, the time-to-testing, and storage temperature, are best devised when driven by empiric data. **Objective:** In this study, we provide stability studies for the Comprehensive Metabolic Panel (CMP) analytes in paired serum and lithium heparin (LiHep) plasma samples. Our findings not only complement preexisting literature data but also help guide the current sample acceptance criteria and policy for receiving satellite clinic samples in our laboratory. **Methods:** Serum and LiHep plasma were collected from healthy volunteers in gel separator tubes. In one set of experiments, samples of both specimen types were all centrifuged within 30 minutes of collection and tested at pre-determined time intervals (2, 4, 6, 12, and 24 hours post collection) using Roche Cobas 8000 system. In a second set of experiments, samples of both specimen types collected from another set of volunteers were kept at room temperature and only centrifuged prior to testing at pre-determined time intervals (2, 4, 6, 12, and 24 hours post collection). Sample storage prior to testing was at room temperature. The percent recovery of each result was calculated relative to the first sample of the series. The CMP includes the following analytes: glucose, sodium, potassium, chloride, carbon dioxide, urea nitrogen, creatinine, calcium, total bilirubin, total protein, albumin, alkaline phosphatase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). **Results:** Glucose in promptly centrifuged (i.e. within 30 minutes of collection) LiHep plasma samples showed acceptable recovery (≥90%) when tested within 12 hours of collection in LiHep gel separator tubes, whereas glucose stability in serum stored at room temperature exceeded 24 hours. In contrast, delayed centrifugation beyond 3 hours led to unacceptably low recoveries in both LiHep plasma and serum sample types. All other analytes included in the CMP were stable beyond 24 hours irrespective of whether the sample type is LiHep plasma or serum, or even if the time-to-centrifugation was up to 24 hours. **Conclusions:** Based on the study, our laboratory has updated our policy to accept pre-centrifuged (i.e. within 30 minutes of collection) LiHep plasma from satellite clinics up to 8 hours from blood collection, which is a 2-hour increase as compared to our prior cut-off acceptability. Given the location of our satellite clinics and unpredictability of sample transport, this increase represents a positive change in reducing outpatient sample rejections while ensuring reliable laboratory results.

A-194

Intravenous fluid contamination of basic metabolic panels is underrecognized by manual workflows

N. C. Spies, C. W. Farnsworth, M. A. Zaydman. *Washington University in St. Louis School of Medicine, Saint Louis, MO*

Background:

Erroneous laboratory results contribute to delays in diagnosis, incorrect treatment decisions, and increased healthcare costs. Of these errors, most occur in the preanalytical phase, before the sample makes it to the laboratory. One particularly common source of pre-analytical error is contamination by intravenous crystalloids (IV fluids). Current workflows for detecting IV fluid contamination rely on manual intervention by technologists, which is labor-intensive, variable between technologists, and may lack sensitivity.

Methods:

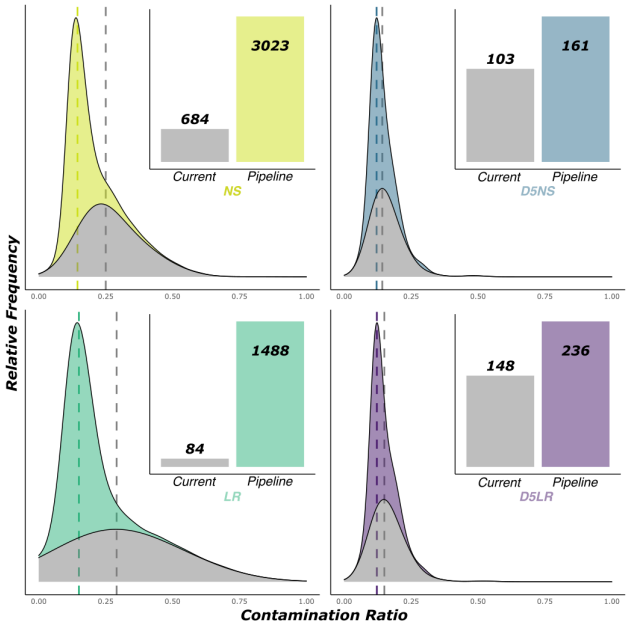
9,627,320 chemistry results were aggregated. IV fluid contamination was simulated by mixing a random sample of results *in silico* with normal saline (NS), lactated Ringer's (LR), and their 5% dextrose-containing counterparts (D5NS and D5LR). This simulation procedure was validated by *in vitro* experiments. Machine learning models were trained to predict contamination and mixture percentage using the simulated results and the prior unaffected result from that patient. A random sample of 100 predicted positives were manually chart reviewed and adjudicated. Predictions from the models on retrospective data were compared to real-world technologist flags.

Results:

The ML pipeline identified 3023, 1488, 161, and 236 results as being likely contaminated with NS, LR, D5NS, and D5LR respectively (Figure 1). Of these, 684 (23%), 84 (6%), 103 (64%), and 148 (63%) had already been flagged by technologists as contaminated. 100/100 manually-reviewed predictions were confirmed. The median contamination ratio for each fluid detected by the pipeline was lower than the current workflow for NS (95% CI of difference: -0.1 to -0.07) and LR (CI: -0.13 to -0.1). The number-needed-to-test to detect one additional contaminated specimen was 125 samples.

Conclusion:

A machine learning pipeline uncovers a substantial number of potentially contaminated results that were previously undetected using manual workflows, especially among non-D5 fluids.



A-195

The Application of Six Sigma Metrics to Assess Quality Control of Syphilis Tests in a Clinical Laboratory

A. H. Utiyama, R. T. Pereira, T. Rodrigues, A. C. Bandeira, D. R. Ramadan, S. Tufik. *AFIP Medicina Diagnóstica, São Paulo, Brazil*

Background Clinical laboratories use quality control processes to monitor and evaluate analytical performance in terms of precision and bias. The periodic measurement of those parameters plays an important role in patient safety and it is a requirement in most accreditation organizations. In recent years, Six Sigma metrics has become one of the most efficient tools to monitor the analytical performance of clinical laboratory systems, since it unifies Total Allowable Error (TEa), random and systematic errors in a single index. However, for many analytes, TEa is not available in the literature based on effect of analytic performance on clinical outcomes or in biological variation. In this case, the TEa must be derived from the "state of art". In this work, it will be described how Six Sigma was established in our laboratory for Syphilis, with TEa derived from external quality assessment program.

Methods A year worth of data was gathered from internal quality control and external quality assessment evaluation to assess the imprecision (in terms of coefficient of variation – CV%) and bias for Syphilis tests processes in four modules of Alinity I (Abbott). Data was split into two groups: "Non-reactive" and "Reactive". TEa was calculated from the external quality assessment for both groups, based on the median CV% of the peer group evaluated in the last 2 years. Six Sigma metrics were calculated from TEa, CV% and bias for each equipment, using the formula Sigma = (TEa-bias)/CV%.

Results TEa for "Non-Reactive" and "Reactive" levels were estimated at 44,25% and 17,7%, respectively. Six Sigma values calculated for "non-Reactive" level were between 4,6 to 5,38 in every equipment. For "Reactive" level, calculated Six Sigma ranged from 4,24 to 5,82. All values found were classified as Good and Excellent according to the Normalized method decision chart.

Conclusion Six Sigma is a powerful tool to monitor the analytical performance of the exam. For Syphilis, it is important to monitor "Non-Reactive" and "Reactive" levels separately for a better control over the analytical system. Abbott's Syphilis test has also a satisfying performance in our laboratory. Also, estimating the TEa from external quality assessment programs is a good alternative for analytes with no quality specifications available in the literature.

A-196

Trend Analysis in Proficiency Testing - Does it Work?

A. C. Bandeira, J. M. Banzato, A. H. Utiyama, V. F. Coutinho, D. R. Ramadan, S. Tufik. *AFIP Medicina Diagnóstica, São Paulo, Brazil*

Background Trend analysis is a powerful tool in any data analysis due to its preventive characteristics. In proficiency testing (PT) it is also important because it can anticipate errors that could impact on patient's results. Therefore, it is a key tool to patient safety. Although this is the current and logical premise, efficacy of trend analysis has not been measured by laboratories. This work aims to share our laboratory's enhancements after implementing trend analysis for PT results.

Methods PT results from a Brazilian national program for two years were analyzed: one year before the implementation of trend analysis (2020) and another year after the process had already been fully established (2022). Qualitative tests were not considered and a total of 432 quantitative analytes were included in the study. Trends were classified by CAP's recommendations for single mailing patterns (Troubleshooting guide for proficiency testing data – ww.cap.org). The percentage of results classified as inadequate and inadequate results with previous trend alert were calculated. Ten results from both years were compared by Chi-square.

Results Over 7,000 results were analyzed and classified. In 2022, overall inadequate results were 37% lower than 2020. Comparing both periods, Chi-square was high (χ^2= 9,74), corroborating that the enhancements observed were statistically significant. Before trend analysis was implemented, 14 inadequate results presented biased results in previous rounds. If trend analysis had not been implemented, we estimate there would be 15 analytes in this situation. However, only 5 were found, which represents a 297,8% improvement. For this metric (inadequate results with previous trend alert), Chi-square from both periods was 5,087.

Conclusion Using a simple trend analysis technique we were able to reach an important improvement in the PT process, contributing to preventive actions for potential problems that could affect patient results and decreasing the number of inadequate results. Hence, in our laboratory trend analysis can be considered worth it.

Lipids, Lipoproteins, and Cardiovascular Risk Factors

A-198

Acute effects of a single moderate-intensity exercise on omega-3 and omega-6 metabolic pathway using whole blood lipidomics

N. Sakane[1], A. Suganuma[1], S. Sakane[1], M. Fujibayashi[2]. *[1]Kyoto Medical Center, Kyoto, Japan, [2]Setsunan University, Osaka, Japan*

Background: Omega-3 fatty acid supplementation can reduce muscle soreness and maintain muscle function following eccentric exercise-induced muscle damage. However, the relationship between the dietary and biological status of omega-3 fatty acids in rugby players is unknown. This study aimed to examine the relationship between the dietary and biological status of omega-3 fatty acids in university rugby players. We investigated acute effects of a single moderate-intensity exercise on omega-3 and omega-6 metabolic pathway using whole blood lipidomics.

Methods: A cross-sectional study of university rugby players and age-matched sedentary controls was conducted. Diets including omega-3 and omega-6 fatty acids were assessed using a validated brief-type self-administered diet history questionnaire. Whole blood lipidomics analysis was performed using the dried blood spot technique before and after a single training session, namely a 2-h shuttle run, and the omega-3 index (EPA + DHA) was calculated. We categorized rugby players based on the proposed omega-3 index risk zones for cardiovascular disease as follows: high risk, < 4%; intermediate risk, 4-8%; low risk, > 8%.

Results: The rugby group (n=29) had a significantly higher intake of omega-3 and omega-6 fatty acids than the control group (n=31). The blood omega-6 relative concentration did not differ between the groups; however, the rugby group had lower omega-3 fatty acid levels than the control group (4.4 ± 1.1 vs. 6.2 ± 1.8%). Approximately 48% of rugby players had an omega-3 index that was considered high risk, and 52% had that considered intermediate risk. None of the patients had a low-risk omega-3 index. A single training session affected the omega-3 and omega-6 metabolic pathways.

Conclusion: Rugby football players may be deficient in omega-3 fatty acids, despite having a higher omega-3 fatty acid intake than controls. The omega-3 index and lipidomics can be used to profile and monitor rugby players. Further nutritional interventions are required to confirm these issues.

A-201

Deriving Information From The Lipid Panel As Spherical Coordinates

J. Cole[1], M. L. Sampson[1], A. T. Remaley[2]. *[1]NIH Clinical Center, Bethesda, MD, [2]NIH National Heart, Lung, and Blood Institute, Bethesda, MD*

Background:

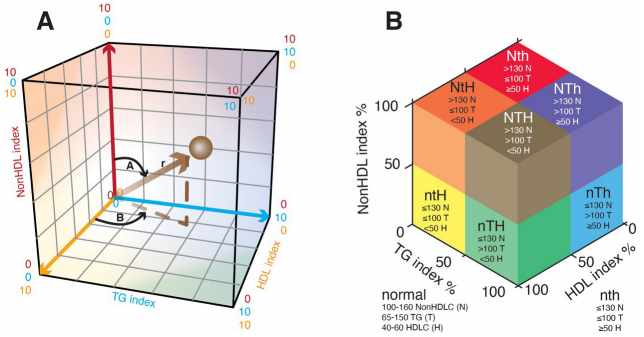
Lipid panel assays are used to calculate LDL-C as a proxy for cardiovascular disease risk. Further investigations are required to classify the patient's dyslipidemia into a phenotype to assist with management. We explored the information contained within the lipid panel with two aims: First, to estimate cardiovascular risk directly, bypassing LDL-C calculation. Second, to provide a novel phenotypic classification system.

Methods:

Various transformations of lipid panel data were explored as inputs to a logistic regression model of cardiovascular risk, and to produce a spherical coordinate system for phenotyping. Area under the receiver operating characteristic (AUROC) curve was used to assess the predictive ability of the model. Clinical utility metrics were calculated and Kaplan-Meier survival curves were used to determine the prognostic value of the model.

Results:

Transforming the lipid panel data into indices allowed derivation of a spherical coordinate system. The spherical coordinates proved to be the best input values for logistic regression to provide the probability of high cardiovascular risk. When we added age, sex and race as variables in the model, the AUROC for predicting cardiovascular events approached that of the American Heart Association's pooled cohort equation (PCE). The final model was more sensitive than the PCE, and provided greater prognostic ability than LDL-C. The spherical coordinate system classified individuals into "normal" or one of eight phenotypes, which were differentially associated with clinical features such as elevated CRP or metabolic syndrome.



Conclusion:

The new model uses all three lipid panel parameters and other routinely available laboratory data to directly predict cardiovascular risk with similar accuracy to the PCE. Conversion of the parameters to a spherical coordinate system allows classification into one of 9 phenotypic groups, which may alert clinicians to underlying or consequent pathological processes to be managed.

A-202

Establishing of lipid-apolipoprotein connectivity map in hyperglycemia

A. A. Ivanova, B. A. Parks, J. Rees, Z. Kuklenyik, J. R. Barr. *CDC, Atlanta, GA*

Background. Hyperglycemia, a condition characterized by a significantly increased glucose level in the blood, can lead to insulin deficiency and pre-diabetes. Lack of insulin production also interferes with lipid metabolism and increases the risk for cardiovascular disease. Thus, diagnosis and treatment of hyperglycemic patients can benefit from advanced proteomics and lipidomics testing approaches. **Methods.** Disease-focused serum samples from 50 specimens were collected and evaluated, including 29 specimens with a glucose level >180 mg/dL and 21 normal lipidemic specimens. The sample proteomics analysis was conducted with a Perfinity IDP workstation (Shimadzu Scientific, Columbia, MD, USA) using on-line protein digestion with an immobilized enzyme reactor (IMER) directly coupled to a HALO-C18 column (Advanced Materials Technology, Wilmington, DE, USA). Lipidomics data were collected with the Lipidyzer platform (AB SCIEX, Framingham, MA, USA). Statistical analysis was conducted using JMP software (SAS Institute, USA) and custom scripts. **Results.** Using targeted lipidomics and proteomics platforms we identified 20 proteins and 575 lipid species across 10 lipid subclasses. We identified specific lipids and lipoproteins dysregulated in hyperglycemic specimens as compared to the normolipidemic samples and linked them to major metabolic pathways. Lipoprotein particles related-proteins apos A1/B/C2/C3/E and lecithin–cholesterol acyltransferase (LCAT) were significantly dysregulated and associated with changes in HDL-mediated lipid transport, very-low-density lipoprotein particle remodeling, acylglycerol homeostasis, and triglyceride homeostasis. **Conclusion.** The conducted integrated analysis of lipid and protein concentrations in hyperglycemia specimens to provide new insights into molecular changes associated with hyperglycemia that may inform new diagnostic and therapeutic strategies. The network analysis can allow improved categorization of patients that can lead to more effective and individualized therapeutic intervention approaches. This study provides new experimental and informatics approaches to better assess underlying metabolic conditions and related diseases, including cardiovascular disease, diabetes, and metabolic syndrome. **Disclaimer.** The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the CDC.

A-203

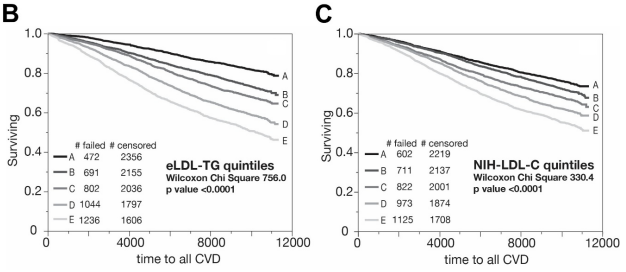
An Equation based on the Standard Lipid Panel for Calculating Low-Density Lipoproteins-Triglycerides

A. Wolska[1], M. Sampson[1], J. W. Meeusen[2], L. J. Donato[2], A. S. Jaffe[2], A. T. Remaley[1]. *[1]National Institutes of Health, Bethesda, MD, [2]Mayo Clinic, Rochester, MN*

Background: There is growing evidence that low-density lipoprotein-triglyceride (LDL-TG) may be a better biomarker to define atherosclerotic cardiovascular disease (ASCVD) risk than LDL-cholesterol (LDL-C), which is currently used to stratify risk and as a treatment goal. Because LDL-TG typically requires additional advanced lipid testing, we developed an equation for estimating LDL-TG (eLDL-TG) from the results of the standard lipid panel. **Methods:** Results from a general dyslipidemic population (N=40,202), which were obtained with beta-quantification (BQ) reference method, were randomly divided into a training and validation datasets and used to develop the equation for estimating LDL-TG shown in **Fig.1A.** Association of *Sampson-NIH*-LDL-C (NIH-LDL-C) and eLDL-TG with ASCVD risk markers was done in the National Heart and Nutrition Examination Survey (NHANES) (N=37,053) and with ASCVD events in the Atherosclerosis Risk in Communities (ARIC) study (N=14,749). **Results:** The comparison between eLDL-TG and measured LDL-TG was only modest (R^2=0.601, slope=0.611), but eLDL-TG **(Fig.1B)** showed better risk stratification than NIH-LDL-C **(Fig.1C)** by survival curve analysis in ARIC. Moreover, receiver-operating characteristic (ROC) analysis showed that eLDL-TG was better than all other lipid-marker tests for ASCVD events (AUC: eLDL-TG=0.633, HDL-C=0.627, sdLDL-C=0.621, remnant-cholesterol=0.613, NonHDL-C=0.613, TG=0.603, apoB=0.598, NIH-LDL-C=0.590, TC=0.568). In NHANES, eLDL-TG was more strongly associated with hypertriglyceridemia, obesity, diabetes, metabolic syndrome and increased high-sensitivity C-reactive protein than NIH-LDL-C. Inclusion of eLDL-TG in a Cox-Proportional Hazards Model along with other conventional risk factors (age, sex, race, diabetes, systolic blood pressure, smoking, HDL-C, and total cholesterol) did not further improve risk prediction. **Conclusions:** Like LDL-C, LDL-TG can also be estimated from the results of the standard lipid panel. It is a better univariate risk marker than LDL-C and hence can be useful for initial ASCVD risk stratification. **Figure 1. A)** eLDL-TG equation; Survival curve analysis in ARIC based on **B)** eLDL-TG, **C)** NIH-LDL-C

A

$$eLDL\text{-}TG = \frac{TG}{38.46} + \frac{NonHDL\text{-}C}{5.75} + \frac{\left(\frac{TG}{NonHDL\text{-}C}\right)}{0.103} + \frac{\left(\frac{1}{HDL\text{-}C}\right)}{0.0041} - 2.95$$



A-204

Associations between Apolipoprotein B/A1 Ratio, Lipoprotein(a) and the Risk of Metabolic-associated Fatty liver Diseases in a Korean Population

K. Park[1], Y. Park[2], H. Park[3], E. You[4], Y. Kim[1]. *[1]Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea, Republic of, [2]Chungnam National University Hospital, Daejeon, Korea, Republic of, [3]Seoul Clinical Laboratories, Seoul, Korea, Republic of, [4]Inje University Busan Paik Hospital, Inje University College of Medicine, Busan, Korea, Republic of*

Background: Metabolic-associated fatty liver disease (MAFLD) is a new nomenclature of the fatty liver condition associated with metabolic dysfunction. Little study regarding the association between apolipoprotein and lipoprotein(a) and MAFLD has been performed. This study aimed to investigate the association between apolipoprotein B/A1 ratio (apo B/A1), lipoprotein(a), and MAFLD based on new diagnostic criteria in a Korean population.

Methods: This cross-sectional study consisted of 14,420 consecutive adults underwent abdominal ultrasonography among the general Korean population visiting at comprehensive health promotion center in Samsung Changwon Hospital from January 2021 to December 2021. Clinical data, anthropometric and biochemical parameters were reviewed. According to the Asian Pacific Association for the Study of Liver guideline, the MAFLD was diagnosed based on hepatic steatosis by abdominal ultrasonography and the presence of overweight/obesity, type 2 diabetes, or the evidence of metabolic dysregulation. Multivariate logistic regression was conducted to analyze the independent association between apo B/A1 ratio and MAFLD.

Results: The prevalence of MAFLD in the general Korean population was 34.5% (4,978/14,420). Multivariate logistic regression analysis revealed that Apo B/A1 ratio (odds ratio: 3.913, P = 0.019) was independently associated with the MAFLD. Lipoprotein(a) levels are inversely associated with the MAFLD, especially in subjects with hepatic fibrosis (P < 0.0001).

Conclusion: Apo B/A1 ratio and lipoprotein(a) have opposite association with the MAFLD in a Korean population. This study suggests that apo B/A1 ratio could be useful biomarkers for MALFD, while lipoprotein(a) should be used with caution as a predictive biomarker for MAFLD.

A-205

Optimized Time Interval for the Measurement of Plasma Lipids for Assessing Cardiovascular Disease Risk

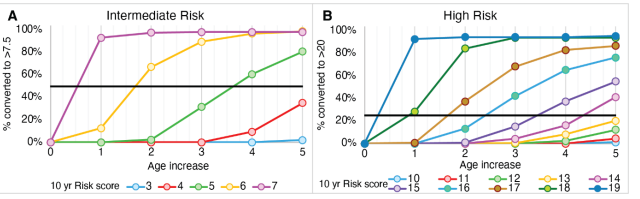
M. Sampson, J. Cole, A. T. Remaley. *NIH, Bethesda, MD*

Background: The measurement of plasma lipids are used to predict atherosclerotic cardiovascular disease (ASCVD) risk and for the management of lipid-lowering therapy. The general recommendation is that plasma lipids should be measured at least every 5 years, but the ideal time may vary depending on the baseline risk, which we examined in this study.

Methods: Using lipid values and other risk factors from the National Health and Nutrition Examination Survey (NHANES) (no diabetes, no lipid medications, n=9704), which is designed to be representative of the US-general population, we calculated a baseline 10-year ASCVD risk with the pooled cohort risk equations (PCE). Future 10-year risk scores were estimated by chronologically increasing age of the subjects and projecting future systolic blood pressure (SBP) and lipid changes, using the mean-percentile age group change in the NHANES population for SBP (no hypertension medications, n=17,329) and the Lifelines Cohort study for lipids (no CVD or lipid medications, n=133,540). Crossing high-risk (>20%/10-years; 25% decison limit) and intermediate risk (>7.5%/10-years; 50% decision limit) PCE thresholds were calculated by baseline age to determine the best time intervals for testing lipids.

Results: As shown in the figure, the likelihood of crossing the intermediate or high-risk threshold depends on the baseline risk. Individuals that are close to one of the risk thresholds are more likely to cross it in the subsequent years. Based on this analysis, we recommend the following time intervals for lipid testing: Baseline risk<5%/10-year: 5-years, baseline risk 5-15%/10-year: 3-years, baseline risk 16-20%/10-year: 1-2 years.

Conclusions: More frequent lipid testing than once every 5 years is desirable, because it would identify at risk patients sooner and allow for earlier therapeutic intervention, which has been shown to increase the effectiveness of statin therapy for reducing ASCVD events.



A-206

Association of Lipids and Glucose with High-sensitivity CRP (hs-CRP) in a Community-based Adult Population

H. Li, N. White Al-Habeeb, P. Catomeris. *Dynacare, Brampton, ON, Canada*

Background: Research and clinical studies have established a pivotal role of inflammation in the development and progression of both cardiac and vascular diseases, responsive to injurious factors such as smoking, hypertension, dyslipidemia, and hyperglycemia. This study analyzed the association of various lipids, HbA1c and glucose with high-sensitivity CRP (hs-CRP), a marker of low-grade chronic inflammation, in a community-based adult population. **Methods:** Data analysis was carried out on within-collection results obtained from adults (>=20 yrs) from 2019 to 2022. Tests included hs-CRP, with cholesterol (n=74560), triglycerides (n=74551), LDL-C (n=73426), HDL-C (n=74458), non-HDL-C (n=69808), ApoB (n=3122), Lp(a) (n=4444), fasting glucose (n=42954) or HbA1c (n=62486). All tests were performed on Roche Cobas instrumentation. Chi square tests were performed to evaluate the association between each lipid and glycemic indicator with hs-CRP. Relative risk (RR) and odds ratio (OR) were calculated to assess the strength of the association. **Results:** Chi-square test demonstrated association of hs-CRP with HDL-C, triglycerides, HbA1c, non-HDL-C, LDL-C, fasting glucose, cholesterol and ApoB, but not Lp(a). Increased risk and odds to have elevated hs-CRP (3.0 -10.0 mg/L) were observed with abnormal glucose and lipid levels. Higher RR and OR were seen with decreased HDL-C and elevated HbA1c, fasting glucose, triglycerides, and non-HDL-C. Slightly increased RR and OR were also observed with elevated LDL-C, ApoB, but not Lp(a).

Analytes	Cutoff/Range	RR	95% CI Low	95% CI High	OR	95% CI Low	95% CI High	Chi-square test P
HDL-C (Female)	<1.3 mmol/L	1.65	1.60	1.70	2.14	2.05	2.24	0.00E+00
HDL-C (Male)	<1.0 mmol/L	1.59	1.52	1.66	1.80	1.70	1.91	3.50E-150
HbA1c	>6.4%	1.53	1.47	1.58	1.76	1.67	1.85	4.45E-217
HbA1c	6.0-6.4%	1.45	1.40	1.50	1.63	1.55	1.72	
Fasting Glucose	>6.9 mmol/L	1.47	1.40	1.55	1.69	1.57	1.81	6.74E-83
Fasting Glucose	6.1-6.9 mmol/L	1.22	1.15	1.28	1.29	1.20	1.39	
Triglycerides	>=1.7 mmol/L	1.37	1.33	1.40	1.54	1.48	1.59	1.55E-304
Non-HDL-C	>=4.2 mmol/L	1.31	1.28	1.35	1.43	1.38	1.49	1.40E-173
ApoB	>=1.2 g/L	1.23	1.06	1.43	1.30	1.07	1.59	5.70E-04
LDL-C	>3.5 mmol/L	1.20	1.17	1.24	1.29	1.24	1.33	2.24E-85
Cholesterol	>=5.2 mmol/L	1.17	1.14	1.20	1.24	1.19	1.28	2.47E-78
Lp(a)	>=75 nmol/L	0.97	0.83	1.13	0.96	0.79	1.17	0.789

Conclusion: Glucose and most lipid parameters were associated with hs-CRP. Stronger association was observed with HDL-C and carbohydrates (glucose and triglycerides), followed by triglycerides-rich non-HDL-C. LDL-C, ApoB and cholesterol had weaker association, and Lp(a) had no association with hs-CRP. This observation from a large data set from a community population may provide helpful insight to prevention and clinical management of low-grade chronic inflammation.

A-207

Analytical Validation of Helena SPIFE Touch to Determine ApoB-Containing Lipoproteins Lp(a)-P, LDL-P and VLDL-P

R. J. Scott[1], L. Donato[2], A. Jaffe[2], J. Meeusen[2], V. Vasile[2]. *[1]Mayo Clinic, ROCHESTER, MN, [2]Mayo Clinic, Rochester, MN*

Background: Elevated Lp(a) is an independent risk factor for atherosclerotic (ASCVD) events and associates with aortic stenosis. Accurate immunochemical measurement of Lp(a) is complicated by heterogeneity of Lp(a) molecular size. The objective of this study was to validate the analytical performance of an electrophoretic assay for measurement of ApoB-containing lipoprotein particles including Lp(a)-P, LDL-P, and VLDL-P and to compare results to other standard clinical methods.

Method: Lp(a)-P, LDL-P, and VLDL-P were quantified by electrophoretic serum separation followed by immuno-staining with an ApoB antibody using the SPIFE Touch (Helena Laboratories, Beaumont, TX). Stability was evaluated by comparison to results at time zero (n=10). Precision was assessed by 5 measurements twice daily for five days. Limit of quantitation (LoQ) was determined by 20 replicate measurements. Linearity was assessed by mixing high and low concentrations at various ratios. Accuracy was assessed by split sample comparison (n=126) with Helena Laboratories. Lp(a)-P and LDL-P results were compared to nuclear magnetic resonance (NMR) using a Bruker Ascend 600 NMR (Bruker, Billerica, MA) with AXINON software (numares, AG, Regensberg, Germany) (n=114). Lp(a)-P is included with LDL-P by NMR. Therefore, the SPIFE Lp(a)-P + LDL-P was compared to NMR LDL-P. SPIFE Lp(a)-P was compared directly to Roche cobas c501 Lp(a) immunoassay calibrated to molar units (Roche Diagnostics, Indianapolis, IN) (n=126).

Results: The analytical performance of Lp(a)-P, LDL-P, and VLDL-P measured on the SPIFE Touch met all acceptance criteria. Bias to the reference method was <20% with R^2>0.98. All analytes were stable 14 days refrigerated and 14 days frozen. Lipoprotein particle measurements on SPIFE Touch correlated to those obtained by both NMR and Roche assays.

Conclusion: The analytical performance of the SPIFE Touch for measuring the concentration of ApoB-containing lipoproteins is acceptable. Further studies are needed to determine clinical implications and utility compared to NMR and Roche assays.

A-208

An Improved Formula for Predicting Low LDL-C Based on an Enhanced Sampson-NIH Equation

T. C. Coverdell[1], M. Sampson[1], J. W. Meeusen[2], L. J. Donato[2], A. S. Jaffe[2], A. T. Remaley[1]. *[1]National Institutes of Health, Bethesda, MD, [2]Mayo Clinic, Rochester, MN*

Background: Low-density lipoprotein cholesterol (LDL-C) is used to assess atherosclerotic cardiovascular disease (ASCVD) risk and to manage lipid-lowering therapy. Proprotein convertase subtilisin/kexin (type 9) serine protease (PCSK9) inhibitors decrease LDL-C by up to 70% when used in conjunction with statins. Due to cost, insurance companies restrict their use. To be eligible for PCSK9 therapy, a patient must have pre-existing ASCVD and LDL-C > 70 mg/dL, while on a maximally tolerated dose of statins. Most clinical laboratories calculate LDL-C by the Friedewald Equation (FWLDL-C), which utilizes results from the standard lipid panel (total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C)). However, this often leads to an underestimation of LDL-C, especially when TG concentrations are elevated. More accurate equations have recently been developed, such as the Martin (MLDL-C), Sampson-NIH (SLDL-C) and extended Martin (extMLDL-C) equations. Using LDL-C determined by the beta-quantification reference method (BQ), we developed the following enhanced version of the Sampson-NIH Equation (eSLDL-C), which includes apoB as an independent variable: eSLDL-C = TC/1.15 - (HDL-C)/1.25 - TG/6.98 - (TG x NonHDL-C)/1115 + TG^2/8903.23 + (TG x ApoB)/1237 + ApoB/4.54 - 4.73

Methods: Results for clinically ordered ApoB, BQ, and lipid panels were used. To test the accuracy of the various equations, we compared BQLDL-C to FWLDL-C, MLDL-C, extMLDL-C, SLDL-C and eSLDL-C by regression analysis (N=12,101, TG<800). Accuracy of each equation was compared to the BQ reference method for classifying patients (LDL-C ≤150, n=9374) as either being below or above the 70 mg/dL treatment decision threshold for PCSK9 therapy.

Results: The eSLDL-C equation performed substantially better than the other equations for estimating low LDL-C (BQLDL-C ≤100 mg/dL, TG 5-800 mg/dL, n=4115), with a mean absolute difference (MAD) of 3.80 mg/dL (compared to SLDL-C: 6.05; FWLDL-C: 8.71; MLDL-C: 6.43; extMLDL-C: 6.39). It also had the best overall normalized Matthew's Correlation Coefficient (nMCC) for the best balance of sensitivity and specificity for identifying patients that are above the 70 mg/dL treatment threshold (eSLDL-C - 94.7% (92.2% spec, 98.5% sen), SLDL-C - 90.7% (94.8% spec , 95.5% sen), FWLDL-C - 88.2% (98.0% spec, 92.7% sen), MLDL-C - 90.3% (88.9% spec, 96.5% sen), and extMLDL-C - 90.1% (88.4% spec, 96.5% sen)). In our dataset with 1125 subjects being below the 70 mg/dL threshold by BQLDL-C, FWLDL-C misclassified an additional 601 subjects below this threshold (Negative Predictive Value 65%). MLDL-C and extMLDL-C misclassified 289 subjects falsely low, whereas eSLDL-C only has 126 subjects falsely classified lower than the 70mg/dL threshold.

Conclusion: We show that the enhanced Sampson-NIH equation, which includes apoB, estimates low LDL-C more accurately than other equations. The use of the new equation would improve access to those patients who might benefit from PCSK9 therapy but have falsely low LDL-C by the FWLDL-C equation or other LDL-C equations. The new equation is also more specific than the MLDL-C and extMLDL-C equations and would, therefore, also reduce the overutilization of PCSK9 therapy by these particular equations for those patients who do not require it, according to the reference BQ method and current guidelines.

A-209

Application of Bilirubin Oxidase for Improvement of False Positive Error Caused by Bilirubin on Cholesterol Efflux Capacity Assay by Immobilize Liposome-Bound Gel Beads method

T. Miyakoshi[1], Y. Mutsuda[1], Y. Horiuchi[1], T. Kameda[1], M. Tozuka[2], R. Ohkawa[1]. *[1]Tokyo medical and dental university, Tokyo, Japan, [2]Life Science Research Center, Nagano Children's Hospital, Nagano, Japan*

Background:

High density lipoprotein (HDL) exerts various atheroprotective functions including cholesterol efflux capacity (CEC). HDL can attenuate lipid burden at atherosclerotic lesion by CEC. Based on the HDL`s role for moderate atherosclerosis, HDL-cholesterol level which indicates the amount of HDL has been evaluated to assess the risk of cardiovascular disease (CVD). Nevertheless, many clinical studies demonstrated that HDL-cholesterol level does not always reflect the risk of CVD, and CEC which represents the quality of HDL is considered as surrogate biomarker for CVD. CEC

is prospective biomarker for CVD risk assessment, however, CEC measurement encounters barriers for clinical application because of its requirements of cultured cell and radioactive substance for conventional CEC assay. To overcome these problems, we invented a novel CEC assay called immobilized liposome-bound gel beads (ILG) method. This method is cell-free assay and utilizes a fluorescence substance as cholesterol tracer. Although the ILG method is useful assay for clinical application, previous study revealed that bilirubin caused false positive error on CEC value. Hence, current ILG method might produce incorrect CEC evaluation for patients with hyperbilirubinemia and even healthy subjects within the range of biological variation of bilirubin. In this study, we improved the influence of bilirubin for ILG method by using bilirubin oxidase (BOD).

Methods:

BOD (Asahi Kasei Corp.) was added to 10 mM Tris-HCl (pH 7.4) containing 150 mM of NaCl and 1 mM of Na_2EDTA (Buffer A). Bilirubin from 'Interference check A Plus' (Sysmex Inc.) was added to serum (free-:10 mg/dL, conjugated-:9.7 mg/dL), and apoB-depleted serum (BDS) was derived from the bilirubin additive serum using polyethylene glycol. After the prepared bilirubin additive BDS was incubated with BOD-containing Buffer A for 16 h, the absorbance spectrum was analyzed. In addition, the time course of bilirubin decrement was monitored during 16 h-incubation. We further measured the fluorescence intensity of bilirubin additive BDS incubated with BOD-containing Buffer A. Using the newly developed BOD-containing Buffer A for ILG method, CEC of bilirubin additive serum was evaluated. Finally, we measured CEC of 4 healthy individuals by conventional and the improved ILG method. The CEC value was standardized by fluorescence intensity of reference sample which was measured in every assay.

Results:

BOD contained in Buffer A could convert bilirubin to biliverdin from results of absorbance spectrum. According to the time course of bilirubin decrement, both free- and conjugated-bilirubin were oxidized to biliverdin in approximately 6 hours. By converting bilirubin to biliverdin, fluorescence light was weakened greatly. We confirmed that there was no significant difference of CEC values between non bilirubin-additive serum and bilirubin additive serum. Compared healthy individual CEC values measured by conventional and improved ILG method, all subjects showed significant decrease of CEC values from the improved method, and false positive error caused by bilirubin was ameliorated.

Conclusion:

In this study, we achieved to improve the positive error caused by bilirubin on CEC assay using ILG method. This improvement would contribute to more accurate CEC assessment by ILG method and advance the clinical application of this method.

A-210

Comparison of Two Lipoprotein(a) Methods: Validation, Sample Stability, and Patient Characteristics

J. Powers Carson[1], M. Corso[2]. *[1]Washington University in Saint Louis, Saint Louis, MO, [2]Washington University in St. Louis-Core Lab Clinical Studies, Saint Louis, MO*

Background: Lipoprotein(a) [Lp(a)] is now thought of as an independent risk factor for coronary heart disease, stroke, and calcific aortic valve stenosis, and drugs specifically targeting Lp(a) are showing promise in clinical trials. Our laboratory recently validated two commercially available methods and used them to characterize a randomly selected group of samples from an outpatient clinic in the United States. Since the assays have different stated sample stabilities, this was also compared. **Methods:** Both the Roche Lp(a) and the Randox Lp(a) immunoturbidimetric methods were run on a Roche cobas c501 platform across several months. In the U.S., the Roche assay is calibrated in mg/dL, while Randox provides both mg/dL and nmol/L units. Assays were tested for reproducibility, intermediate precision and accuracy. Stability of samples and AMR were established. Additionally, 78 residual samples were randomly selected to assess the percentage with high Lp(a) in our patient population. **Results:** Method validation showed acceptable within-run and between-run precision as both assays met or exceeded manufacturer's specifications. The stated AMR for the Roche assay is 6.0 – 80.0 mg/dL; linearity was verified through 90.0 mg/dL using the Randox calibrators. For the Randox assay, linearity was verified for the manufacturer's AMR of 5.2 – 206.0 nmol/L using estimated values assigned to the Roche calibrators. Both assays allow for x3 dilutions to extend the range. Method comparison of the Randox results (nmol/L) was performed with samples sent to Quest. Deming regression results were acceptable (r =0.995, slope 1.2, y-intercept – 2.3). The stated sample stability from Roche is 8 hours at room temperature and 48 hours at 2-8 °C; however, our results suggest 7 hours at room temperature and up to 10 days at 2-8 °C. Our study also extended the number of freeze-thaws to two. The Randox package insert gives no information on freeze-thaws or room temperature stability, but states stability at 4 °C for 14 days. In our assessment, some samples were not stable past 10 days at 2-8 °C. We found samples stable for 2 days at room temperature and up to one month at -20 °C. Three freeze-thaws was acceptable. Our patient population included 49 Caucasians, 19 Blacks, 2 Asians, 3 Hispanics, and 5 with race unknown. The median Lp(a) for Blacks was 151.1 nmol/L (65.1 mg/dL) while for Caucasians it was 17.9 nmol/L (14.5 mg/dL) for samples within range. The extended range with dilutions allowed all high values to be quantified; however, 12 were below the AMR for the Roche method and 8 below the AMR for Randox method. Based on the Roche assay, 20 (25.6%) subjects had Lp(a) >50 mg/dL and would be considered to have increased cardiovascular risk. By the Randox assay, the same 20 had an Lp(a) >100 nmol/L, while 19 had Lp(a) >125 nmol/L. **Conclusions:** Both assays met performance goals and showed no significant difference in % subjects with elevated risk for cardiovascular events using typical cut-offs. Our data suggests greater stability of samples at room temperature and additional stability to freeze-thaws when using the Randox assay.

A-213

Assessing the Function of High-Density Lipoprotein and the Risk of Cardiovascular Disease Using Fully Automated Immunoassay Analyzer, HISCL™

J. Kim[1], K. Murakami[1], A. Harada[1], R. Toh[2], K. Miwa[1], T. Iino[1], C. Suminaka[1], K. Sakaeda[1], M. Nagao[2], T. Ishida[2], K. Hirata[2]. *[1]Sysmex corporation, Kobe, Japan, [2]Kobe University, Kobe, Japan*

Background High-density lipoprotein (HDL) function has earned an attention as a way of assessing the risk of cardiovascular disease. Conventionally, cholesterol efflux capacity (CEC) has been used to measure the function of the HDL, but because of its time-consuming and complex procedure it has been withheld from being widely used in clinical fields. We have previously introduced an immunoassay-based technique that measures cholesterol uptake capacity (CUC) as a novel measure of HDL functionality. There we have demonstrated that CUC is capable of risk stratification in patients with coronary artery disease, in which coronary lesions was inversely associated with CUC (Fujimoto et al., Atherosclerosis 2022; Murakami et al., Scientific Reports 2023). However, since this method uses a specialized washing solution with mild surfactant to prevent delipidation from HDL rather than using the conventional washing solution, it had to be operated as a dedicated device. In other words, CUC could not be measured with automatic analyzer along with the existing immunoassay items. Here we propose an upgraded version that intends for IVD use. **Method** Automated immunoassay system, HISCL™ was used to measure CUC in serum. This rapid and sensitive immunoassay is based on anti-ApoA1 antibody coated magnetic beads that captures HDL particles from the sample, while HDL simultaneously takes in the PEGylated biotin-labeled cholesterol. After the cholesterol uptake reaction, magnetic beads are washed with the HISCL™ washing solution, a commercially available reagent used in conventional immunoassay. Then, using alkaline phosphatase labeled streptavidin and specific substrate, signals are detected. As for the samples, myeloperoxidase-mediated oxidized HDL, recombinant Lecithin-cholesterol acyltransferase (rLCAT) added HDL, and commercially available serum (as control) were measured using above mentioned method to evaluate functional change in HDL. **Results** Despite using the conventional washing solution, we were able to maintain specific signals corresponding to the dynamics of cholesterol probe by optimizing the reaction sequence and reducing the number of washing process. In this condition, the assay was linear between 0 and 50 nL of serum ($R2 > 0.99$), and CUC correlated with CEC ($r = 0.897$, $p < 0.01$). Furthermore, statistically significant difference ($p < 0.05$) in CUC level was seen with HDL in different functional states. In specific, CUC dropped with HDL oxidation while an opposite movement was found in HDL that's been treated with rLCAT, an enzyme that esterifies cholesterol thereby improves cholesterol uptake of HDL. This result suggests that this assay is capable of accurately depicting the function of HDL. In our previous measuring system, CUC demonstrated its utility in coronary risk stratification. Here, we were able to see a significant correlation ($r = 0.973$, $p < 0.01$) between our previous data and our current data with high reproducibility (Coefficient of Variation < 10 %). **Conclusion** Our results suggest that we are inching ever closer to expanding the research of HDL functionality to the clinical settings and diagnosing patients with high risk of cardiovascular disease.

A-214

Modified CDC Beta-Quantification Reference Method for HDL-C and LDL-C Determinations Using Isotope-Dilution Mass Spectrometry - Pilot Report

C. Cho[1], J. Seo[1], H. Lee[1], Y. Yun[2], J. Seo[2], H. Kim[2], S. Park[3], J. Kim[4]. *[1]Division of Chronic Disease Prevention, Korea Disease Control and Prevention Agency, Cheongju, Korea, Republic of, [2]Konkuk University School of Medicine, Seoul, Korea, Republic of, [3]National Cancer Center, Goyang, Korea, Republic of, [4]Yonsei University College of Medicine, Seoul, Korea, Republic of*

Background:

It is known that there is average positive 1.6% bias of total cholesterol by modified Abell-Levy-Brodie-Kendall (AK) colorimetric method, which have been the traceable reference measurement procedure (RMP) used by most of clinical guidelines, comparing to those by isotope-dilution mass spectrometry (ID-MS), the most accurate RMP. The CDC beta-quantification (BQ) reference method is currently the only RMP registered JCTLM database for high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) using the AK colorimetric assay after ultracentrifugation. We compared the modified BQ method using ID-MS assay after ultracentrifugation with the original CDC BQ reference method of HDL-C or LDL-C.

Methods:

For this study, 18 commutable serum pools according to the CLSI 37A and 6 external quality assessment survey materials operated by Cholesterol Reference Method Laboratory Network (CRMLN) were used. We used the same ultracentrifugation procedure as in the original CDC BQ reference method, and the bottom fraction cholesterol (BFC) and HDL-C from supernatant after apo B-containing lipoprotein precipitation using heparin/manganese were measured for each sample using both AK and ID-MS methods, then LDL-C was calculated as the difference between BFC and HDL-C. The comparability analysis between methods at multiple medical decision levels (MDL), and Passing-Bablok regression analysis for each analyte were performed.

Results:

Each Passing-Bablok regression equation of modified CDC BQ reference method *vs.* original CDC BQ reference method for three fractions, BFC, HDL-C, and LDL-C, were as follows; BFC was y (IDMS) = 0.996x (AK) - 0.342 (R = 0.999), for HDL-C was y (IDMS) = 1.007x (AK) - 1.037 (R = 0.995), and for LDL-C was y (IDMS) = 0.990x (AK) + 0.860 (R = 0.999). Both cholesterol results by two methods showed excellent correlation and agreement of the three fractions showing regression R larger than 0.995 and slope or intercept containing 1 and 0 in their 95% confidence interval. The mean differences of IDMS against AK method were -0.56% for BFC, -0.51 mg/dL for HDL-C, and -0.35% for LDL-C, respectively. When the regression prediction at MDLs, 40/50/60 mg/dL for HDL-C and 100/130/160 mg/dL for LDL-C were estimated, all the differences were confirmed to be within allowable level claimed by CRMLN member laboratory accreditation criteria, ±1mg/dL for HDL-C and ± 2.0% for LDL-C, showing equivalent results against CDC BQ reference method, but average negative bias of -0.72%, -1.59%, and -0.28% for BFC, HDL-C, and LDL-C, respectively are still present. These results of the modified BQ method using ID-MS assay may be promising, because the tedious and less accurate AK-assay can be switched to the ID-MS assay, not sacrificing trueness LDL-C or changing clinical guidelines, but quite large negative bias of HDL-C for some samples should require further investigation.

Conclusion:

We got comparable HDL-C and LDL-C results using modified BQ method using ID-MS assay with those by original CDC BQ reference method using AK colorimetric assay. The quite large negative bias of HDL-C for some samples require large-scale additional further multi-center study among CRMLN network laboratories to evaluate the results observed in our study.

A-215

Calculated LDL-cholesterol: Comparability of the extended Martin-Hopkins,Sampson-NIH, Friedewald and four other equations in South African patients

A. Carelse[1], H. M. Rossouw[2], N. Steyn[1], J. Martins[3], T. S. Pillay[3]. *[1]National Health Laboratory Service, Pretoria, South Africa, [2]Ampath Laboratory, Pretoria, South Africa, [3]University of Pretoria, Pretoria, South Africa*

Background: Ultracentrifugation for LDL-C (low-density lipoprotein cholesterol) measurement is the gold standard, but it is unsuitable for routine use. Direct LDL-C assays and predictive equations such as the Friedewald equation are thus used as alternatives. The Friedewald equation is known to be error-prone in hypertriglyceridaemic patients, as well as in LDL-C levels <1.8mmol/L (70 mg/dL) which has become increasingly attainable with the use of pro-protein convertase subtilisin/kexin type 9 inhibitors. This study evaluates the comparability of the Friedewald, extended Martin/Hopkins, Sampson/NIH and four other equations to a direct LDL-C assay to establish a suitable alternative to the Friedewald equation.

Methods: We analysed 44194 lipid profiles from a mixed South African population. The Friedewald, extended Martin/Hopkins and Sampson/NIH LDL-C and four other predictive equations (Vujovic, Puavilai, Delong, Anandaraja) were compared to the Beckman Coulter direct LDL-C assay. Non-parametric statistics were used to analyse the data.

Results: The extended Martin/Hopkins equation displayed the best correlation with direct LDL-C, having the most values fall within the desirable bias (59%) and total allowable error (89%) specifications. In patients with TG values between 4.5 mmol (400 mg/dL) and 9.0 mmol/L (800 mg/dL), the extended Martin/Hopkins equation was more likely to overestimate LDL-C levels, showing a positive median bias of 7.3%, while Friedewald (-21.2%) and Sampson/NIH (-9.8%) showed larger negative median biases as compared to the direct LDL-C assay. The direct LDL-C assay classified 13.9% of patients in the low LDL-C (1.0-1.8mmol/L) (39-70 mg/dL) category, in comparison to the Martin/Hopkins equation (13.4%), the Sampson equation (14.6%) and the Friedewald equation (16.0%). The extended Martin/Hopkins equation performed better than Sampson/NIH equation in the cohort.

Conclusion: We suggest the extended Martin/Hopkins equation as an alternative to Friedewald's equation and direct LDL-C assay. Careful consideration is advised as the choice of analyser platform for the lipid profile used in the equations may influence their performance

A-216

Measuring inflammation and cardiovascular markers at benchtop NMR using diffusion and relaxation edited experiments

J. Wist[1], P. Nitschke[1], R. Masuda[1], S. Lodge[1], H. Schaefer[2], C. Cannet[2], E. Holmes[3], J. K. Nicholson[1]. *[1]Murdoch University, Australian National Phenome Center, Perth, Australia, [2]Bruker, Rheinstetten, Germany, [3]Murdoch University, Center for Computational Systems Medicine, Perth, Australia*

Background:

Metabolic phenotyping is an established tool in systems medicine that captures a profile of one's individual health status and reflects the interaction between genes and external stressors. It uses analytical platforms such as NMR or MS to acquire molecular profiles, and modelling to extract actionable knowledge. Applying these techniques to a cohort of Australians infected by SARS-CoV-2 revealed a strong alteration in regions of 1D NMR spectra associated with lipoproteins and glycoproteins, and referred as Supramolecular Phospholipid Composite SPC (δ = 3.2 ppm) and Glyc (δ = 2.07 ppm). The latter is an established marker of inflammation. These results were later confirmed using larger cohorts from Spain (n = 525) and the UK (n = 1022). The urgent need for very rapid testing, at the early stage of the pandemic, prompted the development of bespoke NMR experiments able to measure this lipoproteins/glycoproteins signature without requiring complex modelling.

Methods:

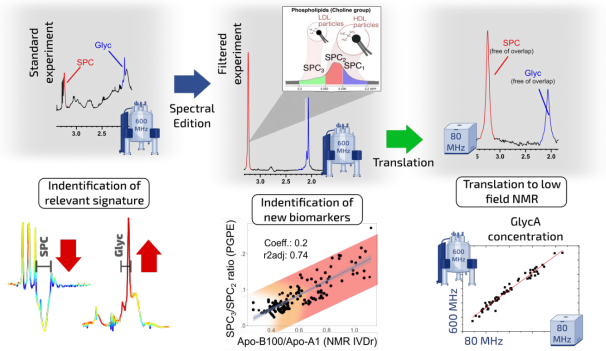
Physico-chemical properties of lipoprotein particles, such as diffusion, transverse and longitudinal relaxation rates, differ from low molecular weight metabolites. Therefore, an edited experiment JEDI (PGPE) was designed that combines diffusion, relaxation and scalar coupling editing blocks to produce a lipoprotein profile devoid of chemical noise (overlapping peaks) and where peaks give quantitative results.

Results:

The SPC peak was further broken down into 3 sub-regions that are related to the main subfraction HDL and LDL (choline headgroups of phospholipids). Interestingly the ratio of SPC_3/SPC_2 highly correlates with the Apo-B100/Apo-A1 ratio, an established cardiovascular risk marker that is increased in patients with COVID19. Longitudinal data suggest this latter remains elevated even 30 days after onset in some patients. Furthermore, the edited experiment performs equally well at low field using a benchtop NMR.

Conclusion:

Translation of a spectral signature relevant for monitoring COVID patients from high to low field NMR is possible using sophisticated editing techniques.



A-218

Analytical Performance Evaluation of Lipid Panel Assays on the Atellica CI 1900 Analyzer

K. Kolewe, S. Singh, C. Tyler, G. Arrode-Bruses, J. Rhea-McManus, J. Snyder. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput, integrated chemistry and immunoassay analyzer utilizing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica CH Creatinine (CREA_2)*, Enzymatic Creatinine (ECre_3)*, Urinary/Cerebrospinal Fluid Protein (UCFP)*, and Microalbumin_2 (µALB_2)* assays on the Atellica CI 1900 Analyzer*.

Methods: The Atellica CI 1900 CH CREA_2, ECre_3, UCFP, and µALB_2 assays use the same reagents and calibrators as the Atellica CH assays. Precision and method comparison (MC) were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 using native and contrived human urine samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. MC studies were performed according to CLSI EP09-A3. Individual native and contrived human urine samples were analyzed using the Atellica CH assays on both the Atellica CH and Atellica CI 1900 Analyzers.

Results: Representative precision and MC results observed from one reagent lot across indicated sample ranges are listed for each assay in the table below. Over the four assays tested, repeatability and within-lab %CVs were <5.1% and <6.0%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1.

Conclusion: Evaluation of the Atellica CH CREA_2, ECre_3, UCFP, and µALB_2 assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on the Atellica CH Analyzer.

	Precision				Method Comparison	
Urine Analyte (Assay)	Unit	Sample Range	Repeatability %CV range	Within Laboratory %CV range	Sample Range	Regression Equation for Comparative Assay
Creatinine (CREA_2)	mg/dL	61.48–190.68	0.3–0.4	1.6–3.2	4.37–239.13	y = 0.97x – 0.64 mg/dL
Enzymatic Creatinine (ECre_3)	mg/dL	43.01–155.37	0.2–0.3	1.0–2.2	3.22–242.92	y = 0.99x – 0.43 mg/dL
Protein (UCFP)	mg/dL	21.1–145.8	0.9–5.1	1.0–5.6	7.0–224.0	y = 0.93X + 1.9 mg/dL
Albumin (µALB_2)	mg/dL	3.3–36.8	0.6–1.2	1.8–5.9	0.3–37.3	y = 1.03x + 0.1 mg/dL

*The products/features mentioned here are not commercially available in all countries. Their future availability cannot be guaranteed.

A-219

A Multiplexed Targeted Assay for Gangliosides Using Liquid Chromatography-Tandem Mass Spectrometry

J. Kim, S. Byeon, D. Oglesbee, D. Matern, M. J. Schultz, A. Pandey. *Mayo Clinic, Rochester, MN*

Background: Gangliosides are a family of complex lipids that are molecules composed of glycosphingolipid with one or more sialic acid linked on the sugar moieties. Gangliosides are recognized to play an important role in many biological processes and the alterations in ganglioside profiles have been observed in some neurodegenerative diseases, neuropathies and lysosomal disorders. Gangliosidoses are a group of inherited lysosomal enzyme deficiencies characterized by an accumulation of incompletely degraded gangliosides in the lysosome. While the diagnosis of gangliosidoses can be accomplished by measurement of enzymatic activities and/or molecular genetic testing, biomarker analysis could facilitate monitoring affected patients, including those enrolled in clinical trials of new treatment approaches (ie., substrate reduction or gene therapies). We developed a rapid and sensitive LC-MS/MS assay that allows simultaneous quantification of multiple gangliosides and other glycosphingolipids and tested it in serum and plasma of patients with a diagnosis of GM2-gangliosidosis.

Methods: Gangliosides and other glycosphingolipids were extracted from 20 µL serum or plasma using chloroform:methanol (1:2, v/v). A multiplexed LC-MS/MS assay was developed that targeted several ganglioside species (GM3, GM2, GM1, GD3, GD2, GD1, GT3, GT2, GT1 and GQ1) along with several glycosphingolipid species (GlcCer, LacCer, Gb3, Gb4 and GA2). This assay was performed on a triple quadrupole mass spectrometer with scheduled MRM in both negative and positive ion modes after internal standards (GM3 18:0-d5, GM1 17:0, GA2 17:0 and Gb3 18:0-d3) were spiked into clinical samples.

Results: The multiplexed targeted assay applied to serum and plasma from controls (47 and 49 samples, respectively) and three GM2-gangliosidosis patients for identification and quantification of the targeted gangliosides and other glycosphingolipids. The abundance of GM2 and GM1 gangliosides inserum and plasma of patients with GM2-gangliosidosis was significantly higher than in the control group ($p < 0.0001$) whereas GM3 levels were significantly lower ($p < 0.005$). Other gangliosides including GD3, GD2, GD1 and GT1 and glycosphingolipids such as GlcCer, LacCer and Gb3 were not significantly different between these patients and controls.

Conclusion: A multiplexed targeted assay for glycosphingolipids including gangliosides was developed. This assay will be further validated for potential use in the diagnosis and monitoring of patients with additional gangliosidoses.

A-221

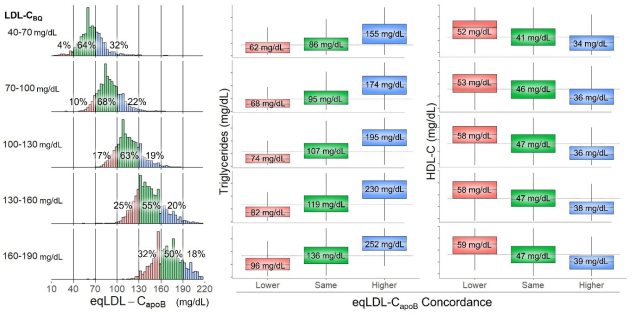
Evaluation of Apolipoprotein B Equivalent Low-density Lipoprotein Cholesterol Measurements

J. M. Hornseth, L. J. Donato, A. S. Jaffe, J. W. Meeusen. *Mayo Clinic, Rochester, MN*

Background: Low-density lipoprotein cholesterol (LDL-C) is the primary biomarker for assessing risk for atherosclerotic cardiovascular disease (ASCVD). However, multiple large studies have demonstrated greater accuracy using Apolipoprotein B (apoB). One obstacle to its widespread use is the lack of guideline endorsed apoB treatment targets. A recently published solution has proposed using population percentiles to calculate LDL-C using apoB values (PMID: 36366949). The LDL-C concentration correlated to an individual's ApoB percentile may more appropriately reflect ASCVD risk compared to lipid panel LDL-C. Here we evaluated this approach using a cohort where LDL-C was measured with the gold-standard beta-quantification method ($LDL\text{-}C_{BQ}$).

Method: Results from clinically ordered apoB, $LDL\text{-}C_{BQ}$, and lipid panel were included (n=18,852). Samples with triglycerides >1000 mg/dL or LpX were excluded. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and apoB were measured using Roche Cobas c501 analyzers. $LDL\text{-}C_{BQ}$ was performed using Beckman LE-80K ultracentrifuge and dextran sulfate-Mg/Cl precipitation. ApoB equivalent LDL-C ($eqLDL\text{-}C_{apoB}$) was calculated according to linear regression of LDL-C percentiles against apoB percentiles described by the equation $eqLDL\text{-}C_{apoB} = 1.45(apoB)-25$. Concordance was determined based on clinical thresholds for $LDL\text{-}C_{BQ}$ and $eqLDL\text{-}C_{apoB}$.

Results: Median (IQR) apoB and $LDL\text{-}C_{BQ}$ was 98 (79-119) mg/dL and 120 (94-150) mg/dL, respectively. $LDL\text{-}C_{BQ}$ and $eqLDL\text{-}C_{apoB}$ were concordant in 60% of samples overall.



Discordance between eqLDL-C_{apoB} and LDL-C_{BQ} shifted from primarily overestimating to underestimating as LDL-C_{BQ} increased. Discordant eqLDL-C_{apoB} was directly associated with TG and inversely associated with HDL-C.

Conclusion: The eqLDL-C_{apoB} calculation is a useful tool to present apoB results in a more familiar context of LDL-C-equivalent units. The concordance findings largely confirm previously published results using eqLDL-C_{apoB}. Furthermore, the pattern of HDL-C and TG suggests that a discordantly elevated eqLDL-C_{apoB} is an indicator of a higher risk lipid profile. More investigation is needed to determine if eqLDL-C_{apoB} is appropriate for widespread adoption.

A-222

Comparative analysis of clinical and laboratory parameters of Brazilian patients with severe hipertriglyceridemia

C. L. Costa[1], M. C. Castelo[2], V. R. Veras[1], L. T. Ramos[1], V. O. Fernandes[1], R. S. Lima[1], M. P. Vasconcelos[1], G. P. Lima[1], J. S. Araujo[1], A. D. Montenegro[1], L. L. Queiroz[1], C. M. Ponte[2], A. C. Flor[1], I. d. Melo[1], A. C. Lopes[3], R. M. Montenegro Junior[1]. *[1]UFC, Fortaleza, Brazil, [2]DASA, Fortaleza, Brazil, [3]DASA, Florianopolis, Brazil*

Background: The presence of elevated and sustained levels of triglycerides (TG) should raise the suspicion of primary causes of hypertriglyceridemia (HT), in which related genetic mutations would be identified. Thus, the aim of this study was to compare clinical and laboratory parameters of Brazilian patients with different etiologies of severe hypertriglyceridemia.

Methods: All patients with severe HT (considered as detection of TG above 880mg/dL on at least 2 occasions) followed in the dyslipidemia outpatient clinic of Federal University of Ceará/Brazil, whose molecular study to investigate genetic variants potentially related to hypertriglyceridemia and pancreatitis has been previously performed (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) were evaluated.

Results: Sixty-six patients were evaluated, identifying 39 (59%) with severe primary HT. Familial Chylomicronemia Syndrome (FCS) was identified in 14 (36%) of the 39 cases of severe primary HT. The main findings are shown in table 1. Compared with other etiologies, FCS patients presented an increased, precocious and recurrent incidence of pancreatitis, earlier detection and higher levels of TG. In patients with severe HT without identification of primary causes, a high incidence of alcoholism and diabetes was observed, with a tendency to higher glycated hemoglobin values among these, in addition to median Body Mass Index compatible with obesity.

Conclusion: FCS patients had the most severe clinical presentation of HT. The high prevalence of secondary factors for HT in patients with a negative genotype reinforces the indication of adequate management of these variables even in cases of primary etiology, aiming at not overlapping pathological mechanisms. In cases of severe HT, metabolic control is still a challenge.

Table 1 – Comparative analysis of clinical and laboratory parameters in different etiologies of severe hypertriglyceridemia.

	All patients (n=66)	Primary Etiologies						Negative genotype (n=27)
		Familial Chylomicronemia Syndrome (n=14)	Familial Combined Hyperlipidemia (n=5)	Type V Hyperlipoproteinemia (n=4)	Congenital Generalized Lipodystrophy (n=6)	Familial Partial Lipodystrophy (n=8)	Primary Hypoalphalipoproteinemia (n=4)	
Age (yo)	41 ± 18 (42)	33 ± 20 (31)	49 ± 21 (61)	54 ± 10 (52)	25 ± 12 (29)	41 ± 13 (37)	49 ± 9 (48)	46 ± 16 (46)
Gender (Female) n (%)	42 (64%)	9 (64%)	5 (100%)	2 (50%)	4 (67%)	5 (83%)	4 (100%)	13 (48%)
Parental consanguinity n (%)	20 (30%)	7 (50%)	3 (60%)	2 (50%)	4 (67%)	1 (17%)	1 (25%)	2 (7%)
Diabetes Mellitus n (%)	37 (56%)	4 (29%)	3 (60%)	1 (25%)	6 (100%)	5 (83%)	1 (25%)	17 (63%)
Glycohemoglobin A1c (%) Diabetics Nondiabetics	8,7 ± 2,0 (8,9) 5,5 ± 0,6 (5,5)	6,5 ± 0,5 (6,3) 5,2 ± 0,3 (5,2)	8,7 ±1,5 (9,4) 5,1 ± 0 (5,1)	9,5 (9,5) 5,9 ± 0,36 (5,9)	7,0 ± 2,2 (6,5) -	9,2 ± 2,3 (8,7) 4,7 (4,7)	11,0 (11) 5,8 ± 0,7 (5,5)	9,5 ± 1,5 (9,6) 5,8 ± 0,6 (5,8)
Body Mass Index (kg/m²)	26 ± 7 (23)	20 ± 5 (20)	25 ± 7 (23)	29 ± 1 (30)	23 ± 2 (23)	26 ± 6 (26)	29 ± 5 (28)	28 ± 5 (30)
Alcoholism n (%)	23 (40%)	2 (14%)	0	1 (25%)	0	1 (33%)	2 (50%)	17 (65%)
Hypertriglyceridemia detection (yo)	28 ± 18 (30)	7 ± 11 (0.9)	35 ± 24 (42)	35 ± 19 (35)	12 ± 8 (15)	29 ± 19 (27)	38 ± 13 (40)	36 ± 13 (35)
Pancreatitis n (%)	22 (33%)	7 (50%)	2 (40%)	1 (25%)	2 (33%)	0	1 (25%)	9 (33%)
Episodes of pancreatitis per affected patient (n)	3 ± 3 (3)	4 ± 3 (3)	2± 1 (2)	3 (3)	2 ± 0 (2)	-	1 (1)	3 ± 2 (3)
Age at first episode of pancreatitis (yo)	28 ± 12 (28)	22 ± 5 (23)	34 ± 9 (33)	34 (34)	17 (17)	-	37 (37)	32 ± 16 (32)
Maximum Triglycerides (mg/dl)	4721 ± 7129 (2392)	10012 ± 13519 (3997)	4383 ± 6012 (1872)	2267 ± 1425 (1910)	3217 ± 3423 (1215)	2112 ± 1844 (992)	3801 ± 12562 (3407)	3453 ± 12508 (2457)
Minimum Triglycerides (mg/dl)	443 ± 510 (290)	792 ± 589 (668)	170 ± 87 (171)	286 ± 73 (284)	311 ± 263 (240)	157 ± 120 (113)	151 ± 57 (143)	473 ± 580 (316)

Continuous variables were expressed as mean ± standard deviation (median) and categorical variables as relative and absolute frequency

A-223

Lipoprotein (a) Profile in Patients with Diabetes and Coronary Artery Disease

M. C. Castelo[1], V. O. Fernandes[2], J. L. Lima Verde[2], M. S. Ballalai[2], A. D. Montenegro[2], R. M. Montenegro Júnior[2], A. C. Lopes[3]. *[1]Diagnósticos da América S.A (DASA), Universidade Federal do Ceará, Hospital de Messejana Dr. Carlos Alberto Studart Gomes, Fortaleza, Brazil, [2]Universidade Federal do Ceará, Fortaleza, Brazil, [3]Diagnósticos da América S.A (DASA), fortaleza, Brazil*

Background: Changes in the lipid profile that cause an increase in cardiovascular risk are basically those that occur with an increase in apolipoprotein B-100 (apoB). This lipoprotein is present in LDL, VLDL, IDL and lipoprotein a or Lp(a). Lp(a) is a molecule similar to LDL being formed by the junction of apoB with apolipoprotein A. Lp (a) levels are virtually independent of diet or environmental factors. Around 90% is defined by the patient's genetic heritage. Lp(a) has the potential to cause atherosclerosis by LDL-like mechanisms (vascular wall impairment, oxidation), However, there are several other mechanisms that are proposed to explain the increased cardiovascular risk caused by increased Lp(a) levels. Some would result in a high risk of thrombogenicity, others of atherogenesis. The aim of this study was to describe the Lp(a) profile in individuals with diabetes and coronary artery disease.

Methods: This is a descriptive cross-sectional study carried out in 2 reference centers in the care of patients with diabetes and high cardiovascular risk in Fortaleza-Ceará, Brazil.We evaluated lp(a) level was and the presence of multivessel coronary artery diseaseThe statistical analysis of the data was performed with the Microsoft Excel program.

Results: Of the 34 patients evaluated, 47% (16) were male and 53% (18) female, with a mean age of 58.3 ± 9.34 years old, The mean time that the coronary event had occurred was 7.85 ± 6.27 years, and the mean age at the first thrombotic event was 51.56± 9.311 years old. There was found 44.1% of dyslipidemia before the first thrombotic and 61.7% had multivessel coronary artery disease. Lp(a) median was 23.5mg/dL (min <3 - max 149).70.5% of the evaluated patients had Lp(a) < 50mg/dL, 20.5% between 50-90mg/dL, and 8.8% had Lp(a) > 90mg/dL.

Conclusion: Most patients with diabetes studied had normal or subnormal levels of lp(a) even though they were patients at very high cardiovascular risk. More studies need to be carried out, but this finding could suggest that in diabetic individuals, as well as LDL-c, we could have lower cutoff points than in the general population.

A-224

Comparative analysis of clinical and laboratory parameters of different subtypes of Familial Partial Lipodistrophy

V. R. Veras[1], M. C. Castelo[2], C. L. Costa[1], L. T. Vasconcelos[1], V. O. Fernandes[1], R. S. Lima[1], M. P. Vasconcelos[1], G. P. Lima[1], J. S. Araujo[1], A. D. Montenegro[1], C. M. Ponte[2], L. L. Queiroz[1], I. d. Melo[1], A. C. Flor[3], A. C. Lopes[4], R. M. Montenegro Junior[1]. *[1]UFC, Fortaleza, Brazil, [2]DASA, Fortaleza, Brazil, [3]UFC, Florianopolis, Brazil, [4]DASA, Florianopolis, Brazil*

Background: Lipodystrophy is a disease characterized by changes in body fat deposits. They are classified according to the extent of fat loss (generalized or partial) and the form of inheritance (inherited or acquired). Family partial lipodystrophy (FPL) are rare forms characterized by distribution of centripetal body adiposity, poorly controlled diabetes mellitus (DM) and severe dyslipidemia.

Methods:Fourty-two FPL patients were evaluated, whose were followed in the lipodistrophy outpatient clinic of the Federal University of Ceará/Brazil. Twenty-two had previous positive molecular test for FPL, among which 19 had an LMNA mutation and 3 had a PPARG mutation. Twenty patients had a negative genetic test but met clinical criteria for lipodystrophy, which were thigh skinfold <20mm for females and <10mm for males or fat mass ratio (FMR) between trunk and lower limbs >1.2. Eight patients in the negative genotype group met criteria for Köbberling Syndrome (Köb index >3.477). The others 12 patients were classified as FPL indeterminate subtype (FPLX).

Results: PFL+ patients perceived the lipodystrophic phenotype before the other groups. All groups had high A1c medians, with the Köbberling group having the highest median (9.1%). However, patients with FPL+ had a higher median insulin dose. Diabetic polyneuropathy was the most prevalent complication in the three groups. Median peak triglycerides were similar for the three groups, with the Köbberling group having the greatest variability. There no pancreatitis episodes in the FPL+ group, but Köbberling e FPLX did. The main findings are shown in table 1.

Conclusion: FPL is a disease with a broad spectrum of phenotype and genotype. It is usually associated with poor DM control, early DM complications and severe hypertriglyceridemia, as our patients demonstrated. In these cases, metabolic control is still a challenge.

Table 1 – Comparative analysis of clinical and laboratorial parameters in patients with partial familial lipodystrophies

		GROUP							
	N	All patients (n=42)	FPL+ (n=22)	Köbberling (n=8)	FPLX (n=12)	p value[2]	FPL+ vs. Köbberling	FPL+ vs. FPLX	Köbberling vs. FPLX
Age (yo)	42	28 ± 12 (25)	20 ± 6 (20)	33 ± 16 (28)	35 ± 11 (30)				
Gender (Female) n (%)	42	(52%)	18 (82%)	8 (100%)	11 (92%)				
Diabetes	42	35 (83%)	16 (73%)	8 (100%)	11 (92%)	0.573	0.10	0.2	0.6
Onset lipodystrophic phenotype until the DM diagnosis	37	9 ± 21 (9)	22 ± 21 (12)	2 ± 16 (1)	-3 ± 14 (0)	**0.004**	0.017	0.001	0.6
Diabetes duration	36	13 ± 10 (10)	10 ± 11 (8)	14 ± 7 (14)	17 ± 10 (18)	0.064	0.3	0.087	0.6
Diabetic retinopathy	41	10 (24%)	3 (14%)	3 (38%)	4 (33%)	0.560	0.9	0.6	0.6
Diabetic polineuropathy	41	20 (49%)	7 (33%)	4 (50%)	9 (75%)	0.076	0.4	0.023	0.3
Coronary arterial disease	41	7 (17%)	1 (4.8%)	1 (12%)	5 (42%)	**0.025**	0.6	0.006	0.078
Diabetic nefropathy	38	9 (24%)	6 (33%)	1 (12%)	2 (17%)	0.420	0.3	0.3	0.8
A1c (%)	40	8.12 ± 2.33 (7.95)	7.58 ± 2.56 (7.30)	8.99 ± 2.39 (9.20)	8.45 ± 1.74 (8.50)	0.141	0.2	0.3	0.6
Insulin (U/Kg)	22	1.47 ± 0.57 (1.52)	1.71 ± 0.62 (1.77)	1.43 ± 0.43 (1.52)	1.35 ± 0.61 (1.24)	0.540	0.4	0.2	0.8
Onset dyslipidemia (yo)	39	38 ± 12 (37)	37 ± 16 (30)	40 ± 8 (44)	37 ± 6 (38)	0.704	0.5	0.9	0.6
Hypertriglyceridemia	41	35 (85%)	17 (81%)	7 (88%)	11 (92%)	0.844	0.7	0.4	0.8
Triglycerides peak (mg/dL)	41	1,007 ± 1,897 (326)	797 ± 1,260 (326)	1,512 ± 3,392 (330)	1,038 ± 1,616 (328)	0.983	0.4	0.7	0.6
Previous pancreatitis	42	4 (9.5%)	0 (0%)	1 (12%)	3 (25%)	**0.038**	0.3	0.018	0.3

Continuous variables were expressed as mean ± standard deviation (median) and categorical variables as relative and absolute frequency

Microbiology and Infectious Disease

A-227

First Brazilian External Quality Assessment Program for Monkeypox molecular tests: results from two surveys

S. Lima[1], G. Barra[2], J. A. Poloni[1], J. Barroso[1], R. Lopes[1], M. Mendes[3], R. Montenegro[1], D. Jerônimo[1], F. Brazão[4], I. Biasoli[1], V. Biasoli[1]. *[1]Controllab, Rio de Janeiro, Brazil, [2]Sabin Medicina Diagnóstica, Brasília, Brazil, [3]Divisão de Laboratório Central do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [4]Laboratório Ruth Brazão, Belém, Brazil*

Background: The WHO declared the current Monkeypox (Mpox) outbreak a public health emergency of international concern. PCR test is the preferred diagnostic assay. External Quality Assessment Programs (EQAP) provide an independent assessment of the effectiveness of analytical systems, especially with emerging pathogens. There are a few references about EQAP for Mpox. Here, we report the results of the first Mpox EQAP conducted by a Brazilian EQAP provider, which follows the criteria of ABNT NBR ISO/IEC 17043:2011.

Methods: The quality control samples were inactivated lyophilized suspension of Vero cells (BCRJ 0245/ATCC CCL-81) infected with viable Mpox virus particles and cultured under BSL-3 conditions. The EQAP surveys were conducted in September and October/2022. In the first round, two samples were sent - one negative and one positive [cycle threshold (CT) of 23]. In the second round, 5 samples with different viral loads were sent (CT of 24.9/negative/26.5/34.8/34.6). The percentage of correct results (%CR), sensitivity (SE), specificity (SP), false positive (FP), and false negative (FN) were calculated for each sample, and the performance of laboratory-developed test (LDT) or in vitro diagnosis (IVD) was compared.

Results: A total of 49 laboratories (38 from Brazil) submitted 319 datasets with qualitative results. In the first round, 30 laboratories used LDT methods and 11 IVD methods, and in the second round, it was 32 and 14, respectively. The %CR ranged from 81.4-100%, SE from 83.3-100%, SP from 95.45-97.7%, FP from 2.3-4.55%, and FN from 2.3-16.3%. Samples with higher FN have lower viral loads (p=0.00044).The overall % of CR, SE, SP, FP, and FN for LDT methods were 76.7-100%, 79.3-100%, 93.3-100%, 0-6.7%, and 0-20%, and for IVD methods were, 90.9-100%, 90.9-100%, 90.9-100%, 0-3.1%, and 0-7.7%, respectively. No statistical difference was found between methods regarding these parameters.

Conclusion: This is the first report of EQAP of Mpox molecular testing from Brazil. The EQAP showed that Mpox PCR had an overall good accuracy and performance. FN rates ranged from 2.3% to 16.3%, suggesting there is room for assay improvement, especially with samples with low viral loads. LDT and IVD tests presented similar performance.

A-228

Performance Evaluation of the LabGenius C-CT/NG-BMX assay for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Y. Lim, O. Kweon, M. Lee. *Chung-Ang University, Seoul, Korea, Republic of*

Background: The LabGenius C-CT/NG-BMX assay (LabGenius CT/NG; BIOMEDUX, Gyeonggi-do, Republic of Korea) is a recently developed real-time polymerase chain reaction assay that can simultaneously detect sexual transmitted pathogens (Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG)) in vaginal swab specimen or urine specimen.

Methods: To evaluate the analytical performance, a total of 343 vaginal swab and 167 urine specimens were selected to compare the LabGenius CT/NG with the BD MAX™ CT/GC/TV (Becton, Dickinson and Company, NJ, USA). In addition, using binomial logistic regression analysis, we estimated the limit of detection which 95% (Lod95) detection rate were achieved in the LabGenius CT/NG.

Results: The LabGenius CT/NG showed almost perfect agreement with the BD MAX™ CT/GC/TV (κ=0.826 for CT, κ=0.944 for NG), and overall agreements between two assays were 91.6% for CT and 97.8% for NG, respectively. The LoD_{95} values using vaginal swab and urine specimens were 9.9 and 7.8 copies/uL for CT, and 7.6 and 5.1 copies/uL for NG, respectively.

Conclusion: In this study, LabGenius CT/NG demonstrated acceptable analytical performance compared to BD MAX™ CT/GC/TV, and this assay could be a cost-effective option for routine and follow-up test for detection of sexual transmitted pathogens.

A-229

Immune Biomarkers Associated with COVID-19 Disease Severity in an Urban, Hospitalized Population

A. B. Chambliss[1], M. Aljehani[2], B. Tran[2], X. Chen[2], E. Elton[2], C. Garri[2], N. Ung[2], N. Matasci[2], M. E. Gross[2]. *[1]University of Southern California, Los Angeles, CA, [2]Lawrence J. Ellison Institute for Transformative Medicine, Los Angeles, CA*

Background: We sought to identify immune biomarkers associated with severe Coronavirus disease 2019 (COVID-19) in patients admitted to a large public county hospital during the early phase of the SARS-CoV-2 pandemic. We hypothesized that we could identify clinically relevant immune markers at the time of initial hospital admission that could be used to predict the course of COVID-19 illness.

Methods: The study population consisted of SARS-CoV-2 positive patients admitted for COVID-19 (n=58) or controls (n=14) at the Los Angeles County University of Southern California Medical Center between April-December 2020. Immunologic markers including chemokine/cytokines (IL-6, IL-8, IL-10, IP-10, MCP-1, TNFα) and serologic markers against SARS-CoV-2 antigens (including spike subunits S1 and S2, receptor binding domain (RBD), and nucleocapsid (N)) were assessed in serum collected on the day of admission using custom MILLIPLEX® immunoassay panels. Result values were computed using mean fluorescent intensity in individual samples fit to a standard curve using a 5PL logistic formula with power law variance. Comparison of patient demographic, clinical, cytokines and immunoglobulins characteristics between mild vs. moderate/severe COVID-19 groups were conducted using Wilcoxon tests for continuous variables and Chi-square tests for categorical variables. Linear support vector machine models were fitted to perform the binary classification task of predicting mild vs. moderate/severe COVID-19 using the python library scikit-learn.

Results: SARS-CoV-2 antibody levels were significantly elevated in patients with the highest COVID-19 disease severity, with IgM S1, IgG N, IgG RBD, IgG S1, and IgG S2 showing statistical significance between mild vs. moderate/severe disease group medians (p=0.037, 0.032, 0.007, 0.003, and 0.015, respectively). Of the chemokines/cytokines tested, only IP-10 showed significance across the disease groups (medians 640.8 pg/mL in mild, 493.3 pg/mL in moderate/severe, and 259.9 pg/mL in control, overall p=0.005). The linear support vector machine model achieved an accuracy of 64% and an AUROC of 0.81 in predicting COVID-19 severity status. The most important clinical variables for predicting disease severity were white blood cell count, diastolic blood pressure, and platelet count, while the most important serologic markers were IgG anti-SARS-CoV-2 N, S1, S2, TNF-α, IP-10, and IL-10.

Conclusion: Our results suggest that IP-10 and anti-SARS-CoV-2 antibody measurements could be useful to identify patients most likely to experience the most severe forms of the disease. Strengths of this study include a focus on a racially and ethni-

cally diverse patient population and a combined analysis of both cytokine/chemokine and immune response (antibody) biomarkers. However, we emphasize that our subjects were enrolled at a time before widespread vaccination against SARS-CoV-2.

A-231

Prediction of Severe COVID-19 Based on Routine Biomarker Assessment

H. Kapoor[1], C. Bi[1], A. E. Salm[1], J. Szymanski[2], Y. D. Goldstein[2], L. R. Wolgast[2], G. Rosenblatt[2], A. S. Fox[2], M. H. Kroll[1]. *[1]Quest Diagnostics, Secaucus, NJ, [2]Montefiore Medical Center, Bronx, NY*

Background: Disease courses in COVID-19 patients vary widely. Prior studies did not focus on initial values of various laboratory biomarkers, instead used data throughout the disease course. Prediction of disease severity based on parameters at the initial diagnosis would aid appropriate management. Objective of our study was to develop predictive models of COVID-19 severity based on demographic, clinical, and laboratory data collected at initial patient contact after diagnosis of COVID-19.

Methods: We evaluated de-identified demographic, clinical, and routine biomarkers data from 14,147 patients with COVID-19 diagnosed by polymerase chain reaction SARS-CoV-2 testing at Montefiore Health System from March 2020 to September 2021. We generated models predicting severe disease (death or more than 90 hospital days) vs. mild disease (alive and fewer than 2 hospital days), starting with 58 parameters, by backward stepwise logistic regression.

Results: Of the 14,147 patients, 18% had severe, 24% had mild outcomes and the rest were moderate (cases who were alive but in the hospital for 2-89 days). We identified 4 proficient models in predicting patient outcomes: Inclusive model A, with the largest number (37) of parameters; Receiver Operating Characteristics (ROC) Model B, including 9 parameters with the highest Area under Curve (0.85); the Specific Model C, including 10 parameters with the highest specificity (81%) and Positive Predictive Value and the Sensitive Model D including 9 parameters with the highest sensitivity (91%) and Negative Predictive Value (Table 1 and 2). The parameters that remained in all models were age, albumin, diastolic blood pressure, ferritin, lactic dehydrogenase, socioeconomic strength, procalcitonin, b-type natriuretic peptide, C reactive protein, d dimer and platelet count.

Conclusions: These findings suggest that certain clinical parameters and routine laboratory biomarkers within the specific and sensitive models could benefit healthcare providers, in their assessment of disease severity and clinical management of COVID-19 infection.

Table 1: The parameters included in each model

	Inclusive Model	AUC Model	Specificity Model	Sensitivity Model
Parameters included in Each Model:	A	B	C	D
Age	X	X	X	X
Albumin	X	X	X	X
Diastolic BP	X	X	X	X
Ferritin	X	X	X	X
Lactate dehydrogenase	X	X	X	X
Social Economic Status	X	X	X	X
Procalcitonin	X	X	X	X
b natriuretic peptide	X	X	X	
Platelet count	X	X		X
C Reactive Protein	X		X	X
d dimer	X		X	
Parameters included only in model A:				
Alanine aminotransferase				
Alkaline phosphatase	Bilirubin total	Creatinine	Lymphocyte count	Respiratory rate
Anion gap	BMI	eGFR	Monocyte count	Systolic BP
Aspartate aminotransferase	Calcium	Ethnicity	Neutrophil count	Sodium
Bilirubin conjugated	Carbon dioxide	Gender	Potassium	Temperature
Urea nitrogen	Chloride	Glucose	Pulse O2	Total protein

AUC, Area Under Curve; BMI, body mass index; DBP, diastolic blood pressure; ROC, receiver operating characteristics; RR, respiratory rate; SBP, systolic blood pressure; SES, socioeconomic strength.

Table 2. Prediction performance of selected models

Model	AUC	Sensitivity	Specificity	PPV	NPV	Parameters	Training set #	Test set #	Prevalence	Accuracy
A: Inclusive	0.81	78%	73%	72%	79%	37	311	134	47%	75.4%
B: ROC	0.85	86%	68%	71%	84%	9	353	150	47%	76.7%
C: Specific	0.83	77%	81%	78%	80%	11	348	149	47%	79.2%
D: Sensitive	0.80	91%	60%	62%	90%	9	529	226	42%	73.0%

AUC, Area Under Curve; PPV, positive predictive Value; NPV, Negative predictive value

A-233

Novel microfluidic system for antimicrobial susceptibility testing

S. Bajer-Borstyn, G. Zurawska. *Scope Fluidics SA, Warsaw, Poland*

Background Aiming to enhance antibiotic susceptibility testing (AST), BacterOMIC combines microdilution AST with cutting-edge microfluidics to quantitatively evaluate all clinically relevant antibiotics on a single disposable panel. In this study, we evaluated the performance of BacterOMIC in determining minimum inhibitory concentrations (MICs) of selected agents against Gram-positive and Gram-negative bacteria and in detecting the presence of extended spectrum beta-lactamase (ESBL) produced by Enterobacterales. **Methods** Using the BacterOMIC UNI Panel, we characterized bacterial strains collected from Polish hospitals in 2020-2022 . We inoculated the BacterOMIC panels with bacterial suspensions in cation-adjusted Mueller-Hinton broth at a density of 5*10^5 CFU/mL. The panels then incubated in an analyzer for 16 h, and results were automatically calculated via built-in analytical software. For comparison, we obtained reference MIC data from laboratories contracted for determining strain MIC values via microdilution. We calculated the essential agreement (EA%) and bias according to ISO20776.We also qualitatively assessed several antibiotics via our panel. Categorical agreement (CA%) and discrepancies were determined for these antibiotics according to ISO20776.To assess ESBL detection, we compared BacterOMIC results with data obtained in our internal laboratory using the MIC Test Strip ESBL (Liofilchem). **Results** With the BacterOMIC system, CA% values exceeded 90% for amoxicillin, benzylpenicillin, ceftazidime/avibactam, ciprofloxacin, erythromycin, gentamicin, meropenem, moxifloxacin, tedizolid, and vancomycin. EA% values exceeded 90% for the following antimicrobials, with a bias of less than 30%.

By comparing with our reference method, we found that BacterOMIC detected ESBL with a specificity of 92% (confidence interval [CI] 95%; 81%-98%) and sensitivity of 97% (CI 95%; 84%-99%). **Conclusions** Our data demonstrate that BacterOMIC provides reliable MIC and ESBL results for assessing the drug susceptibility of Gram-positive and Gram-negative bacteria against 25 agents in a single panel. Thus, the BacterOMIC system provides a valuable tool for automated AST systems.

Antibiotic	Bacteria group	EA%	Bias%
Amikacin	Enterobacterales	95.24	-8.48
Amoxicillin-clavulanic acid	Enterobacterales	90.72	12.36
Ampicillin	Enterobacterales	92.38	13.19
Azithromycin	Staphylococcus	97.89	22.79
Cefazolin	Enterobacterales	91.01	2.00
Cefepime	Pseudomonas	95.83	8.91
Ceftriaxone	Enterobacterales	90.38	16.86
Cefuroxime intravenous	Enterobacterales	91.59	4.98
Clarithromycin	Staphylococcus	91.04	24.17
Clindamycin	Enterococcus	100.00	-4.17
Levofloxacin	Staphylococcus	100.00	9.71
Linezolid	Staphylococcus	91.92	24.73
Ofloxacin	Staphylococcus	92.75	15.89
Tobramycin	Enterobacterales	90.83	-8.40

A-234

Self-collected vaginal swabs for high-risk hpv testing in the us: validation of pre-analytical variables

M. Qi[1], A. J. Duque[2], T. S. Lorey[2], A. Naranjo[1], M. Rummel[1], J. M. Schapiro[2], B. J. Suh-Burgmann[3], D. N. Greene[1]. *[1]LetsGetChecked Laboratories, Monrovia, CA, [2]The Permanente Medical Group, Kaiser Permanente Regional Reference Laboratory, Berkeley, CA, [3]The Permanente Medical Group, Department of Research, Oakland, CA*

Introduction: Several clinical trials have illustrated that primary HPV screening with unsupervised self-collected vaginal swabs is clinically appropriate; however, there is little data regarding quantitative changes imposed by pre-analytical components when samples are self-collected. Here we took a detailed analysis of the variables related to self-collected vaginal swabs to calculate analytical acceptability metrics and to identify limitations that can be improved to aid in modern cervical cancer screening pathways. **Methods:** HPV was evaluated in self-collected dry vaginal swabs or paired self-collected vaginal/provider-collected endocervical swabs using an assay that is FDA-approved for provider-collected HPV primary screening. The assay includes amplification of endogenous hemoglobin subunit beta gene (HBB;beta-globin) as an internal control. Paired provider/self-collected samples (n=144) were compared for HPV and beta-globin detection. Self-collected dry vaginal swabs (n=15 participants;5 swabs/participant) were used to calculate intra-individual variability in cycle threshold (Ct) between swabs. A second set of self-collected swabs (n=20 participants;3 swabs/participant) were challenged using winter or summer simulation with temperature cycling designed to mimic extreme seasonal fluctuations. Ct values for seasonal challenges were compared to unchallenged swabs. Various approaches were used to optimize collections: (1) a time course to see if the number of hours a self-collected vaginal swab remained dry before suspending in buffer influenced detection; (2) an alternate internal control (protein only RNase P catalytic subunit gene, PRORP) was amplified to exclude sample-specific inhibitor(s); (3)participants were provided an instructional video to aid collection. **Results:** Most samples were concordant between

provider and self-collect (n=60 HPV positive; n=60 HPV negative). There were 20 discrepant samples: 12 negative for HPV with provider-collected, but positive for HPV using self-collected swabs and 4 positive for HPV with provider-collected, but negative for HPV using self-collected swabs. All discrepancies were due to low viral load; for provider and self-collected swabs the average Ct (SD) in discrepant samples was 31.4 (3.5) and 33.1 (2.7), respectively, for HPV and 25.8 (2.1) and 27.1 (2.6), respectively, for beta-globin. The average self-collected intra-individual % CV for HPV and beta-globin was 3.5% and 2.7%, respectively. The average HPV Ct for swabs that cycled through the summer, winter or unchallenged conditions was 28.3 (SD=5.9), 27.2 (SD=7.1), and 25.7 (SD=3.1), respectively. The average beta-globin Ct for swabs that cycled through the summer, winter or unchallenged conditions was 26.8 (SD=2.7), 26.7 (SD=3.0), and 26.0 (SD=2.2), respectively. The time a sample remained dry did not influence detection. RNAse P amplification indicated results with invalid beta-globin were a function of cellularity not inhibition. The instructional video improved collection by ~8.5%; 61/64 (95.3%) 125/144 (86.8%) were valid with and without the video, respectively. **Conclusions:** Self-collected vaginal samples are a convenient and discreet way to screen for cervical cancer. Understanding the pre-analytical/analytical limitations can improve HPV detection.

A-235

Results and Interpretation: Serology Testing Platforms and Assays Compatibility Assessment for MAS™ SARS-CoV-2 IgG Quality Controls

H. Wang, N. Krishnaswami, D. Crandall, R. Hu. *Thermo Fisher Scientific, Fremont, CA*

Background: Serology testing of antibodies against SARS-CoV-2 viruses serves many purposes: disease diagnosis, on-going and surveillance, epidemiologic and vaccine studies, etc. There was a critical need to provide standardized quality control materials for development and harmonization across different test methods and IVD platforms. The MAS™ SARS-CoV-2 IgG Controls are human plasma-based materials containing IgG antibodies against SARS-CoV-2. The control offers commutability by mimicking patient samples and is intended to be used for monitoring serological assays, providing confidence in routine test results, test methods troubleshooting and system errors identification. We report here the compatibility assessment of MAS™ SARS-CoV-2 Controls testing on different assays and platforms.

Methods: The platforms and assays compatibility was assessed for the MAS™ SARS-CoV-2 IgG Positive and Negative Controls across all 11 assays and platforms listed as below. These serology assays target either total antibodies (IgA, IgM, IgG), IgM/IgG, or IgG against either the SARS-CoV-2 nucleocapsid (N), RBD or spike (S) proteins. Replicated samples were tested on each instrument for statistical confidence.

Manufacture/ Platform	Assay Description	MAS™ SARS-CoV-2 IgG Positive Control	MAS™ SARS-CoV-2 Negative Control
Abbott™ ARCHITECT™ ci8200	SARS-CoV-2 IgG	Assess the assay and platform compatibility: Divide the number of positive test results by the sum of true positives and false negatives.	Assess the assay and platform compatibility: Divide the number of negative test results by the sum of true negatives and false positives.
Abbott™ ARCHITECT™ ci8200ci8200	AdviseDx SARS-CoV-2 IgG II (Semi-quant)		
Abbott™ ARCHITECT™ ci8200ci8200	AdviseDx SARS-CoV-2 IgM		
Roche™ cobas® E601E601	Elecsys Anti-SARS-CoV-2		
Ortho™ VITROS® ECiQ	Anti-SARS-CoV-2 IgG/Total		
Abbott™ ALINITY® CiCi-Series	SARS-CoV-2 IgG		
Beckman Coulter™ Access 2® System	Access SARS-CoV-2 IgG		
bioMérieux™ VIDAS®	Anti-SARS-CoV-2 IgG		
Diasorin™ Liaison®	SARS COV 2 TrimericS IgG		
Siemens™ ADVIA™ Centaur	SARS-CoV-2 Total		
Siemens™ Atellica™ IM	IM SARS-CoV-2 Total (COV2T)		

Results: All results demonstrated conclusively the comparable performance of MAS™ SARS-CoV-2 IgG controls across different clinical diagnostic assays and platforms manufactured by Abbott, Roche, Ortho, DiaSorin, Beckman Coulter and bioMérieux. The positive control showed 100% expected reactive results (IgG Positive) and Negative control at 82% non-reactive results, respectively. Furthermore, both controls were compatible towards assays detecting IgG antibodies targeting specific regions (N, S or RBD).

Conclusion: SARS-CoV-2 serology testing remains unstandardized with numerous assays and platforms receiving FDA EUA. While tested on multiple commonly used assays and platforms, MAS™ SARS-CoV-2 IgG Positive and Negative Controls were found to be platform agnostic. To conclude, MAS Controls will contribute to the harmonization of the SARS-CoV-2 Serology assay offerings, monitor the clinical laboratory analytical performance, streamline the workflow on test method development, validation and verification, and routine assessment.

A-239

Comparison between blood culture and MeMed BV test results

A. ZURETTI[1], M. CHRISTIAN[2], S. MOTOV[2], M. KOGAN[2]. *[1]SUNY DOWNSTATE, BROOKLYN, NY, [2]MAIMONIDES MEDICAL CENTER, BROOKLYN, NY*

Background: The rate of cultures ordered for patients in which infection is suspected is approximately 40% on Emergency Department patients at Maimonides Medical Center and represents a significant financial burden for our institution. Most blood cultures in clinical practice do not grow organisms suggesting that suboptimal blood culture collection practices (e.g., suboptimal blood volume) or suboptimal selection of patients to culture (patients with low likelihood of bacteremia may be occurring.

•BV is a new host-protein test based on TRAIL, TNF-related apoptosis inducing ligand protein, IP-10, interferon gamma -induced protein 10 and CRP, C- reactive protein that produces a score between 1-100 indicating the likelihood of a bacterial versus viral infection. It has been validated in multiple studies to have sensitivity and specificity of more than 90%.• In this study we compare blood culture and BV results.

Methods:

BV test were taken at ED physician discretion from March 2022 to December 2022

• BV score 0-35 is viral, 35-65 is equivocal and 65-100 is bacterial.

• BV test was measured using MeMed BV (MeMed, Israel) and MeMed Key (MeMed, Israel) from serum samples.

• This sub analysis focuses on patients presenting at the ED for whom blood culture was ordered and there was a record of the result.

Results:

• BV test was taken for 545 patients during their ED visit, of these, 389 had blood cultures drawn and results recorded.

• There were 233 cases with BV score bacterial, 42 with BV score equivocal and 114 with BV score viral

• Out of the BV score viral, there were 7 with positive findings, of which 5 were considered contaminants

Conclusion:

There is a high agreement between viral BV results and negative cultures. Ongoing collection of this data in real time will help establish this finding further in order to provide guidance for our clinicians to reduce the financial burden of unwarranted cultures as well as the unnecessary administration of antibiotics.

A-240

Rapid and Reliable Identification of Microorganisms from Early Growth of Positive Blood Cultures Using MALDI-TOF Mass Spectrometry

B. L. Roemmich, M. L. Yarbrough, K. K. Alvarado. *Washington University in St. Louis, Saint Louis, MO*

Background: Rapid identification of organisms causing bloodstream infections is critical for treatment. Traditional workflows rely on 18-24h growth of isolated colonies. Our objective was to evaluate identification of early growth using Bruker and VITEK® MS PRIME MALDI-TOF systems. Materials: Positive blood cultures (BACT/ALERT® FA/FN Plus; bioMérieux VIRTUO®) were included if bottles were positive ≤8h and had time-to-positivity of ≤40h. Broths were sub-cultured to blood agar and incubated at 35°C/5% CO_2. MALDI-TOF identification of 6-8h growth (short incubation, SI) and 18-24h growth (standard incubation, SDI) was performed by a high (HEU, >1 year) and low (LEU, <1 month) experience user. Each identification was performed using standard set-up for Bruker and VITEK MS PRIME, and target set-up using VITEK PICKME™ pen. Identification level and time-to-result (TTR) were measured and compared using Fisher's exact and t-test. Results: 53 organisms (36 Gram-positive, 15 Gram-negative, 2 yeast) were isolated from 50 blood cultures. The HEU achieved species-level identification for 87% on VITEK MS PRIME using routine set-up and PICKME after SI versus 74% on Bruker, while 66% and 70% of organisms were identified on VITEK MS PRIME versus 75% on Bruker by the LEU (Table). After SDI, ≥94%(HEU) and ≥87%(LEU) of organisms were identified, with no differences noted by set-up or user experience (p>0.05). Species-level identification after SI was higher for Gram-negative (100%) versus Gram-positive (67-86%) organisms for the HEU. PICKME pen usage increased identifications to 100% from 67% for Gram-negatives for the LEU. For mixed cultures (n = 3), only 1 of 2 organisms was identified after SI. TTR was significantly longer(p<0.05) for SI vs SDI and Bruker vs VITEK MS PRIME for the HEU. Conclusions: Identification of microorganisms after short incubation on solid media achieves early, reliable identification for a majority of positive blood cultures without need for additional hands-on time or extraction.

	High Experience User						Low Experience User					
	VITEK PRIME Standard		VITEK PRIME PICKME Pen		Bruker		VITEK PRIME Standard		VITEK PRIME PICKME Pen		Bruker	
	Short Incubation	Standard Incubation	Short Incubation	Standard Incubation	Short Incubation	Standard Incubation	Short Incubation	Standard Incubation	Short Incubation	Standard Incubation	Short Incubation	Standard Incubation
Genus level ID, n(%)												
Gram positive	0 (0)	1 (3)	0 (0)	0 (0)	7 (19)	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)	4 (11)	0 (0)
Gram negative	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7)	2 (13)
Yeast	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	1 (50)
Overall	0 (0)	2 (4)	0 (0)	1 (2)	7 (13)	1 (2)	0 (0)	0 (0)	1(2)	1 (2)	5 (9)	3 (6)
Species level ID, n(%)												
Gram positive	31 (86)	35 (97)	31 (86)	36 (100)	24 (67)	35 (97)	28 (78)	32 (89)	24 (67)	34 (94)	29 (81)	33 (92)
Gram negative	15 (100)	15 (100)	15 (100)	15 (100)	15 (100)	15 (100)	10 (67)	15 (100)	15 (100)	15 (100)	13 (87)	13 (87)
Yeast	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	1 (50)	0 (0)	0 (0)
Overall	46 (87)	50 (94)	46 (87)	52 (98)	39 (74)	50 (94)	38 (72)	49 (92)	39 (74)	50 (94)	42 (79)	46 (87)
No identification, n(%)												
Gram positive	5 (14)	0 (0)	5 (14)	0 (0)	5 (14)	1 (3)	8 (22)	4 (11)	11 (30)	2 (6)	3 (8)	3 (8)
Gram negative	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (33)	0 (0)	0 (0)	0 (0)	1 (7)	0 (0)
Yeast	2 (100)	1 (50)	2 (100)	0 (0)	2 (100)	1 (50)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	1 (50)
Overall	7 (13)	1 (2)	7 (13)	0 (0)	7 (13)	2 (4)	15 (28)	4 (8)	13 (25)	2 (4)	6 (11)	4 (8)
Average TTR, h (Set-up to identification)	0.53	0.48	0.55	0.53	0.65		0.47	0.44	0.56	0.62	0.61	

A-242

Integrating respiratory metagenomics and metatranscriptomics for diagnosis of lung cancer and infection in patients with pulmonary diseases

D. Han[1], F. Yu[2], Y. Chen[2]. *[1]The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Chin, Hangzhou, China, [2]the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, Hangzhou, China*

Background: Advances in unbiased metagenomic next generation sequencing (mNGS) technologies have enabled the study of microbial and host genetic material (DNA and RNA) in one test. In this study, we aimed to develop machine learning-based differential diagnostic models (MLBDDMs) using the metagenomic and human transcriptomic data generated by an affordable bronchoalveolar lavage fluid (BLAF) mNGS assay and investigated their clinical utility for early differential diagnosis of lung cancer and pulmonary infection in patients with pulmonary diseases. **Methods:** We recruited 775 patients with respiratory disease, including 160 pathologically diagnosed lung cancer and clinically diagnosed 615 infectious causes (131 tuberculosis, 172 fungal pneumonia and 312 bacterial pneumonia). An affordable mNGS assay on BALF samples collected from these patients on admission were performed. Using the generated mNGS data, we compared the differences in microbial diversity and host gene expression between lung cancer patients and pulmonary infection patients. The BLAF mNGS datasets of lung cancer group and each infection group were then randomly divided into a training dataset and a validation dataset at a ratio of approximately 3:1 for developing optimal MLBDDMs that can be used to distinguish lung cancer from various pulmonary infections. **Results:** By comparing the BALF mNGS data of lung cancer (n=160) and pulmonary infection (n=615), we found that the infection group had higher microbial diversity than lung cancer group (p-value<0.05). Respiratory colonizing microorganisms (e.g., *Corynebacterium propinquum* and *Bacteroides uniformis*) and pathogen (*Mycobacterium tuberculosis* and *Cryptococcus neoformans*) were found as differential microbes (adjusted p-value<0.05, LDA score>2). From BALF gene expression data, we detected 175 genes enriched in NOD-like receptor signaling pathway and chemokine signaling pathway differentially expressed between lung cancer and pulmonary infection groups (False Discovery Rate, FDR<0.05). Cell composition analysis revealed that macrophage M1 was higher in lung infection group (p-value<0.001), whereas mast cell activated and DCs activated were higher in lung cancer group (p-value<0.001, p-value=0.016). We integrated the metagenomic (microbial composition and human copy number variation) and transcriptomic data (host differentially expressed genes and cell composition) generated by the BALF mNGS assay with eleven machine learning classifiers to establish diagnosis models for distinguishing lung cancer from pulmonary infection (we named LC/PI model). The results showed that a Random Forest diagnostic model (the RF-LC/PI model) had optimal performance, with a sensitivity and specificity of 86.7% and 87.8%, respectively, in distinguishing lung cancer from pulmonary infection (area under the receiver operating characteristic curve [AUC]=0.838 in the training dataset; AUC=0.79 in a held-out validation dataset). Similar to the establishment of the LC/PI model, we further developed three diagnostic models for distinguishing lung cancer and tuberculosis (LC/TB model), lung cancer and fungal pneumonia (LC/FP model), and lung cancer and bacterial pneumonia (LC/BP model), respectively. The AUC of these three models were 0.91, 0.88, 0.91, respectively, showing a high differential diagnosis accuracy. **Conclusions:** We have established MLBDDMs using BALF metagenomic and metatranscriptomic data and achieved superior accuracy for differentiating lung cancer and pulmonary infections, which could promote early diagnosis of pulmonary diseases and benefit more patients with one test.

A-244

Performance Comparison of the Alinity i Hepatitis C core antigen assay in routine laboratory testing vs HCV RNA testing

I. L. Gunsolus[1], J. Prostko[2], S. Pearce[2], B. Degaga[1], S. Eickstead[1], S. Thapa[1], C. Radman[1], R. Taylor[2], J. Grieshaber[2], K. Richard[2], A. Hoffman[2], A. Pekalska[2]. *[1]HealthPartners, Bloomington, MN, [2]Abbott Diagnostics, Abbott Park, IL*

Background: The elimination of Hepatitis C by 2030 is a goal established by the WHO and other country specific health organizations worldwide. This goal is enabled by the availability of effective treatment regimens. A recent surprise in the US has been the increase in HCV infections during the COVID 19 pandemic, which may be caused by increased needle sharing. HCV Antibody assays have been used to screen for HCV, but confirmation of acute infection is relegated in the current US guidelines to PCR which often takes multiple days and may result in a loss to follow up and

treatment, especially in high prevalence populations. HCV Core Antigen is a new immunoassay on the Alinity i system. The aim of the study was to evaluate the precision, linearity, and sensitivity of this research use assay in a US-based population.

Methods:Alinity i assay HCV Ab is an automated chemiluminescent immunoassay built to measure total antibody to the virus. Similarly, the Alinity i HCV Core Ag is a research use only automated chemiluminescent immunoassay for detection of HCV Core antigen in human plasma and serum. Precision (repeatability, and reproducibility) was determined over 3 days (4-10 replicates/day) using control material consisting of a negative human plasma control, and positive controls made with recombinant core Ag in buffer. Linearity was evaluated by preparing 4 dilutions of a remnant specimen with detectable HCV RNA viral load. Clinical sensitivity was determined using 90 remnant specimens with positive HCV Ab results. Specimens were confirmed positive for Hepatitis C via HCV RNA quantitation using the Aptima HCV Quant Dx transcription-mediated amplification (TMA) assay on the Panther System. Correlation between the HCV Ag assay and TMA was evaluated using specimens submitted for either Hepatitis C infection screening and monitoring of known infection.

Results: The repeatability of the HCV Ag assay ranged between 3.7% and 11.1% CV depending on the sample S/CO level tested. Dilution recovery averaged 98.2% (range 90.2%-107.3%) and a plot of HCV Ag concentration vs dilution factor showed a correlation coefficient R =0.998 The Hepatitis C core antigen assay had a correlation coefficient R = 0.92 with the TMA. The HCV assay demonstrated a clinical sensitivity of 96.7% vs TMA.

Conclusion: The performance characteristics of the Alinity i HCV Core Ag assay highlight an opportunity to speed time to treatment by reflexing on the same instrument from HCV Ab to Ag and confirming effectiveness of treatment.

A-245

Reagent Lot Variation - When should I be Concerned?

G. VINCINI, W. Dimech. *NRL, Melbourne, Australia*

Background: Reagent lot variation is often considered a major source of concern for infectious disease serology testing. Regular review of quality control testing data for specific assays confirms that, when using traditional statistical methods, such as using small datasets to establish mean/SD and then applying Westgard rules, reagent lot variation is the most common cause of variation observed. To investigate every incident is time consuming, costly and likely unnecessary in the majority of cases. But how do we create an alternative to monitoring QC data that accepts normal reagent lot variation yet highlight unacceptable variation? Applying the results from two assays using the same multimarker QC sample to traditional and an alternative QC monitoring method such as QConnect highlights when we should be concerned with lot variation and when the variation is normal.

Methods: QC testing data from 14 lots of the multimarker QC 'Optitrol Blue' over a three year period tested on two assays - Abbott ARCHITECT Anti-HCV (n=54,102) and Abbott ARCHITECT HIV AgAb Combo (n=62,682) were reviewed for lot variation using traditional and alternative QC monitoring methods.

Results: Numerous examples of lot variation were identified from the two assays over the three-year period. Applying traditional QC methods resulted in frequent recalculation of mean/SD to accommodate the lot variation, effectively making the lot variation part of the routine, negating its importance, and making the clinical significance of reagent lot variation difficult to ascertain. Using an alternative QC monitoring method such as QConnect, which incorporated normal lot in calculations to create a constant acceptance range, removed the uncertainty and highlighted instances where variation was outside 'normal', triggering further investigation by using meaningful QC ranges. A clear example was observed where two similar looking Levey-Jennings charts (one for ARCHITECT HCV and one for ARCHITECT HIV) demonstrated reagent lot variation that was detected as an outlier whereas Traditional methods identified all reagent lots as being outliers.

Conclusion: In infectious disease serology testing, reagent lot variation is frequent, normal, and rarely a cause for concern. However, it is difficult to determine how much variation is acceptable when using traditional QC methods, because most lot changes are flagged as "rejections". QC processes that incorporate normal reagent lot variation, derived from historical data, allows for rapid identification of unacceptable variation, saving money and time (particularly when all reagent lot variation identified as outliers need unnecessary investigation), while reducing risk of poor results.

A-246

Desialylation profiles of monocytes and macrophages upon LPS stimulation

M. A. Aljohani, X. A. Sun, Y. A. Zhao. *Cleveland State University, Cleveland, OH*

Background: Cell surface expresses a dense layer of glycans terminated with sialic acids (Sias), known as sialylation, which often represent different cellular statuses. Meanwhile, the removal of Sias from the glycans catalyzed by sialidase (desialylation) is also involved in many biological processes. Previous studies have established that lipopolysaccharide (LPS) can induce the expression of endogenous sialidases in monocytes and macrophages, leading to desialylation of the LPS receptor TLR4. This desialylation of TLR4 has been shown to impact the LPS/TLR4 signaling pathway. In this study, we conduct a profiling of desialylation in THP-1 monocytes and THP-1 macrophages in response to LPS stimulation. This will provide insights on the intricate connection between sialylation, desialylation, and LPS-induced signaling. **Methods:** The activity of sialidases was assessed by utilizing 4-methylumbelliferyl-N-acetylneuraminic acid (4-MU-NANA) and ganglioside GM3 as substrates. The total sialic acid in the THP1 was quantified using liquid chromatography tandem mass spectrometry (LC-MS/MS). Flow cytometry was used to analyze the exposure of galactose on THP-1 monocytes and macrophages after LPS stimulation (100 ng/mL LPS for 24 hours), using PNA-FITC staining. The expression level of Neu1 and Neu3 sialidases in cell lysates of THP-1 monocytes and macrophages were assessed by western blot technique after LPS stimulation. ELISA was used to measure the levels of sialidases in the cell supernatant of THP-1 monocytes and macrophages upon LPS stimulation **Result:** We found a significant reduction in total sialic acid levels in both THP-1 monocytes and macrophages upon LPS stimulation. This was further confirmed by the significant increase in total sialidase activities which measured by both 4-MU-Neu5Ac and GM3 substrates in THP-1 monocytes and macrophages upon LPS stimulation. Surprisingly, we also found that sialidase activities significantly increased in the cell supernatant of both THP-1 monocytes and macrophages, indicting sialidases secretion upon LPS stimulation. Western blot analysis further confirmed the high expression and secretion of both Neu1 and Neu3 sialidases from THP-1 monocytes and macrophages after LPS stimulation. **Conclusion:** The present study conducted the first systematic profiling of desialylation and sialidase expression in monocytes and macrophages upon LPS stimulation. This results demonstrate that endogenous sialidase expression and secretion play a role in the LPS/TLR4 signaling pathway and subsequent cellular processes and functions in the context of inflammation and immune response.

A-248

Evaluation of NextGene™ MTB/NTM Detection Kit for detection of *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria in sputum and culture specimens

J. Ahn[1], D. Kim[1], W. Lee[2]. *[1]Gachon University Gil Medical Center, Incheon, Korea, Republic of, [2]Kyung Hee University Hospital at Gangdong, Seoul, Korea, Republic of*

Background: Tuberculosis, an infectious disease, is a major cause of deteriorating health, and, until SARS-CoV-2 (COVID-19) became a pandemic disease, a major cause of death worldwide. The recently developed NextGene™ MTB/NTM Detection Kit is designed for detection of *Mycobacterium tuberculosis* complex (MTB) using IS6110 genes and MPB64 genes, and for detection of nontuberculous mycobacteria (NTM) using 16S rRNA genes for resolution of false positive and false negative results, and for development of more sensitive and specific real-time PCR products. The purpose of this study is to examine the clinical sensitivity and specificity of the NextGene™ MTB/NTM Detection Kit in order to verify its clinical efficacy.

Methods: A total of 446 residual clinical specimens were obtained from 274 sputum and 172 culture samples collected from July to August 2021. Diagnostic tests were performed in order to confirm that these residual samples were positive and negative; 344 specimens were positive for MTB or NTM, and 102 specimens were confirmed as negative for both MTB and NTM. Measurement of positive/negative percent agreement values and kappa coefficients was performed for comparison of diagnostic performance between the NextGene™ and Advansure assays. Positive and negative controls were included in each run.

Results: Of the 446 samples (274 sputum and 172 culture samples), 344 specimens showed a positive result and 102 specimens showed a negative result. Results of the comparison tests were all consistent for detection of MTB and NTM. Both MTB and

NTM met the criterion for evaluation of target validity of kappa value 0.8 or higher in sputum and culture samples. Results of the evaluation of clinical sensitivity and specificity were consistent with both clinical diagnoses.

Conclusion: This study was conducted in order to examine the clinical sensitivity and specificity of the NextGene™ MTB/NTM Detection Kit in order to verify its clinical validity. Residual samples from sputum and culture specimens from patients who were suspected being infected that were confirmed positive or negative for MTB and NTM were used in this study with the goal of establishing the already commercially available licensed product as a control reagent for comparative analysis of the clinical performance of the NextGene™ MTB/NTM Detection Kit. Compared to existing kits that are widely used, consistent results from comparison of the NextGene™ MTB/NTM Detection Kit have been reported with comparable clinical sensitivity and specificity, thus it is regarded as showing the same performance.

A-249

Theranostic algorithm: sensitivity and specificity of a composite index test for guiding treatment in severe COVID-19

N. V. De Vos[1], M. Bruyneel[2], C. Duterme[1], A. Roman[3], H. Dahma[4], S. Alard[5], S. Andre[6], D. Barglazan[1], A. Chirumberro[2], F. Cotton[1]. *[1]Department of Clinical Chemistry, Laboratoire Hospitalier Universitaire Bruxelles – Universitair Laboratorium Brussel (LHUB-ULB), Université Libre de Bruxelles, Brussels, Belgium, [2]Department of Pulmonology, CHU Saint-Pierre, Brussels, Belgium and Université Libre de Bruxelles, Brussels, Belgium, [3]Department of Intensive Care Medicine, CHU Saint-Pierre, Brussels, Belgium and Université Libre de Bruxelles, Brussels, Belgium, [4]Department of Microbiology, Laboratoire Hospitalier Universitaire Bruxelles – Universitair Laboratorium Brussel (LHUB-ULB), Université Libre de Bruxelles, Brussels, Belgium, [5]Department of Radiology, CHU Saint-Pierre, Brussels, Belgium and Université Libre de Bruxelles, Brussels, Belgium, [6]Department of Pulmonology, CHU Brugmann, Brussels, Belgium and Université Libre de Bruxelles, Brussels, Belgium*

Background: Since the outbreak of the pandemic, severely affected COVID-19 patients receive standard of care at intensive care unit (ICU), but clinical trials are still ongoing for new expensive treatments. There is a need for guiding therapeutic decisions to restrain the public healthcare costs and to target the most efficient therapy. Based on the pathophysiology of SARS-CoV-2 on its ACE2-receptor, the angiotensin pathway is activated and circulating biomarkers of interest can be measured. This diagnostic test accuracy study describes how to classify COVID-19 patients in severe versus mild cases with the intention of guiding treatment decisions, to prevent long term sequels like pulmonary fibrosis. A theranostic approach is proposed, which combines in vitro diagnostics and personalized therapeutics. **Methods**: N=17 severely affected COVID-19 with acute respiratory distress syndrome (ARDS) at ICU were included and compared to N=7 mild or asymptomatic COVID-19 cases and to N=10 healthy controls. Follow-up samples have been collected during pulmonology consultation at three time points during one year post-ICU (N=39). Non-parametrical statistics were used; data are expressed as [median (IQR)] and $p<0.05$ is considered to be statistically significant. The biological markers angiotensin-(1-7) (Ang-(1-7)), transforming growth factor beta (TGF-β), ferritin, C-reactive protein (CRP from Roche) and Krebs von den Lungen 6 (KL-6 from Fujirebio) were measured in plasma samples. Those biomarkers represent a disequilibrium in lung protection, fibrogenesis, inflammation and hyperplasia of alveolar epithelial cells type II. Cut-off for a composite index was obtained through receiver operating curve (ROC). **Results**: COVID-19 patients show higher Ang-(1-7) concentrations than healthy controls ($p<0.05$). Critical patients at ICU show Ang-(1-7) deficit [124 pg/mL (81-154)], compared to asymptomatic COVID-19 cases [147 pg/mL (138-201)] ($p<0.05$). COVID-19 patients with insufficient Ang-(1-7) are eligible for substitution therapy and novel expensive therapies beside standard of care, while the subpopulation of high Ang-(1-7) is analyzed with a composite index test including TGF-β, ferritin and CRP. A composite index of <34 differentiates asymptomatic COVID-19 from severe COVID-19 at ICU having acute respiratory distress syndrome (sensitivity=100%, specificity=80%, AUC=0.933, LR=5). The patients without risk for pulmonary sequels are not eligible for expensive treatment.After 1 year, KL-6 (<275 U/mL) and TGF- β (<34968 pg/mL) normalise in only 25%, respectively 33% of severe COVID-19 patients. Within the first year of FU post-ICU, the mean KL-6 value in the patient population does not significantly change at different measuring moments ($p=0.63$), while TGF-β decreases gradually at 3, 6 and 12 months post-ICU ($p<0.0001$). **Conclusion**: This theranostic algorithm predicts whether a patient infected by SARS-CoV-2 is at risk of developing clinical complications and supports clinical decision making concerning therapy. A supported therapeutic choice, based on objective biomarker measurements, could diminish long-term sequelae like pulmonary fibrosis. Clinical trials using companion drugs (e.g. Ang-(1-7) substitution therapy, MAS-receptor agonist), anti-fibrotics, interferon therapy, or monoclonal antibodies, could also rely on those biomarkers. The measurement of Ang-(1-7) for example, could categorize patients in the targeted subpopulation before administrating Ang-(1-7) substitution therapy, aiming at obtaining better treatment efficacy.

A-250

Rapid molecular identification of herpes simplex, varicella-zoster, and enteroviruses from cerebrospinal fluid

J. Bialas[1], L. Joki[2], M. Hermanowski[1], K. Brea-Sellheim[1], H. Leibhan[1], M. Jacobs[3], M. Lång[4], J. Vainio[2], J. Martelin[2]. *[1]Labor Berlin - Charité Vivantes Services GmbH, Berlin, Germany, [2]Uniogen, Turku, Finland, [3]Klinikum der Stadt Ludwigshafen am Rhein GmbH, Ludwigshafen, Germany, [4]Department of Clinical Microbiology, Fimlab Laboratories, Tampere, Finland*

Background: Accurate and efficient molecular testing of viral meningitis/encephalitis is crucial for proper patient care. Here we describe the clinical evaluation of the GenomEra® HSV-1/2, VZV + EV Assay Kit (Uniogen, Finland) allowing simultaneous detection of four key viruses responsible for causing meningitis/encephalitis, namely HSV-1 and HSV-2, VZV, and enteroviruses.

Methods: The study was carried out at three European institutions in 2022, with a total of 193 frozen, anonymized leftover cerebrospinal fluid (CSF) specimens analyzed retrospectively. For the GenomEra HSV-1/2, VZV + EV Assay Kit, 50 µL of CSF was mixed with lysis buffer in a 1:1 ratio, allowed to incubate for 1 min at room temperature, and then filtered by a 1-min centrifugation through an Extraction Column utilizing size-exclusion chromatography. A 35-µL sample of the flow-through was applied into the Test Chip and analyzed using the GenomEra CDX System. The cotemporally performed comparison tests included the FilmArray Meningitis/Encephalitis (ME) panel (bioMérieux), Xpert® EV Assay (Cepheid), artus VZV assay (Qiagen), and a well-established, validated non-commercial real-time RT-PCR assay for the detection of HSV-1/2, VZV, and EV.

Results: The GenomEra HSV-1/2, VZV + EV Assay Kit produced results in 70 minutes with a sensitivity and specificity of 92.2% (95% CI: 82.7-97.4%) and 99.9% (95% CI: 99.2-100%), respectively, as compared to the reference tests. Of the discordant results, five tested negative with the GenomEra assay, whereas the reference tests produced low positive results (Ct values ≥ 35). In addition, one specimen yielded a positive signal for VZV while remaining negative with the reference tests.

Conclusion: The GenomEra HSV-1/2, VZV + EV Assay Kit showed a high overall specificity and a high level of sensitivity particularly for herpes simplex and varicella-zoster viruses. For enteroviruses, while the specificity reached 100%, the sensitivity was not as high as with the reference methods. The high clinical performance, together with the simple sample preparation protocol and short turnaround time make the GenomEra assay a competitive molecular test especially for small and medium-sized laboratories that may not have a specialist in molecular biology. In addition, the GenomEra assay only requires a small amount of sample, conserving the valuable specimens for additional testing.

A-251

Comparison of Antimicrobial Susceptibility Profiles of *Enterobacteriaceae* spp. Isolates from Retail Meat Products Imported from USA and Brazil or Domestically Produced on Caribbean Islands of St. Kitts and Grenada

T. Luthra, K. Varghese, A. Martens, O. Fakayode, J. Harrington. *St. George's University, St. George's, Grenada*

BACKGROUND: *Enterobacteriaceae* species, including *Escherichia coli, Klebsiella* and *Salmonella,* are endogenous colonizers of gastrointestinal tract of livestock animals, which pose potential health threats to humans, particularly for individuals with compromised immune systems (Guentzel 1996). The emergence of drug-resistant strains due to the overuse of antibiotics in agricultural practices have increased the urgency to evaluate zoonotic bacteria and to assess potential impact on human and animal health (Llor 2014). PROJECT AIM and METHODS: The aim of this study is to compare the microbial prevalence and antimicrobial resistance profiles of *Enterobacteriaceae* isolates present on pork and poultry products domestically produced in the Caribbean Islands, St. Kitts and Grenada as compared to poultry imported from USA and Brazil. This study was conducted over a 4-year time period at University of Medicine and Health Sciences (UMHS) in St Kitts and at St. George's University (SGU), Grenada. Microbial colonies were isolated on MacConkey agar after 24-hour incubation at 36°C in Buffered Peptone Water and identified using standard biochemi-

cal assays and API-20E test kit. Antimicrobial susceptibility was evaluated using the Kirby-Bauer disc diffusion on Mueller-Hinton plates. RESULTS: A total of 44 meat products from Caribbean Islands and 49 meat samples from Brazil and USA were processed to yield 64 and 60 bacterial isolates, respectively. Bacterial colonies isolated from imported meat products displayed higher incidence of antibiotic susceptibility to multiple classes of drugs (MDR = Multiple Drug Resistant) as compared to domestically produced meats. The prevalence of MDR bacteria was 45% MDR isolates from USA-imported chicken as compared to 13% MDR bacteria isolated from chicken domestically raised. CONCLUSIONS: Imported meats exhibit a higher prevalence of antimicrobial resistance, particularly in the beta-lactam class of antibiotics, including amoxicillin and cephalosporins and fluroquinolones. The overuse of subtherapeutic antibiotics in mass scale livestock production is likely creating selective pressure for the overabundance of MDR strains in the gastrointestinal tract of animals. The findings of this study will be communicated to relevant community leaders and organizations in order to inform the development of strategies aimed at reducing the prevalence of antimicrobial resistance and identifying appropriate treatment options for associated infections.

A-252

Differentiation of SARS-CoV-2, Influenza A and B, and Respiratory Syncytial Virus Using a Single Swab: A Clinical Performance Evaluation of the BD MAX System Multiplex PCR Assay

M. Faron[1], N. Ledeboer[2], B. Van Der Pol[3], T. Davis[4], E. Lockamy[5], S. Paradis[5], J. Andrews[5]. *[1]the Medical College of Wisconsin, Milwaukee, WI, [2]The Medical College of Wisconsin, Milwaukee, WI, [3]University of Alabama, Birmingham, AL, [4]Sidney and Lois Eskenazi Hospital, Indianapolis, IN, [5]Becton, Dickinson and Company, Sparks, MD*

Background: Some shared clinical features of SARS-CoV-2, influenza (flu), and respiratory syncytial virus (RSV) infection often pose a diagnostic challenge for clinicians. In that context, the use of molecular diagnostic systems multiplex assays, using a nasopharyngeal or nasal swab, facilitates the detection of the causing virus by differentiating between the RNA of each disease. Our study evaluated the clinical accuracy of one such multiplex PCR assay, the BD Respiratory Viral Panel for BD MAX™ System (BD MAX RVP).

Methods: Symptomatic patients that had a nasopharyngeal and nasal specimen prospectively collected from 6 geographical locations in the US were enrolled in the study between January and April 2022 along with nasopharyngeal swabs retrospectively collected between December 2019 and January 2022. All specimen testing was performed using the BD MAX System. Hologic® Flu A/B/RSV assay on Panther Fusion™ System was the comparator for flu A, flu B, and RSV while a composite of two out of three assays authorized under FDA Emergency Use Authorization (Roche cobas® SARS-CoV-2 on cobas® 6800 System, Hologic® Aptima® SARS-CoV-2 on Panther® System, and Quidel Lyra® SARS-CoV-2 on Applied Biosystems® 7500 Fast Dx) was used as comparator for SARS-CoV-2. Positive and negative percentage agreements (PPA and NPA, respectively) were calculated to determine performance. Reproducibility and precision assessments were also performed as part of the study.

Results: Tested specimens included 252 nasopharyngeal and 254 nasal swabs, prospectively collected, and 240 retrospectively acquired nasopharyngeal specimens. For prospectively collected SARS-CoV-2 nasopharyngeal specimens, PPA of 98.8% (95% CI 93.6, 99.8) and NPA of 98.2% (95% CI 94.9, 99.4) were observed while for flu A, PPA was 100% (95% CI 61.0, 100) and NPA 99.6% (95% CI 97.7, 99.9). PPA and NPA for prospectively collected SARS-CoV-2 anterior nasal specimens were 98.8% (95% CI 93.3, 99.8) and 98.3 (95% CI 95.1, 99.4), respectively. For flu A, PPA of 100% (95% CI 61.0, 100) and NPA of 99.6% (95% CI 97.8, 99.9) were observed. Lack of enrolled positive patients with flu B or RSV precluded the PPA calculation for those viruses. However, NPA for both flu B and RSV was 100% for the two specimen types. In retrospectively obtained nasopharyngeal specimens, PPA was 100% (95% CI 93.7, 100) for flu A, 100% (95% CI 93.8, 100) for flu B, and 98.4% (95% CI 91.5, 99.7) for RSV while NPA was 98.9% (95% CI 96.1, 99.7) for flu A, 98.9% (95% CI 96.1, 99.7) for flu B, and 100% (95% CI 97.9, 100) for RSV.

Conclusion: The BD MAX RVP assay showed a strong clinical performance in detecting and differentiating between the RNA of SARS-CoV-2, flu A and B, and RSV.

A-253

Overcoming Pre-Analytical and Analytical Challenges Associated with Home Collected STI Specimens

B. Hockman[1], M. Qi[1], H. Rotblatt[2], L. Borenstein[3], S. Rajagopalan[3], D. N. Greene[4]. *[1]LetsGetChecked, Monrovia, CA, [2]Los Angeles County Department of Public Health Division of HIV and STI Programs, Los Angeles, CA, [3]Los Angeles County Department of Public Health Laboratory Division of Serology, Virology, & Molecular Diagnostics, Los Angeles, CA, [4]LetsGetChecked Laboratories, Monrovia, CA*

Introduction: *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are common sexually transmitted infections (STIs) that are often asymptomatic, delaying diagnosis and treatment which can cause health consequences and increased transmission. The CDC suggests that self-collected (SC) samples (urine; vaginal, rectal, oropharyngeal swabs) are reasonable alternatives to provider-collected (PC) samples. However, only urine and vaginal swabs are commercially offered for within-clinic self-collection leaving a gap for (1)detecting extra-genital infections (common among LGBTQ+ community) and (2)improving access by allowing for at-home self-collection. The objective of this study was to elucidate analytical and pre-analytical variables associated with at-home SC STI testing with the intention of improving inclusion and access to sexual healthcare.

Methods: Paired PC/SC rectal (n=164) and throat (n=159) swabs were compared for CT/NG detection; reproducibly equivocal results (n=3) were considered positive. Limit of detection (LoD) for CT/NG was performed by serial dilution to determine a concentration at the point of failure below which detection becomes unreliable (n=20). Concentrations of 2-10x the LoD were used in subsequent experiments. Interference testing was performed to determine if hand contaminants (1% v/v) would impact assay performance (n=5/contaminant). To evaluate shipping stability, samples were challenged using winter or summer simulation with temperature cycling designed to mimic extreme seasonal fluctuations. Additional experiments were performed on urine samples to evaluate the impact of underfilling/overfilling or removing the transport medium from the sample tube. CT/NG detection was performed using the Hologic Aptima Combo 2 Nucleic Acid Amplification Assay.

Results: Relative to provider collected samples, rectal swabs demonstrated a positive agreement of 95.5% for CT (n=22 (13.4%) positive PC) and 100% for NG (n=9 (5.5%) positive PC) with an overall agreement of 97.5% for CT and 98.1% for NG. Throat samples demonstrated a positive agreement of 100% for both CT and NG (CT: n=2 (1.3%) positive PC; NG: n=11 (6.9%) positive PC) with an overall agreement of 100% for CT and 96.8% for NG. Although there was one false negative rectal SC relative to PC, almost all swab/organism combinations had positive results by SC that were negative by PC: 3+ SC for rectal CT; 3+ SC for rectal NG; 5+ SC for throat NG. LoDs were 0.03-0.06 IFU/mL for CT and 0.06-0.5 CFU/mL for GC using urine or vaginal swabs. Hand contaminants did not interfere with CT/NG detection, and samples demonstrated ≥ 95% agreement after winter and summer shipping stability challenges. Urine results were unaffected by underfilling (≥ 0.5 mL urine), overfilling (≤ 4.5 mL urine), or removal of transport medium (≥ 0.5 mL buffer volume).

Conclusions: Self-collection of extra-genital swabs for CT/NG detection may offer better sensitivity than physician collected samples without compromising the analytical prowess of the assay. Home collected STI specimens are a viable option for improving access to STI screening and offer a non-stigmatizing approach to sexual health.

A-256

Clinical Performance of Presepsin for Sepsis in the Emergency Department Patients in a Tertiary Hospital in Korea

E. Ji, S. Lee. *Pusan National University Yangsan Hospital, Yangsan, Korea, Republic of*

Background: Sepsis is a life-threatening condition that requires prompt recognition and treatment. Presepsin is a new diagnostic biomarker of sepsis that has been implemented as a selective reimbursement in Korea and was introduced in our hospital in October 2022. This study aimed to evaluate the clinical performance of the Presepsin Assay (Sysmex, Kobe, Japan) on the Sysmex HISCL-5000 system in the southeast population in Korea.

Methods: The study is a retrospective study that included emergency department (ED) and ED-based intensive care unit (EICU) patients tested for presepsin from August 18, 2022, to February 11, 2023. We calculated the patients' sepsis-related organ failure assessment (SOFA) score at the time of blood collection for presepsin from the electronic medical record. Presepsin, procalcitonin, and high-sensitivity C-reactive

protein (hsCRP) were also investigated. The sepsis markers for sepsis prediction were evaluated using receiver operating characteristic (ROC) curve analysis and area under the curve (AUC).

Results: A total of 81 patients, including 66 ED and 15 EICU patients, were tested for presepsin. The presepsin results ranged from 101 to 8,788 pg/mL (Mean 1,005.9 pg/mL, Standard deviation 1,509.4). In ROC curve analysis, three biomarkers - presepsin, procalcitonin, and hsCRP generated an area under the curve (AUC) value of 0.698, 0.670, and 0.694 for predicting sepsis, respectively, for the threshold ≥7 of SOFA score to screen the risk of sepsis.

Conclusion: The clinical performance of presepsin in the ED-based setting in Korea was found to be comparable in detecting the risk of sepsis. Further research is needed to determine the optimal use of presepsin in the other clinical areas of the hospital, such as intensive care units, and to assess its impact on patient outcomes.

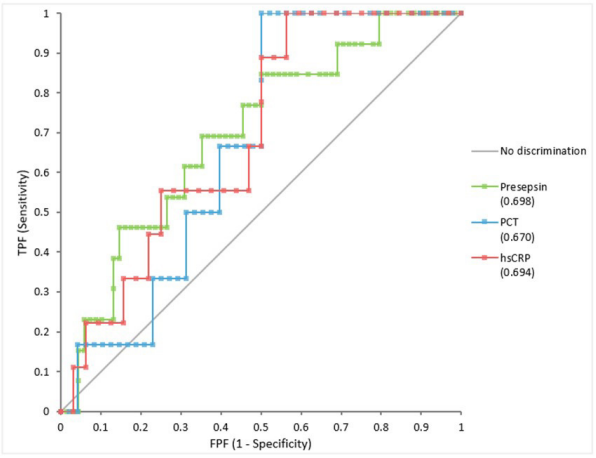


Figure 1. ROC curve analysis of presepsin, procalcitonin, and hsCRP for sepsis prediction

A-257

Association Between Matrix Metalloproteinase-3 and its Post-Transcriptional Regulator miR-17-5p in the Pathogenesis of Tuberculous Meningitis Patients

A. Aggarwal[1], S. R. Sharma[2], M. Modi[3], K. Sharma[2], I. Verma[2], N. Singla[3], M. Konar[2], M. Kaur[2], K. Sharma[2]. *[1]Post Graduate Institute of Medical Education and research, Chandigarh, India, [2]Post graduate institute of medical education and research, Chandigarh, India, [3]Post graduate institute of medical education and research, chandigarh, India*

Background: Tuberculosis, a communicable disease caused by *Mycobacterium tuberculosis* is one of the top 10 causes of death worldwide. Tuberculous meningitis (TBM), an extra-pulmonary central nervous system tuberculosis is the most severe form with maximum mortality. To combat the complications of disease, host immune response gets activated which initially protects the host but later an exaggerated response leads to aggravation of the disease. This causes tremendous neuroinflammation which further increases the complications of the disease. Matrix metalloproteinase (MMPs) like MMP-9, 2 and 3 are known to cause damage to the blood brain barrier and have a role in the pathogenesis of disease particularly increasing the neuroinflammation. MMPs are regulated at post-transcriptional level by microRNAs (miRNA) that alter their levels by targeting various genes. Based on available literature we found miR-17-5p to be a key regulator of MMP3. In this study, we assessed the levels of MMP3 and its post-transcriptional regulator miR-17-5p in the cerebrospinal fluid (CSF) of TBM patients, healthy controls (HC) and non-infectious neuroinflammatory disease patients (NID). **Methods**: In this study, 100 TBM patients were recruited based on the Lancet scoring system and CSF samples were collected. For NID control group, 25 patients with other non-infectious neuroinflammatory diseases like Guillain-Barre syndrome, multiple sclerosis were recruited. Twenty five HC CSF samples were collected from patients undergoing spinal anaesthesia for any lower limb surgery. The samples were stored at -80ºC and processed when required for ELISA and quantitative real time PCR. **Results:** We found a significant increase in levels of median MMP3 levels in the CSF of TBM patients compared to HC group (2.719ng/ml vs 1.696ng/ml). The MMP3 levels were also significantly elevated in the NID control group as compared to HC group (3.116ng/ml vs 1.696ng/ml). A stagewise comparison in TBM group showed an increase in levels of MMP3 with respect to HC but within stages results were comparable. The expression of MMP3 regulator miR-17-5p showed an upregulation in the CSF of TBM group as compared to NID and HC group. **Conclusion:** An increase in levels of MMP3 was observed in both TBM and NID group which infers that MMP3 might have a role in neuroinflammation in neurological conditions. In our study groups we did not find any relation between miR-17-5p expression and MMP3 levels, indicating that there might be some other regulatory pathway altering the levels of MMP3 in neurological conditions. Hence, further studies can be done to highlight the explicit role of MMP3 in neuroinflammation which can then be targeted therapeutically to relieve the associated complications.

A-259

Detecting Human RNase P Gene Prevents False-Negative of SARS-CoV-2

F. Chuang. *Chiayi Chang Gung Memorial Hospital, Puzi City, Chiayi County, Taiwan*

Background: Real-time Reverse transcriptase - polymerase chain reaction (RT-PCR) is a regular technique for detecting SARS-CoV-2 infection. Human RNase P sequence is using as internal control for PCR assay usually. This article discusses the correlation between SARS-CoV-2 PCR result and human RNase P gene result.

Methods: Nasopharyngeal swabs were collected from two patients within three consecutive days. We detected for SARS-CoV-2 target gene and human RNase P gene by using real-time RT-PCR assay, which SARS-CoV-2 target gene includes E gene, Rarp gene and N gene . And the threshold cycle values (Ct) were determined by cobas® z 480. Evaluate the results of SARS-CoV-2 and RNase P gene detection. All samples were also analysis for SARS-CoV-2 infection in Taiwan Centers for Disease Control.

Results: Results of SARS-CoV-2 PCR assay were consistent in both laboratories. SARS-CoV-2 target genes were detected from specimens of both patients on day1. Ct values of E gene were 29.63 and 29.50. Ct values of Rdrp gene were 32.23 and 32.50. Ct values of N gene were 33.09 and 32.68. Ct values of RNase P gene were 21.21 and 21.22. On day 2 and 3, all specimens were negative detected for SARS-CoV-2 PCR. Ct values of RNase P gene were 25.73 and 26.16 on day 2 and 29.82 and 30.28 on day 3.

Conclusion: The Ct values of human RNase P gene detection can be correlated with the numbers of collected cells. Comparing the results of two patients for three consecutive days, the Ct value of human RNase P gene related to SARS-CoV-2 PCR result. Collection technique can be a factor to affect Ct value of human RNase P gene detection. Develop acceptance range for Ct value of RNase P gene detection can be a quality standard for sample collection to prevent false-negative of SARS-CoV-2.

A-262

Analytical and Clinical Validation of a Multiplex RT-PCR based Mpox Assay

P. Upadhyay[1], J. Reddy[1], V. Singh[2]. *[1]HealthTrackRX, Denton, TX, [2]HealthtrackRX, Denton, TX*

Background: The 2022 non-endemic spread of Mpox in the western hemisphere was a unique public health challenge in the backdrop of the COID19 pandemic. To address the public health emergency where Mpox transmission was unlike previous instances, HealthtrackRX was one of the first independent diagnostics labs to develop a multiplexed RT-PCR based test for Mpox. In the present study analytical and clinical validation results for the Mpox RT-PCR test are presented.

Methods: The multiplexed pan-orthopox/pan-Mpox RT-PCR test was validated using inactivated *Mpox* (Clade I and II) viral samples establishing the limit of detection (LoD). Clinical validation of the test was performed against the FDA approved test at CDC and a commercially available pan-Orthpox assay. Sensitivity and specificity performance against interfering substance, pathogens and other poxviruses was also established. All statistical analysis were performed using R version 3.6.0.

Results: The multiplexed pan-Orthopox/pan-Mpox RT-PCR test successfully detected both Clade I and II Mpox and the LoD was established at 10^2 RNA copies/µl.The in-house developed Mpox test displayed 100% concordance against the FDA approved CDC pan-orthopox test and the pan-orthopox RT-PCR test from Thermo Fisher. The test showed no interference from a number of sexually transmitted pathogens and could successfully discern Mpox from several other poxviruses. With the exception of ethanol, no substances (from the FDA recommended list) interfered with the test performance. A total of 1712 patient samples were collected (July 25th, 2022, to February 1st, 2023) and tested for the presence of Mpox.

Conclusion: We have demonstrated the development, validation, and commercialization of a multiplexed RT-PCR based pan-orthopox/pan-Mpox diagnostic test for Mpox. Our data shows the feasibility of rapidly developing and deploying a PCR based test against an emerging infectious disease.

A-263

Prevalence and Antimicrobial Susceptibility Profiles of Escherichia coli Isolated from Retail Meat Products Imported from USA and Domestically Produced on Caribbean Islands of St. Kitts and Grenada

O. O. Fakayode, A. Martens, K. Varghese, T. Luthra, J. Harrington. *St. George's University School Of Medicine, St. George's, Grenada*

Background: *Escherichia coli (E. coli)* is a commensal flora of the gastrointestinal tract of humans and livestock animals. However, pathogenic and opportunistic variants are becoming a significant cause for concern in human healthcare (CDC, 2014). The emergence of these resistant strains is attributed to the overuse of antibiotics in agricultural practices (Llor, 2014). These microbes carry antimicrobial resistance (AMR) genetic markers that cause severe complications in treating intestinal and extra-intestinal infections in humans (Crecencio, 2020). The role of global meat industrialization and trade as a potential reservoir and source of transmission for AMR bacteria is considered a priority for OneHealth Initiatives, with collaborations between researchers, physicians, veterinarians, and food industries.

Methods: This study aims to compare the prevalence and antimicrobial susceptibility profiles of E. coli isolated from pork and poultry products imported from the USA to those domestically produced on the Caribbean Islands of St. Kitts and Grenada. This study was conducted over a 6-year period at the University of Medicine and Health Sciences (UMHS) in St Kitts and St. George's University (SGU) in Grenada. Meat product samples were collected from retail stores around the Caribbean Islands. These meat samples were incubated at 36°C in Buffered media for 60 minutes and 24 hours, then spread onto MacConkey agar to select for Gram-negative bacterial isolates. Lactose fermenting colonies were isolated and subcultured onto Nutrient agar. Species identification was performed using standard biochemical assays and Biomerieux API-20E test kits. Isolates were introduced to Mueller Hinton agar, and antimicrobial susceptibility was tested using the Kirby-Bauer disc diffusion.

Results: 100 meat product samples (50 domestic, 50 imported) were processed for investigation on the Caribbean Islands. On the Island of St. Kitts, 64 and 60 bacterial colonies were isolated from the domestic and imported samples, respectively. The prevalence of E. coli was 16% (n=20) for imported samples and 12% (n=15) for domestically produced samples. As Multiple drug resistance to >3 classes of antibiotics in E. coli isolates was 50% of imported isolates and 27% domestically produced. Resistance to amoxicillin was observed in 60% and 33% of E. coli isolates from Imported and Domestic origins, respectively. Resistance to trimethoprim-sulfamethoxazole was 25% and 13% in imported and domestic, respectively. Resistance to ciprofloxacin was 35% and 27% for imported and domestic, respectively. Resistance to cephalosporins was 60% in isolates from imported meat products and 33% in isolates from meat products domestically produced on the island of St. Kitts. Investigations are still ongoing for bacteria colony isolates from meat product samples from Grenada.

Conclusion: Imported meats products exhibit a higher prevalence of resistant *E. coli*, particularly in the beta-lactam class of antibiotics, including amoxicillin and cephalosporins. Therefore, the risk of importing meat products is a concern for introduction of AMR genetic markers to human populations. Active surveillance must be conducted to better understand the risk of exposure to humans from contaminated retail meat products to inform antibiotic therapy and resistance control continuously.

A-264

Comparison of the ZEUS Scientific Lyme Assays to the DiaSorin Liaison Lyme Assays Using a Modified Two-Tiered Testing Algorithm for Lyme Disease

M. E. Walsh, D. J. Sietsma, I. W. Martin, L. A. Brunelle. *DHMC, Lebanon, NH*

Background: Lyme borreliosis is the most common vector-borne disease in the United States. Modified two-tiered testing (MTTT) strategies have recently been developed that use enzyme immunoassays as the second tier test in place of an immunoblot. We directly compared two sets of MTTT assays with samples obtained from the northeastern United States, a Lyme disease endemic area. **Methods:** Remnant specimens from 120 patients initially tested by the ZEUS Scientific Lyme ELISAs in accordance with an MTTT algorithm were analyzed with the DiaSorin Lyme chemiluminescent immunoassays. The ZEUS assays were performed on a DYNEX DS2 and the DiaSorin assays were performed on the DiaSorin Liaison XL. Analytical concordance was evaluated. **Results:** Of the 25 samples that were negative by the ZEUS VlsE1/pepC10 IgG/IgM assay, 100% (25/25) were negative by the DiaSorin Lyme Total Antibody Plus assay. Of the 95 samples that were presumptive positive or equivocal by the ZEUS IgG/IgM assay, 64% (61/95) were presumptive positive or equivocal and 36% (34/95) were negative by the DiaSorin Lyme Total assay. Samples that tested presumptive positive or equivocal on the ZEUS IgG/IgM or DiaSorin Lyme Total were evaluated by the respective IgG and IgM assays. Positive IgG results and negative IgM results demonstrated >90% agreement between the ZEUS and DiaSorin assays (Table 1). Overall clinical interpretation was concordant between the ZEUS and DiaSorin MTTTs in 57% (54/95) of patients who tested presumptive positive or equivocal on the ZEUS IgG/IgM. Twenty-four samples that tested positive for IgM by the ZEUS assay tested negative for DiaSorin Lyme Total or IgM assays. **Conclusion:** Comparison of the DiaSorin Lyme assays to the ZEUS Lyme assays using an MTTT algorithm suggests that the DiaSorin assays would result in fewer positive screening tests, decreasing unnecessary secondary testing, and may result in fewer patients treated with antibiotics.

A-265

Disparities among SARS-CoV-2 Antibody Positivity in Healthcare Workers

K. E. Mullins, R. Christenson. *University of Maryland School of Medicine, Baltimore, MD*

Background: Early in the COVID-19 pandemic disparities in hospitalizations and mortality rates were seen between race/ethnicities. Currently, the CDC reports that there is a 2.1x and 1.6x increase in risk of hospitalizations and deaths, respectively, for Black non-Hispanic (NH) persons when compared to White NH persons. Additionally, studies have found that Black and Hispanic individuals are more likely to test positive for SARS-CoV-2 as compared to other ethnicities/races. Many socioeconomic factors have been associated with increased risk, however, few if any, studies have investigated the disparities in SARS-CoV-2 exposure for healthcare workers.

Methods: To gain a better understanding of the risk of SARS-CoV-2 exposure in healthcare workers, EDTA plasma samples were collected from healthcare workers associated with the University of Maryland Medical System between June 1st, 2020, and August 31st, 2020. Plasma samples were tested for Anti-SARS-CoV-2 Spike Ig/M/A Antibodies using an in-house developed Enzyme-linked Immunoassay and the Ortho Vitros COVID-19 Total Antibody assay.

Results: 7,850 healthcare workers were enrolled (82.3% female). 26.7% were Black NH and 75.3% were White NH individuals. No differences were seen between age or sex between the two groups. Overall, the positivity rate for Anti-SARS-CoV-2 antibodies was 3.5%, however, when stratified by race/ethnicity 6.7% of Black NH individuals and 2.4% of White NH individuals were positive for Anti-SARS-CoV-2 antibodies. When stratified by sex there were no statistically significant differences.

Conclusion: These findings suggest that Black NH healthcare workers were 2.6 times more likely to have been exposed to SARS-CoV-2 when compared to White NH individuals, irrespective of sex. Further understanding of the geographic and socioeconomic factors that play a role in these findings is needed. However, these findings suggest that heath disparities are prevalent and need to be addressed not only in the communities that our healthcare workers treat, but within our healthcare worker communities themselves.

	Overall N (%)	Black N (%)	White N (%)
N	7850	1937 (26.7%)	5913 (75.3%)
Male	1392 (17.7%)	297 (15.3%)	1095 (18.5%)
Female	6458 (82.3%)	1640 (84.7%)	4818 (81.5%)
Mean Age	47.1	47.8	46.8
Positive for Anti-SARS-CoV-2 Antibodies	274 (3.5%)	130 (6.71%)	144 (2.43%)
Male	*47 (3.37%)*	*15 (5.03%)*	*30 (2.74%)*
Female	*228 (3.5%)*	*115 (7%)*	*114 (2.36%)*

A-266

Evaluation of an Antibody Index Assay for Assessment of Intrathecal Anti-*Treponema pallidum* IgG Synthesis for the Diagnosis of Neurosyphilis

D. Granger, E. S. Theel. *Mayo Clinic, Rochester, MN*

Background: Diagnosis of neurosyphilis (NS) is challenging due to nonspecific clinical symptoms and the lack of a sensitive and specific laboratory test. The non-treponemal Venereal Disease Research Laboratory (VDRL-CSF) assay is a commonly used test in diagnosing NS. An important limitation of the VDRL-CSF is limited specificity, in part due to the inability to differentiate between intrathecally produced antibodies versus passive antibody diffusion into the CSF due to blood-brain barrier (BBB) disruption. Here, we compared the intrathecal synthesis antibody index (AI) of anti-Treponema pallidum IgG antibodies to results from the VDRL-CSF assay in routinely submitted clinical samples for evaluation of suspected NS. **Methods**: Paired serum and CSF residual clinical samples from 98 patients with previous VDRL-CSF results were collected. For each pair, the serum and CSF were drawn within 24 hours. No additional clinical information and limited supporting clinical test results were available. Thirty-one positive and 67 negative paired samples were tested by an anti-*T. pallidum* IgG ELISA (Euroimmun, Lübeck, Germany). The AI was calculated using Reiber's method, with intrathecal total IgG synthesis corrected using the Reiber recommended albumin quotient calculation. Results were compared for positive, negative and overall agreement between methods. **Results**: The anti-*T. pallidum* AI showed a positive, negative and overall agreement of 71.0% (22/31), 100% (67/67) and 90.8% (89/98), respectively, compared to VDRL-CSF. Of note, all nine discordant samples showed abnormally elevated albumin levels in CSF (range: 27.4 to 215 mg/dl; median = 41.7 mg/dl; normal value = <27 mg/dl), indicating a compromised BBB which likely resulted in false-positive VDRL-CSF results. The range and median value for CSF albumin in the 22 concordant samples was 12.4 to 168 mg/dl and 37.2 mg/dl, respectively. In total, 83.9% (26/31) of VDRL-CSF positive samples showed elevated CSF albumin levels which suggests a high percentage of BBB impairment in this patient population. This demonstrates the advantage in specificity that is offered by AI testing compared to use of the VDRL-CSF assay, given the ability of AI to differentiate intrathecally produced versus passively diffused syphilis-specific antibodies. No potential increase in sensitivity was observed in AI testing as 100% negative agreement was obtained. **Conclusion**: Our data suggests that establishment of an AI using a treponemal assay to detect intrathecal antibody synthesis provides improved specificity compared to the current commonly performed VDRL-CSF antibody test as an aid in the diagnosis of NS. Further evaluation of sensitivity is warranted using samples from patients with clinically diagnosed NS.

A-267

Evaluation of the Quidel Sofia 2 rapid Lyme test in a modified two-tier testing protocol

K. Lewandrowski, J. Branda, S. Turbett, E. Lee-lewandrowski. *MGH, Boston, MA*

Background: Several Modified two-tier testing algorithms (MTTT) for Lyme disease involving sequential enzyme immunoassays (EIA) have been approved by the FDA as an alternative to conventional two-tiered testing using EIA followed by immunoblot. The MTTT algorithms are reported to be more sensitive than conventional two-tiered algorithm's especially in early Lyme disease. The Quidel Sofia 2 Lyme assay utilizes lateral flow with fluorescence detection (FIA) providing a rapid on-demand instrument-read alternative to central laboratory batch mode testing We compared the Quidel assay as a first-tier test in an MTTT algorithm to the Zeus Scientific VlsE1/pepC10 IgG/IgM enzyme immunoassay (EIA) followed by the Zeus monovalent IgM and IgG EIA as the confirmatory test. **Methods:** Serum specimens (n=179) were obtained from consecutive samples sent to our clinical laboratory for Lyme serology testing. For the first-tier the samples were tested using the Zeus EIA VlsE1/pepC10 lgG/IgM test and the Quidel Sofia 2 Lyme FIA assay. Positive or equivocal first-tier tests were then tested using the Zeus monovalent IgM and IgG EIA as the confirmatory test. **Results:** The positive percent agreement was 100%, the negative percent agreement 97.9 % with an overall agreement of 98.3%. The Kappa statistic was 0.945 (95% confidence interval 0.883-1.000 indicating "almost perfect" agreement). **Conclusion:** The Quidel Sofia Lyme test performs well when compared to the Zeus test in a two-tiered testing MTTT algorithm. Obtaining samples to evaluate Lyme serologic tests can be challenging. Our data should prove useful for facilities considering the use of the Quidel assay.

A-268

Evaluation of a Real-Time Multiplex PCR Assay for Simultaneous Detection of *Chlamydia trachomatis, Neisseria gonorrhoeae,* and *Trichomonas vaginalis* in Urine Specimens

L. Branson, D. Southard, A. Jaime, W. Katrangi, R. Alturkmani. *Labtech Diagnostics, San Antonio, TX*

Background: Infections with *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) are among the most prevalent and treatable sexually transmitted diseases in the US. Currently, most available testing methods require patient samples to be split between CT/NG and TV assays. Here, we evaluate performance characteristics of the Seegene Novaplex STI Essential Assay, a real-time multiplex polymerase chain reaction assay for concurrent amplification and detection of multiple pathogens including CT, NG, and TV.

Methods: To prepare urine samples for extraction, 1 mL of each sample was centrifuged at 13,000 rpm for 15 minutes. The supernatant was discarded and the pellet was rehydrated in 190 µL of saline. Nucleic acid extraction was performed on a CyBio® FeliX liquid handler (Analytik Jena, Germany) using MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher Scientific, MA). PCR amplification and detection were performed using Seegene Novaplex STI assay (Seegene Inc, South Korea) on a CFX96™ System (Bio-Rad Laboratories, CA). The assay utilizes Seegene MuDT™ technology that allows detection of multiple targets in a single channel without melting curve analysis. For method validation, precision studies were performed within-run and over 5 days. Limits of detection were verified by spiking known negative urine samples with CT/NG standards from Exact Diagnostics (Bio-Rad Laboratories, CA) and TV standards from ZeptoMetrix® (Antylia Scientific, IL). Method comparison was carried out by extracting and analyzing split patient urine samples against cobas® CT/NG assay (Roche Diagnostics, IL) and Solana® Trichomonas assay (Quidel, CA) performed by a reference laboratory. Additional accuracy studies were performed by spiking 20 negative patient samples with variable concentrations of the three pathogens.

Results: Within-run and between-day precision studies demonstrated consistent and reproducible results for all pathogens. Limit of detection was established at 10 copies/mL for CT and NG and 10 cells/mL for TV. Results of the spike-and-recovery studies were consistent with expected results for all three targets across evaluated concentrations. Patient correlation studies demonstrated 100% agreement with results obtained by the reference laboratory for both NG (32 total samples, 11 positive) and TV (22 total samples, 4 positive), and 96% agreement for CT (29 total samples, 10 positive).

Conclusion: The Seegene Novaplex STI Essential Assay offers a rapid and reliable method for detecting CT, NG, and TV in urine samples. The capability of the assay to detect multiple targets in a single sample well improves efficiency and turnaround time.

A-269

Post-pandemic Opportunities for Commercial Laboratories in Infectious Disease Diagnostics and Public Health

D. Nall, X. Thacker, B. Sengsouk, M. Hardison, C. Clark. *Aegis Sciences Corporation, Nashville, TN*

Background: Aegis launched COVID-19 testing with a daily capacity of 3000 tests and scaled to over 100,000 tests/day in one year. The lab has performed over 14.2M COVID-19 tests to date, servicing a broad network of physicians, urgent care centers, nursing homes, public schools, departments of health, and retail pharmacies across the United States. As SARS-CoV-2 infections reach an equilibrium, Aegis recognized the opportunity to address broader public health needs and leveraged the partnerships, technologies, and infrastructure developed to build the Infectious Disease Testing (IDT) menu aimed at limiting the spread of health risks within the community. Methods: Real-time polymerase chain reaction (RT-PCR) tests for common and rare bacterial, viral, and parasitic pathogens associated with genital health (GH) and sexually transmitted infections (STI), as well as infections of the urinary (UTI), gastrointestinal (GI), and respiratory tracts (RTI) were validated. These tests are performed in 384-well custom PCR plates, pre-spotted with primers and probes capable of detecting the presence of DNA or RNA (LOD = 10 copies/µL) from 30 different organisms in up to 12 patient samples simultaneously. Syndromic panels were developed for ordering based on similar intended use, and providers have the option to order panels or stand-alone tests based on medical necessity. Following receipt into the lab, the total nucleic acid is extracted from the primary specimen and pipetted into the PCR assay plate with Taq DNA polymerase master mix, and analysis and reporting of ordered targets are complete within nine hours, on average. Results and de-identified demographic data are parsed from the laboratory information management system (LIMS)

to Microsoft PowerBI software to internally track testing trends, positivity rates, and outbreaks of infectious diseases. Results: Historical data including ordered profiles, matrices, and pathogens detected combined with de-identified demographic data such as patient age, gender, race, residing state, and collection month were imported into PowerBI and used to create interactive visuals for a dashboard to monitor trends and predict future testing patterns. The IDT panels ordered most frequently include STI (27.1%), upper RTI (23.6%), bacterial vaginosis (21.0%), GI bacterial (15.1%), candidiasis (13.7%), and GI viral (13.0%). The majority (74%) of GH/STI tests were ordered for patients between the ages of 15 to 34 years, and the most prevalent infections detected were bacterial vaginosis (35%), mycoplasma hominis (22%), and chlamydia trachomatis (4%). Sixty-one percent (61%) of upper RTI tests were ordered for patients between the ages of 0 to 14 years, and fluctuations in positivity rates followed seasonal trends for pathogens such as rhinovirus, influenza, adenovirus, RSV, and enterovirus D68. GI profiles were predominantly ordered for the younger (0-14 years, 35%) and the older (>65 years, 18%) population with e. coli O157 (11%), norovirus (9%), sapovirus (6%), and astrovirus (4%) infections detected at the highest rates. Conclusion: The COVID-19 pandemic underscored the importance of molecular diagnostics for SARS-CoV-2 infection and surveillance. Experience gained during the COVID-19 response allowed Aegis to repurpose the resources, capacity, and infrastructure to create business development opportunities aimed at laboratory growth in infectious diseases and public health.

A-271

Verification of a multiplexed STI Pathogen Microarray Test with Saliva Collected With SDNA-1000 Kit

C. R. Quinter[1], J. Serrett[1], M. M. Jensen[2], D. Vigerust[3], R. Gupta[3], J. Peek[4]. *[1]Alimetrix, Huntsville, AL, [2]Spectrum Solutions, Inc., Draper, UT, [3]Spectrum Solutions, LLC., Draper, UT, [4]Microarray, Inc., Huntsville, AL*

Background:

Sexually transmitted infections (STI) spread through oral sex potentially cause infections of the mouth, throat, genitals, or rectum. Saliva may be an important source of transmission of STI following oral sex. Multiplexed STI assays are useful diagnostically for patients with risk factors for STI as co-infections occur frequently.

Methods:

Alimetrix STI is a multiplexed laboratory developed test for the identification of STI pathogens present in clinical specimens. DNA was collected in SDNA-1000 devices, extracted, purified, underwent multiplex PCR, and labeled amplicons were hybridized to a microarray with gene-specific 50mer capture probes. Fluorescently tagged amplicons were detected on a microarray scanner. Verification included analytical sensitivity (Limit of Detection (LOD), competition, and inclusivity), stability, and analytical specificity (exclusivity, precision and accuracy). Pathogens included in the multiplexed microarray included N. *gonorrhea,* C. *trachomatis,* T. *vaginalis,* T. *pallidum , herpes 1 and 2, M. genitalium,* U. *urealyticum*, and monkeypox.

Results:

LOD for each pathogen in the Spectrum Solutions SDNA Collection System, throat swab, and first catch urine is found in Table 1. Competition of organisms showed the ability to co-detect up to 5 organisms from a single sample. Saliva collected using Spectrum Solutions SDNA Collection System was stable for 35 days at room temperature enabling discreet at home collection. Inclusivity showed related strains were detectable at 1x LOD, while maintaining 100% precision, 100% accuracy, and 97% exclusivity for high concentrations of closely related analytes in normal flora.

Conclusion:

Control of STI associated with oral sex is dependent upon the demonstration of all pathogens present. Saliva collected in the SDNA-1000 device a high degree of sensitivity, precision, and accuracy, as well as the ability to detect multiple analytes (co-infection) within a given sample. Saliva is a clinically informative specimen source useful for the diagnosis, monitoring, and management of patients with oral and systemic disease.

Table 1: Limit of Detection in Clinical Matrices

STI Target (Strain)	Analytical LoD	Urine (pooled male/fel	Saliva	Throat Swab
Neisseria *gonorrhoeae (z017)*	30 CFU/mL	30 CFU/mL	250 CFU/mL	30 CFU/mL
Chlamydia *Trachomatis* (Z054,D-UW)	50 EB/mL	50 EB/mL	500 EB/mL	50 EB/mL
Trichomonas (Z070)	5 trophs/ml	5 trophs/ml	5 trophs/ml	Not Detected
Herpes Simplex Virus 1 (Macintyre)	50 $TCID_{50}$/mL	50 $TCID_{50}$/mL	50 $TCID_{50}$/mL	Not Detected
Herpes Simplex Virus 2 (MS)	50 $TCID_{50}$/mL	50 $TCID_{50}$/mL	50 $TCID_{50}$/mL	50 $TCID_{50}$/mL
Muycoplasma *genitalium* (SEA-1)	150 CCU/mL	150 CCU/mL	1500 CCU/mL	Not Detected
Ureaplasma *urealyticum* (ATCC 27618)	1500 CCU/mL	1500 CCU/mL	1500 CCU/mL	Not Detected
Treponema *pallidum (CDC N8k9Q2CL)*	500 Treponemes/mL	500 Treponemes/mL	5000 Treponemes/mL	500 Treponemes/mL
MPox_85PRB (USA/_ MA001/2022)	150 copies/mL	150 copies/mL	150 copies/mL	150 copies/mL
Mpox_N3RPB (USA/_ MA001/2022)	1500 copies/mL	1500 copies/mL	1500 copies/mL	1500 copies/mL

A-272

Challenges in designing an antibiotic gene resistance panel for clinical use.

L. Dedhia. *Accureference medical lab, linden, NJ*

Background:

We faced 3 major challenges in our journey of designing a custom panel for antibiotic resistance genes (ABR).Challenge 1# Fast, robust, and affordable antimicrobial resistance testing (ABR) In general, qPCR-based methods are efficient, reliable, and fast with high specificity and sensitivity, and with the test results available within 2-3 h. We offer urinary tract infection identification by qPCR and wanted to supplement this test with detection of antibiotic resistance genes. we narrowed our choice of method to be qPCR based, Applied Biosystems™ OpenArray™ technology on the Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System. Challenge2# Genes to be tested. Antibiotic resistance occurs via various mechanism. Based on the literature review, we included the following gene in our custom panel 1. Aminoglycosides: aac(6')-Ib-cr6, aac(6')-Ib-cr, aac(6')-Ib-cr4 2. Tetracycline: tet(M), tet(B) 3. Sulfonamides: sul1, sul2 4. Macrolide-lincosamide-streptogramin B-ketolides-oxazolidinones: erm C, erm A, erm B 5. Quinolone/(fluoroquinolones): qnrS , qnrA, aac(6')-Ib-cr6, aac(6')-Ib-cr, aac(6')-Ib-cr4 6. Glycopeptides: VanA, VanB 7. Beta-lactam Class A: SHV, KPC, CTX-M 8. Beta-lactam Class B: NDM, VIM, IMP 9. Beta-lactam Class C: CMY, DHA, FOX, KPC 10. Beta-lactam Class D: OXA1, OXA-23, OXA-48, 11. Methicillin resistance: mecA

Results:

Validation of the ABR panel Accuracy: 50 isolates were ordered from CDC AR isolate bank and were used to test the accuracy. The accuracy was detected to be 98% concordant. Sensitivity: The assay was sensitive to detect all urinary pathogens upto 10^3 CFU/mL. Precision: the inter and intra reproducibility was shown to be 99% concordant Specific: when tested against the CDC controls, the assays were 100% specific

Challenge 3# Genotype - Phenotype correlation and prevalence of antibiotic resistance genes in USA. We compared 100 consecutive clinical specimens (urine) for genotype -phenotype co-relation. Data from the current qPCR panel and Microscan instrument was compared. It was observed for the pathogens that were phenotypically resistant to certain antibiotics, the corresponding gene association was not found in (2-50%) of antibiotic groups. This can be explained due to the limited number of genes in each drug class. Resistance to some antibiotics such as nitrofurantoin drug (100% missed) and sulphonamide drug resistance (50% missed) is induced by point mutations, which are not captured in open array genotyping qPCR assay. To overcome this

gap between genotype and phenotype correlation and to understand the prevalence of antibiotic resistance genes in USA, we initiated a new project. We aim to test for 300-400 clinical samples which known resistance phenotypes and check for antibiotic resistance gene in a new expanded panel. We were successful in designing the new panel to include 88 genes (vs 30 genes in the old panel).

Conclusion: The current assay was approved by the CLIA authorities for clinical testing. In general, qPCR-based methods are efficient, reliable, and fast with high specificity and sensitivity, and with the test results available within 2-3 h.

A-273

Multicenter Assessment of the Accuracy of MIC Results for Piperacillin/Tazobactam with MicroScan Dried Gram-negative MIC Panels using CLSI Breakpoints

A. Chipman[1], G. Omai[2], A. Harrington[3], M. Traczewski[4], S. DesJarlais[3], D. Beasley[4], J. Diaz[1], R. Brookman[1], C. Hastey[1], J. Chau[1]. *[1]Beckman Coulter Microbiology, West Sacramento, CA, CA, [2]UCLA David Geffen School of Medicine, Los Angeles, CA, [3]Loyola University & Medical Center, Maywood, IL, [4]Clinical Microbiology Institute, Wilsonville, OR*

Background: MIC data from MicroScan Dried Gram-negative MIC (MSDGN) Panels with piperacillin/ tazobactam were evaluated with CLSI-M100-ED32 breakpoints for *Acinetobacter*, Enterobacterales, Other-Non-Enterobacterales, and proposed CLSI breakpoints for *Pseudomonas aeruginosa* from a multicenter clinical study. MIC results were compared to results obtained with CLSI frozen broth microdilution panels.
Materials/Methods: The study included a total of 683 clinical isolates tested using Prompt and turbidity during efficacy and challenge phases. MSDGN panels were evaluated at three clinical sites, comparing MIC values obtained using MSDGN panels to MICs utilizing CLSI reference panel. MSDGN panels were incubated at 35±1°C and read on WalkAway System, autoSCAN-4 instrument, and read visually at 16-20 hours. Frozen reference panels were prepared according to CLSI methodology, incubated for 16-20 hours for Enterobacterales, Other Non-Enterobacterales, and *Pseudomonas aeruginosa* and for 20-24 hours for *Acinetobacter* and read visually. CLSI breakpoints(mg/L) used for interpretation of MIC results were: ≤16/4 S, 32/4-64/4 I, ≥128/4 R for *Acinetobacter* and Other Non Enterobacterales, ≤8/4 S, 16/4 SDD, ≥32/4 R for Enterobacterales, and ≤16/4 S, 32/4 I, >64/4 R for *Pseudomonas aeruginosa*.
Results: When compared to frozen reference panel results, essential and categorical agreements for all isolates tested are as follows with these recommendations: Due to elevated major error rates for *S. marcescens* and WalkAway reads with Prompt, results should be confirmed visually prior to reporting. Due to performance with *P. rettgeri* and *S. liquefaciens* complex, do not report drug, therapy, or MIC.

Organism Group	Prompt EssentialAgreement %		Prompt Categorical Agreement %		Prompt Major Error %		Prompt Very Major Error %	
	Walk-Away	Manual	Walk-Away	Manual	Walk-Away	Manual	Walk-Away	Manual
Acinetobacter spp.	96.0 (48/50)	96.0 (48/50)	96.0 (48/50)	100 (50/50)	0 (0/33)	0 (0/33)	0 (0/15)	0 (0/15)
Enterobacterales	93.4 (510/546)	96.5 (527/546)	92.5 (505/546)	95.6 (522/546)	3.8 (17/452)	0.4 (2/452)	1.3 (1/75)	1.3 (1/75)
Other Non-Enterobacterales	100 (15/15)	100 (15/15)	100 (15/15)	100 (15/15)	0 (0/14)	0 (0/14)	--	--
P. aeruginosa	92.9 (65/70)	94.3 (66/70)	95.7 (67/70)	95.7 (67/70)	4.1 (2/49)	2.0 (1/49)	0 (0/19)	0 (0/19)
All Organisms	93.7 (638/681)	96.3 (656/681)	93.3 (635/681)	96.0 (654/681)	3.5 (19/548)	0.6 (3/548)	0.9 (1/109)	0.9 (1/109)

Conclusion: Piperacillin/tazobactam MIC results for gram-negative clinical isolates obtained with the MSDGN panel correlate with MICs obtained using frozen reference panels with updated CLSI interpretive criteria.

A-274

Validation of Cefepime-Taniborbactam MIC Antimicrobial Susceptibility Test for MicroScan Dried Gram-Negative MIC Panels from a Multicenter Assessment of Enterobacterales and *Pseudomonas aeruginosa*

O. Garner[1], A. Harrington[2], C. Emery[3], S. DesJarlais[2], J. Diaz[4], A. Chipman[4], C. Hastey[4]. *[1]UCLA David Geffen School of Medicine, Los Angeles, CA, [2]Loyola University & Medical Center, Maywood, IL, [3]Indiana University School of Medicine, Indianapolis, IN, [4]Beckman Coulter Microbiology, West Sacramento, CA, CA*

Background: Cefepime-taniborbactam is an investigational agent with activity against carbapenem and multidrug-resistant Enterobacterales and *Pseudomonas aeruginosa*.[1] Given the need for accurate antimicrobial susceptibility testing results to support patient care decisions, a multicenter study was conducted with Enterobacterales and *Pseudomonas aeruginosa* clinical isolates on investigational MicroScan Dried Gram-negative MIC (MSDGN) panels with cefepime-taniborbactam.[2]

Materials/Methods: MSDGN panels were evaluated at three clinical sites by comparing MIC values obtained using MSDGN panels to MICs utilizing CLSI broth microdilution reference panels. The study included 490/491 Enterobacterales and 65 *Pseudomonas aeruginosa* clinical isolates tested using Prompt® and turbidity methods of inoculation during the combined efficacy and challenge phases. MSDGN panels were incubated at 35±1°C and read on the WalkAway System, the autoSCAN-4 instrument, and visually at 16-20 hours. Frozen reference panels were prepared according to CLSI/ISO methodology, incubated for 16-20 hours and read visually.

Results: Essential agreement was calculated compared to MIC results from frozen reference panels for isolates tested in efficacy and challenge and found in the following table.

Read Method	Enterobacterales Essential Agreement (EA) % (n/N)		*Pseudomonas aeruginosa* Essential Agreement (EA) % (n/N)	
	Prompt	Turbidity	Prompt	Turbidity
WalkAway	90.0 (441/490)	99.2 (487/491)	100.0 (65/65)	100.0 (65/65)
autoSCAN-4	94.3 (462/490)	99.0 (486/491)	98.5 (64/65)	98.5 (64/65)
Visually	92.7 (454/490)	99.2 (487/491)	100.0 (65/65)	100.0 (65/65)
Prompt = Prompt inoculation method, Turbidity = Turbidity inoculation method				

Conclusion: Cefepime-taniborbactam MIC results for Enterobacterales and *Pseudomonas aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels in this multicenter study.

1. Pending approval by the US Food and Drug Administration; not available for in vitro diagnostic use in the US. Not for Distribution in the US.

2. Pending submission and clearance by the US Food and Drug Administration; not yet available for in vitro diagnostic use in the US.

For Investigational Use Only. Performance characteristics of this product have not been established.

A-275

Clinical evaluation of SMARTCHEK® SARS-CoV-2 Detection Kit and SMARTCHEK® SARS-CoV-2 Fast Detection Kit for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal swabs

J. Ahn[1], W. Lee[2], S. Yoon[3], S. Cho[4]. *[1]Gachon University College of Medicine, Incheon, Korea, Republic of, [2]School of Medicine, Kyung Hee University, Seoul, Korea, Republic of, [3]Korea University College of Medicine, Seoul, Korea, Republic of, [4]Ajou University School of Medicine, Suwon, Korea, Republic of*

Background: Rapid and accurate diagnosis of COVID-19 is very important in our daily lives. SMARTCHEK® SARS-CoV-2 Detection Kit (Genesystem, Daejeon, Korea) and SMARTCHEK® SARS-CoV-2 Fast Detection Kit (Genesystem) are rapid PCR kits for SARS-CoV-2 using a microfluidic chip-based PCR method. These two kits can provide results in 40 minutes and 20 minutes, respectively, enabling rapid

point-of-care diagnostics. In this study, the diagnostic performance of SMARTCHEK® SARS-CoV-2 Detection Kit and SMARTCHEK® SARS-CoV-2 Fast Detection Kit using UF-340 Four-in-One Real-time PCR System (Genesystem) were compared to STANDARD M nCoV Real-Time Detection kit (SD Biosensor, Korea) using CFX96 Dx System (Bio-Rad, USA) in nasopharyngeal swabs.

Methods: Nasopharyngeal swabs were collected and frozen (at ≤ -70°C) using 500ul of the residual samples of 64 patients who underwent COVID-19 PCR at Ajou University Hospital. After thawing, RNA was extracted with TANBead OptiPure Nucleic Acid Auto Plate (Taiwan Advanced Nanotech Inc., Taoyuan, Taiwan) using SLA-E13200 (Taiwan Advanced Nanotech Inc.), and real time PCR was performed according to the manufacturer's instructions. For correlation evaluation, positive, negative, and overall concordance rates were calculated.

Results: Among 64 samples, results from STANDARD M nCoV Real-Time Detection kit showed 49 negative (77%) and 15 positive (23%) results. Ct value exceeding 30 was observed in 4 samples (27% of positive samples). The positive, negative, and overall concordance rates of the STANDARD M nCoV Real-Time Detection Kit and SMARTCHEK® SARS-CoV-2 Detection Kit were all 100%. One positive sample with the STANDARD M nCoV Real-Time Detection Kit (Ct value: E gene 34.5, RdRp gene 35.5) was excluded from the analysis because the results could not be determined even after repeated testing with SMARTCHEK® SARS-CoV-2 Detection Kit. The positive, negative, and overall concordance rate of the STANDARD M nCoV Real-Time Detection Kit and SMARTCHEK® SARS-CoV-2 Fast Detection Kit were 87%, 100%, and 97%, respectively. Both of the two discrepant samples had a Ct value close to 35.

Conclusion: SMARTCHEK® SARS-CoV-2 Detection Kit and SMARTCHEK® SARS-CoV-2 Fast Detection Kit can quickly and accurately diagnose COVID-19 in nasopharyngeal swabs and is especially useful in small to medium-sized laboratories or areas where space or equipment is limited.

A-276

Clinical performance evaluation of SMARTCHEK® SARS-CoV-2 Detection Kit and SMARTCHEK® SARS-CoV-2 Fast Detection Kit for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in saliva

S. Park, J. Yoo, S. Cho. *Ajou University School of Medicine, Suwon, Korea, Republic of*

Background: Saliva is a good alternative to the nasopharyngeal swab because it is non-invasive and does not require separate collection personnel. SMARTCHEK® SARS-CoV-2 Detection Kit (Genesystem, Daejeon, Korea) and SMARTCHEK® SARS-CoV-2 Fast Detection Kit (Genesystem) are rapid PCR kits for SARS-CoV-2 using a microfluidic chip-based PCR method. These two kits can provide results in 40 minutes and 20 minutes, respectively, enabling rapid point-of-care diagnostics. This study compared the diagnostic performance of SMARTCHEK® SARS-CoV-2 Detection Kit and SMARTCHEK® SARS-CoV-2 Fast Detection Kit using UF-340 Four-in-One Real-time PCR System (Genesystem) to the STANDARD M nCoV Real-Time Detection kit (SD Biosensor, Korea) using CFX96 Dx System (Bio-Rad Laboratories, Inc., USA) in saliva.

Methods: Saliva was collected in a sterile urine specimen cup after obtaining consent from subjects who were scheduled to perform a COVID-19 PCR within 24 hours or had already performed it within 24 hours at Ajou University hospital. RNA was extracted by SLA-E13200 (Taiwan Advanced Nanotech Inc., Taoyuan, Taiwan) and Saliva:Direct™ Extraction Buffer (Genesystem), respectively. The Saliva:Direct™ Extraction Buffer was able to extract RNA within 10 minutes without additional equipment. Real-time PCR was performed according to each manufacturer's guidelines. The STANDARD M nCoV Real-Time Detection kit after RNA extraction by SLA-E13200 was tested as the control method. The positive, negative, and overall concordance rates were calculated for evaluation of correlation with the control method. This study was approved by Ajou University Hospital IRB.

Results: Among 66 samples, the control method identified 50 negative (76%) and 16 positive (24%) samples, two of which showed Ct values exceeding 30 (13% of positive samples). When RNA was extracted with SLA-E13200, the positive, negative, and overall concordance rates of SMARTCHEK® SARS-CoV-2 Detection Kit and SMARTCHEK® SARS-CoV-2 Fast Detection Kit were 100%, 100%, 100%, and 94%, 100%, 98%, respectively. A positive sample with a Ct value exceeding 30 showed discrepant results with SMARTCHEK® SARS-CoV-2 Fast Detection Kit. After RNA extraction with Saliva:Direct™ Extraction Buffer, positive condordance rates of STANDARD M nCoV Real-Time Detection Kit, SMARTCHEK®SARS-CoV-2 Detection Kit, and SMARTCHEK® SARS-CoV-2 Fast Detection Kit compared to the control method were 67%, 75%, and 81%, respectively. All negative concordance rates were 100%, and overall concordance rates were 94%, 94%, 95%, respectively. Four out of 16 positive samples by the control method showed inconclusive results with the STANDARD M nCoV Real-Time Detection Kit when RNA was extracted with Saliva:Direct™ Extraction Buffer.

Conclusion: SMARTCHEK® SARS-CoV-2 Detection Kit and SMARTCHEK® SARS-CoV-2 Fast Detection Kit can quickly and accurately diagnose COVID-19 in saliva after RNA extraction with SLA-E13200, and may be useful especially in small to medium-sized laboratories or areas lacking in space or equipment. Although the RNA extraction process with the Saliva:Direct™ Extraction Buffer is fast, simple, and doesn't require supplementary equipment, some improvement is warranted before use in clinical settings due to relatively low positive concordance rates.

A-277

Health economic impact of use of GCAL calprotectin immunoassay for early detection of infection in intensive care patients

M. Jaquemar[1], A. Larsson[2], A. Havelka[1]. *[1]Gentian AS, Moss, Norway, [2]Department of Medical Sciences, Clinical Chemistry, Akademiska University Hospital, Uppsala, Sweden*

Background:

Early diagnosis of bacterial infections in critically ill patients is challenging, as the clinical manifestation is non-specific. Neutrophil activation is a major response to bacterial infection and calprotectin an important marker for neutrophil mediated inflammation. Earlier studies have shown the ability of calprotectin to predict bacterial infections before onset of clinical symptoms. With early diagnosis of bacterial infections delayed treatment will be avoided as well as deterioration due to severe infections/sepsis.

Methods:

A decision tree model is employed to estimate the impact of calprotectin analysis for early detection of bacterial infection and thus, the earlier start of antibiotic treatment compared to other diagnostic comparators such are white blood cell count, procalcitonin, C-reactive protein, and no testing. The analysis is based on patients admitted to an ICU in a Swedish hospital. The model allows for different diagnostic outcomes based on correctly and incorrectly diagnosis of bacterial infection and timing of antibiotic treatment: patient survival, length of stay in ICU and in general ward and total costs.

Results:

The base-case results show that predictively measuring of calprotectin in an ICU, using GCAL® assay reduces total costs by approximately 13,000 - 18,000 EUR per patient, overall mortality rate by 0.11, and mean length of stay in an ICU and general ward by 1.3 - 2 days and 6 - 8 days, respectively.

Conclusion:

The base-case scenario identified GCAL® Calprotectin Immunoassay as cost-effective for a patient cohort presenting in a Swedish ICU. Compared to the comparators, GCAL® saves total costs, reduces the mean duration of in-patient care, and reduces in-hospital mortality in those patients. In the sensitivity analysis, when key model inputs are varied, GCAL® Calprotectin Immunoassay remains the dominant option.

A-278

Development and validation of platform independent, turbidimetric Sars-CoV-2 total antibody assay

M. Jaquemar[1], T. Nilsen[1], A. Safdari[1], A. Haukebø[1], E. Trosten[1], A. Havelka[1], Ø. Olsvik[2]. *[1]Gentian AS, Moss, Norway, [2]. Department of Medical Microbiology, Faculty of Medicine, UiT Norwegian Arctic University, Tromsø, Norway*

Background:

The outbreak of the SARS-CoV-2 pandemic and continuous presence of the virus in the community require efficient tests for monitoring the anti-SARS-CoV-2 levels and management of the vaccination response in the population. Gentian AS, in collaboration with the University of Tromsø, has developed a quantitative SARS-CoV-2 antibody assay that captures the full immune response detecting antibodies targeting the S1-subunit with high sensitivity and specificity and is calibrated against the WHO international standard.

Methods:

The immunoassay was developed and validated on turbidimetric instruments Cobas c501 from Roche and BS240 BS/240Pro from Mindray. Pre-pandemic samples from

healthy donors or patients, were used for evaluation of clinical specificity. Samples from patients infected with Sars-CoV-2 virus or vaccinated against the virus were used for evaluation of clinical sensitivity.

Results:

The assay has a calibration range from 0 to 1000 BAU/mL. No antigen excess was observed up to 2500 BAU/mL. Precision achieved a CV <20 % for samples with a concentration ≤ 165 BAU/mL, and <10 % for samples >165 BAU/mL. No clinically relevant cross reactivity towards 13 antibodies, including antibodies against other Corona viruses was observed. The assay was able to detect antibodies generated in response to different vaccines and Alpha, Beta, Gamma, Delta and Omicron virus variants. Sensitivity and specificity of the assay met the acceptance criteria set out from the MDCG 2021-21 guideline, ≥90% and >99% respectively.

Conclusion:

The Gentian SARS-CoV-2 total antibody assay is a platform independent high-throughput assay with high sensitivity and specificity for assessment of antibody response after a natural infection and/or vaccination. The assay can ensure effective and reliable monitoring of the immunity and can be used for planning and management of the vaccination response in the community.

A-279

Comparison of Direct Identification and Short Incubation Time Methods of MALDI-TOF MS from Positive Blood Vials

S. KUO[1], L. Pan[1], H. You[2]. [1]*Chiayi Chang Gung Memorial Hospital, Chiayi, Taiwan,* [2]*Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan*

Background: MALDI-TOF analysis after a short incubation time of positive blood culture is currently one of the most widely used methods for rapidly identifying microorganisms. This method can identify most pathogenic bacteria within 4 to 6 hours after blood culture. This experiment aims to use direct identification for MALDI-TOF analysis, which can shorten the identification time of bacteria and analyze the advantages and disadvantages of the two methods. Method: We collected from January 2022 to December 2022, a total of 295 bottles of a single infection. After pretreatment methods such as removing cells and high-speed centrifugation to collect bacteria, carry out direct identification by MALDI-TOF then culture for 5 hours for short incubation time method. The culture medium was continued incubated for 18-20 hours, to confirm the final identification results of the strains and compare the identification accuracy of direct identification and short incubation time methods. Definition: confidence score cut-off values >=1.7 of direct identification method. Result: The identification rate of score> =1.7 were 67%(199/295). Gram-negative bacilli were identified in 89%, followed by anaerobes in 60% and Gram-positive species in 56%. The yeast could not be identified either by the short incubation time method or the direct identification method. The short incubation time method identified 90% of Gram-negative bacilli, followed by 79% of Gram-positive bacteria and 7% of anaerobic bacteria. The rapid identification of anaerobic bacteria was significantly better than the identification results of the short incubation time method (60%:7%) (P value = 0.01). In addition, the results can be obtained within one hour by direct identification, which can save at least 5-fold times required for the short incubation time method of about 5 hours, although the effect is slightly worse than short incubation time method (67 %:76%). Especially for Gram-negative bacilli, there is no statistically significant difference between direct identification and short incubation time methods; it proves that direct identification method can indeed replace short incubation time method. Conclusion: Patients with septic shock who were treated with antibiotics within one hour had an 80% chance of survival; however, for every hour of delay within six hours of onset, the survival rate decreased by 7.6%. Compared with the short incubation time method, the direct identification method can report the correct strain to clinician 5-fold times earlier. This rapid, simplified extraction direct identification method is cost-effective, time-saving, and easy to use. The SSC sepsis guidelines in 2016 pointed out that extending the course of antibiotic treatment not only has no additional benefits but also is prone to drug resistance, resulting in side effects of antibiotics. Therefore, the direct identification method can significantly improve the optimal utilization of antibiotics for effective targeted treatment and reduce mortality, especially for the identification of anaerobic bacteria, which can provide faster identification results. Therefore, it is recommended that clinical laboratories include a direct identification method for routine identification of blood culture, and if they cannot be identified, they can be supplemented by a short incubation time method, to shorten the time of blood culture identification and effectively improve the treatment of bloodstream infections.

A-280

Impact of 2022 CLSI Revised Piperacillin/Tazobactam Clinical Breakpoints on *Enterobacterales* Isolates Identified at a tertiary medical center in southern Taiwan

S. KUO. *Chiayi Chang Gung Memorial Hospital, Chiayi, Taiwan*

Background Piperacillin-tazobactam (TZP) is one of the most common antibiotics administered to hospitalized patients. Its broad activity against gram-negative, gram-positive, and anaerobic pathogens. The Clinical and Laboratory Standards Institute (CLSI) publishes revised TZP breakpoints in 2022. The purpose of this study was to evaluate the impact of changing the TZP breakpoint in a regional teaching hospital in southern Taiwan. Methods We retrospectively evaluated TZP for 1347 Enterobacterales isolates identified by MALDI-TOF (Bruker Daltonik, Bremen, Germany) between September 1, 2022, through December 31, 2022, at Chang Gung Memorial Hospital in Chiayi. Blood antimicrobial susceptibility testing was performed using an NMIC-411 panel on the BD *Phoenix™ M50*. The antibiotic susceptibility test of Enterobacterales from other sources of infection used the Disk diffusion method (Kirby-Bauer Method). Susceptibilities were assessed based on historic CLSI breakpoints in 2021 as well as the current CLSI breakpoints from the most recent CLSI MIC breakpoints, the 32th edition of document M100, published in 2022. Results We evaluated 1347 Enterobacterales isolates. Most isolates identified were *Escherichia coli* [n=768 (57%)] and *Klebsiella pneumoniae* [n=286(21.2%)]. The new TZP breakpoints reduced the percentage of strains that were previously judged to be susceptible by CLSI M100-S31 from 80.9%(1090/1347) to 50%(674/1347). The majority of isolates 674(50%) were susceptible; 416 (30.9%) isolates were susceptible-dose dependent and 257 (19.1%) were resistant to piperacillin/tazobactam using the new CLSI breakpoints. The results of the analysis of the source of infection found that the bacteria that had the greatest impact on the respiratory tract decreased from 43.6% to 12.7%, followed by the genitourinary tract infection decreased from 80.4% to 27%, bodily fluid infection decreased from 69.8% to 24.5%, and abscesses,wounds,pus decreased from 72.7% to 28.1%; The most insignificant are blood infection strains, from 83.1% to 81.3%. Conclusion Our results indicate an increase in resistance to Enterobacterales isolates tested. Non- susceptible to piperacillin/tazobactam from 19.1% to 50% using the revised CLSI breakpoints. The decreased susceptibilities make it clear that microbiology laboratories should accept new MIC breakpoints. Although our study has demonstrated decreased susceptibilities, there is no correlation with clinical outcomes.In conclusion, changes in breakpoints had a significant impact on the susceptibility of TZP for Enterobacterales isolates in our study. Understanding and evaluating the impact of the breakpoint changes is of paramount importance. Institutions should ensure that their breakpoints are up to date to allow for the most optimized treatment.

A-281

Analysis of Concurrent HBV Surface Antigen and HBV DNA Test Results

G. W. Pratt, L. V. Rao. *Quest Diagnostics, Marlborough, MA*

Background: HBV surface antigen (HBsAg) is the serological hallmark of both acute and chronic infection. HBV DNA levels are monitored clinically in chronic HBV patients to evaluate HBV replication, candidacy for antiviral therapy and monitor patients on therapy. Although HBsAg has an intrinsic association with HBV DNA, it does not necessarily correlate with HBV DNA, especially in the natural course of infection. This study evaluates concurrent HBsAg and HBV DNA testing with a focus on natural infection serological markers such as detection of HBV core antibody (HBcAb), HBV e antigen (HBeAg), and HBV e antibody (HBeAb).

Methods: Clinical laboratory results from 1,604 specimens tested over one year for both HBsAg and HBV DNA were analyzed. The HBsAg test is a qualitative assay, but concurrent quantitative HBV DNA results were available. HBV DNA results spanned the measurable range of the assay (lower limit-of-detection: 3.5IU/mL, lower limit-of-quantitation: 10IU/mL). HBsAg-positive/HBV DNA-negative results were further investigated to clarify under what circumstances such discordant results are more likely to be observed.

Results: HBsAg-positive and HBV DNA-negative results, which accounted for approximately 18% of the data set (284 out of 1,604 samples), are more likely to occur when the specimen is HBeAg-negative (Table). A limitation of this study is a lack of information regarding treatment status of the study population. Additionally, HBsAg can persist during HBV treatment. This discordance between HBsAg and HBV DNA test results could also be due to increased integration of HBV into the host genome that produces HBsAg in the absence of measurable viral load in treatment-naïve individuals.

Conclusion: HBsAg-positive and HBV-DNA negative discordance is most often seen when HBeAg is negative.

Additional testing results of HBsAg positive, HBV DNA negative specimens						
HBsAg-Positive / HBV DNA-Negative						
	HBeAg +	HBeAg -	HBeAb +	HBeAb -	HBcAb +	HBcAb -
Percent (N/Total)	9 (7/77)	91 (70/77)	65 (50/77)	35 (27/77)	81 (25/31)	19 (6/31)

A-282

Coinfection Rates of SARS-CoV-2, Influenza, and Respiratory Syncytial Virus

G. W. Pratt, M. Platt, C. Wong, L. V. Rao. *Quest Diagnostics, Marlborough, MA*

Background: In late 2022, a severe spike of respiratory syncytial virus (RSV) cases in the United States alongside seasonal influenza and the ongoing SARS-CoV-2 crisis underscored the potential consequences for respiratory coinfections. Coinfections of respiratory viruses can complicate treatment and increase the likelihood of severe cases. It is not well understood how prevalent respiratory coinfections were during this time in the general and pediatric populations. In this retrospective analysis we examine respiratory molecular diagnostic testing results to estimate the rates of coinfection in the study population.

Methods: Respiratory sample results from a clinical laboratory for a 107-day period in autumn 2022 were analyzed. Assays were performed on two platforms: the Roche cobas® SARS-CoV-2 & Influenza A/B Test and the Cepheid Xpert ® Xpress CoV-2/Flu/RSV plus assay. Overall positivity and coinfection rates were calculated. Coinfection results of the pediatric population were further investigated.

Results: A total of 26,657 respiratory testing results were analyzed. Cases of coinfection of SARS-CoV-2, influenza A, influenza B, and RSV were seen. Positivity ranged from 0.03% to 21.86% and coinfection rates ranged from 0.00% to 2.28% depending upon the pathogen. Overall, 9800 samples (36.76%) came from pediatric patients (age ≤21). Pediatric positivity ranged from 0.04% to 24.70% and coinfection rates ranged from 0.00% to 6.00% depending on the pathogen (Table).

Conclusion: Overall, coinfections were observed in 0.55% of samples comprising 1.33% of all positive results. In this study population, pediatric samples accounted for a disproportionately high percentage of positive results for influenza A, RSV, and SARS-CoV-2 coinfections when compared to the overall percentages from the complete study population.

Positivity and coinfection rates of SARS-CoV-2, influenza A (FluA), influenza B (FluB), and RSV						
	% Positivity	% of SARS-Cov-2 Positives coinfected with FluA	% of SARS-Cov-2 Positives coinfected with FluB	% of SARS-Cov-2 Positives coinfected with RSV	% of FluA Positives coinfected with SARS-CoV-2	% of FluA Positives coinfected with RSV
SARS-Cov-2	21.86% (5,828/26,657)	1.65% (96/5,828)	0.00% (0/5,828)	0.38% (22/5,828)	2.28% (96/4,206)	0.64% (27/4,206)
FluA	15.78% (4,206/26,657)					
FluB	0.03% (7/26,657)					
RSV	13.43% (1,146/8,531)					
Pediatric Population						
SARS-Cov-2	7.48% (733/9,800)	6.00% (44/733)	0.00% (0/733)	2.59% (19/733)	1.82% (44/2421)	0.95% (23/2421)
FluA	24.70% (2,421/9,800)					
FluB	0.04% (4/9,800)					
RSV	17.15% (882/5,144)					

A-285

Performance Evaluation of LIAISON® QuantiFERON®-TB Gold Plus Assay and Its Use in Long-Term Care Facilities.

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

Background: Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis with 7,860 cases reported in 2021 in the United State and estimated 13 million people are living with Latent TB infection (LTBI); Early detection of TB infection is crucial to prevent the spread of the disease; there are two types of tests for TB and LTBI: Tuberculin Skin Test (TST) and Interferon-Gamma Release Assay (IGRA). Although TST has good sensitivity but false-negative reactions occur in certain population and the specificity of is decreased among persons with prior Bacillus Calmette-Guérin (BCG) vaccination, especially those vaccinated post-infancy and those with repeat vaccination. Similarly, persons living in areas where nontuberculous mycobacteria are common are at increased risk of having false-positive TST reactions. IGRA is blood-based laboratory test that measures responses to TB-specific peptide antigens in whole blood. Like the tuberculin skin test (TST), it is an indirect test for M. tuberculosis infection, but is more specific than TST and is unaffected by prior BCG vaccination and most environmental mycobacterial infections.

Methods: TThe LIAISON® QuantiFERON®-TB Gold Plus assay is an in vitro diagnostic test for the detection of interferon-γ (IFN-γ) in human lithium heparin plasma by chemiluminescence immunoassay (CLIA) using the LIAISON ® XL Analyzer. The clinical specificity and sensitivity were 96.9% and 84.4% respectively. Clinical studies have shown Positive Percent Agreement (PPA) of 97.4% and Negative Percent Agreement (NPA) of 98.6%. We evaluated the precision, reproducibility, and correlated patient results to our reference lab results. 452 samples collected from Long-Term Care facilities resident were run over 9 months. Statistical analyses were done using Analyse-it.

Results: The percentage agreement for precision, reproducibility, and accuracy were 100%. The correlation with the reference laboratory was 100% agreement. We found 14.8% of samples tested were positive, 83.2% were negative and 2.0% were indeterminate.

Conclusion: The LIAISON® QuantiFERON®-TB Gold Plus assay offers the benefit of a fully automated with high sensitivity and specificity. The faster turnaround will aid the physician in the diagnosing M. tuberculosis, eliminates the need for two-step testing, and is preferred in patients who received BCG vaccination. In addition, with the growing geriatric population, the availability of this test will give the physician the opportunity to follow the 2016 American Thoracic Society (ATS)/Infectious Disease Society of America (IDSA)/CDC clinical practice guidelines for using IGRAs in older patients.

A-286

Performance Evaluation of Molecular Clostridium Difficile Assay using BD Max™ System.

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

Background: *Clostridium difficile* (*C.Diff*) is a gram positive anaerobe producing Enterotoxin and Cytotoxin (toxin A and B respectively); toxin B has been reported to be essential for its virulence. It is estimated that *C. Diff* will cause over half a million infection yearly and one in 11 people over the age of 65 diagnosed with healthcare-associated *C.Diff* infection die within one month. It is the most common cause of acute diarrhea; residents in Long-Term Care Facilities (LTCF) are more prone to have *C. Diff* infection (CDI) because of their age, the frequent use of antibiotics, fragilities, co-morbidity, and living environment. Diagnosing CDI is based on clinical signs and symptoms as well as laboratory tests. Several tests available for diagnosing the infection which include: enzyme immunoassays, tissue culture cytotoxicity, anaerobic culture, glutamate dehydrogenase antigen and nucleic acid amplification. The BD MAX™ System is a PCR based assay that provides the sensitivity, specificity and faster turnaround.

Methods: The MAX™ System is an automated in vitro diagnostic test for the direct qualitative detection of the *C. difficile* toxin B gene (tcdB) in human liquid or soft stool specimens from patients suspected of having *Clostridium difficile* Infection (CDI).The test is performed directly on the specimen, utilizing real-time polymerase chain reaction (PCR) for the amplification of *C. difficile* toxin B gene (tcdB*).* The test utilizes fluorogenic sequence-specific hybridization probes for detection of the amplified DNA. The test was validated for accuracy, precision, and patient correlation to enzyme-immunoassay using Premier Toxins A & B is an enzyme immunoassay

for the direct detection of *Clostridium difficile* toxin A and toxin B in stool samples. Statistical analyses were done using Analyse-it. **Results**: The percentage agreement for precision, reproducibility, and accuracy were 100%. We had 100% agreement for positive patients on EIA and 85% for negative patients on EIA; however, upon investigation we found the discrepancies were due to false negative EIA results.

Conclusion: The BD Max gave the benefit of a fully automated with high sensitivity and specificity for the presence of toxin B producing C. Diff. The faster turnaround gives the physician the ability to diagnose CDI and prevent spreading of the disease to other patients. It is very important to remember that C.diff infection can be prevented with appropriate use of antibiotics and implementing antibiotic stewardship and infection control plan to prevent and stop spreading of the infection.

A-287

Evaluation of Urinalysis Reflex to Culture in Long-Term Care Facilities: Is It Worth it?

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

Background: Urinary Tract Infection (UTI) is one of the second common infections among the geriatric population. It is more common in women than men and the incidence increases with age. UTI can cause serious complications and it is a significant cause of morbidity and death. The high mortality rate is largely due to delayed presentation and the development of bacteremia/sepsis. Physicians rely heavily on urinalysis to diagnose UTI, and it is one of the most frequently requested tests; however, the need to reflect to urine culture became more in demand to cut the cost of test not needed without the sacrificing of patient care.

Methodology: 4,307 urine samples were collected for urinalysis and culture from residents in Long-Term Care Facilities (LTCF) over a period of 5 weeks. Urinalysis and microscopy were done using IQ WORKCELL automated system. All the specimens were cultured; the culture was done using MicroScan Walkaway96 conventional panels. We used urine culture as the reference standard, no growth or <10,000 colony-forming unit/mL were considered negative, cultures with > 50,000 colonies colony-forming unit/mL were considered positive. UA is considered positive if it was positive leukocyte esterase, WBC>5, nitrate, or had ≥moderate bacteria. We compared positive UA to the culture outcome. Statistical analyses were done using Analyse-it.

Results: Women represented 64.7% of the samples tested; the positivity rate was 49.2% (52.3% for women and 43.4% for men) and 7.0% of the all the cultures had mixed flora. Urinalysis identified 3,038 as positive UA, only 1,941 (63.9%) had positive culture and 846 (43.6%) had negative culture. Urinalysis identified 1,269 samples as negative, 176 samples (13.9%) of them had positive culture.

Conclusion: The use of negative urinalyses to reflex to culture would help eliminating the majority of the cultures that are not necessary. However, avoiding the culture will risk missing infection in 13.9% of the negative urinalysis samples. In addition, more than one third of the positive urinalysis will require unnecessary cultures. Although the use of reflex will help eliminating the unnecessary culture, we have to remember that urinalysis and microscopy still lack the sensitivity and specificity to be used alone to diagnose urinary tract infection; urinalysis should not be the only marker for UTI, it should be used in conjunction with patients clinical diagnosis. The high positivity rate for UTI in LTCF adds to the complication of the reflex testing. More work needed to establish better parameters and algorithm to be used when screening patients for UTI using urinalysis.

A-288

Molecular detection of *Mycobacterium tuberculosis* in pulmonary and extra-pulmonary samples. Data from a Brazilian clinical laboratory

J. M. Nurmberger, G. C. de Carvalho, B. B. Perez, M. N. Santos, D. R. Ramadan, S. Tufik. *Associação Fundo de Incentivo a Pesquisa (Afip - Medicina Diagnostica), São Paulo, Brazil*

Background: *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), is among the leading infectious causes of death in developing countries. In humans, TB presents as a progressive granulomatous infectious disease that affects lungs causing pulmonary tuberculosis, but can also affect intestines, meninges, bones, joints, lymph nodes, skin and other body tissues causing extra-pulmonary tuberculosis. The aim of this study was to evaluate the frequency of tuberculosis cases detected during the COVID-19 period and the emergence of cases with extrapulmonary diagnosis.

Methods: This study was a retrospective analysis from January 2017 to December 2022 based on the analysis of 3105 clinical samples processed by the molecular biology section of AFIP Laboratory. All clinical samples were isolated from pulmonary and extra-pulmonary sites recovered from inpatients admitted in several Brazilian tertiary hospitals. In Brief, the DNA extraction was performed according to the type of patient's samples and, then submitted to Real-Time PCR (qPCR) method using a commercial kit, which contains specific primers and probes to the *M. tuberculosis* genome.

Results: Of the 3105 samples tested, 222 (14%) samples were positive for the presence of *M. tuberculosis*. Out of which, 120/1405 (8,5%) were detected in the pre-Covid-19 period (2017-2019) and 102/1700 (6%) were detected in Covid-19 period (2020-2022). The prevalence of *M. tuberculosis* infection was 64.5% and 35.5% among men and females, respectively. The main age range was 41 to 50 years (44.7%), while over 60 years comprised 13.5% of cases (Fig. 1). Between the isolate sites, 67.1% of the *M. tuberculosis* cases were detected in pulmonary and 32.8% in extra-pulmonary body sites, of which, cerebrospinal fluid and blood are among the most frequent, followed by cervical lymph node, ascitic fluid, gastric lavage, urine and others. A total of 95% of *M. tuberculosis* was detected in patients assisted by public health service. We did not have access to patient's symptoms, clinical history or further diagnostic procedures.

Conclusion: Comparing the two periods of the study, a reduction in the positivity rate of new cases was observed in the pandemic period, perhaps this fact can be associated with restrictions on access to the diagnosis of other pathologies in the pandemic period. Despite no information about the severity of the infection, male gender prevalence and age range from 41 to 50 years group need further attention.

A-289

Emergence of unusual non-fermentative Gram-negative bacilli associated to COVID-19 period at Brazilian hospitals

J. M. Nurmberger, F. M. Inoue, A. T. Lobo, D. R. Ramadan, S. Tufik. *Associação Fundo de Incentivo a Pesquisa (Afip - Medicina Diagnóstica), São Paulo, Brazil*

Background: Since the global pandemic of coronavirus disease (COVID-19), some studies have reported on the emergence of opportunistic nosocomial pathogens associated with co-infection on the outcome of COVID-19 patients. The aim of this study was to describe the increase of six unusual species of Non-fermentative Gram-negative bacilli (NF-GNB) clinical isolates recovered from inpatients in several Brazilian hospitals comparing pre-Covid-19 (January 2018 to December 2019) period *versus* Covid-19 (January 2020 to December 2021) period.

Methods: This study design was a retrospective, observational analysis based on microbiological data of emergent NF-GNB isolates, detected in several Brazilian tertiary hospitals. The bacterial identification was performed by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) and the susceptibility of antibiotics was determined using disk diffusion method**.**

Results: During the 48 months period, a total of 1665 (2018; 349), (2019; 351), (2020; 485), (2021; 480) emergent NF-GNB isolated from blood (n=744), respiratory tract (n=239), skin and soft tissue (n=264); urine (n=119), catheter (n=101), fluid and secretions (n=113), Cerebrospinal fluid (n=12) and others (n=73) were analyzed. The frequency of NF-BGN according to species level identification was *Stenotrophomonas maltophilia* (1040; 62.5%), *Burkholderia cepacea* complex (457; 27.4%), *Elizabethkingia meningoseptica* (64; 3.8%), *Elizabethkingia miricola* (31; 1.8%), *Ochrobactrum anthropi* (69; 4.1%) and *Ochrobactrum intermedium* (4; 0.2%). The prevalence of these NF-BGN detected in pre-Covid-19 period (2018-2019) and Covid-19 period (2020-2021) were 42% and 58%, respectively. The incidence of *Stenotrophomonas maltophilia, Burkholderia cepacea* complex, *Elizabethkingia* spp. and *Ochrobactrum* spp. infection was (47.7% and 52.3%), (42.9% and 57.1%), (43.2% and 56.8%) and (50.7% and 49.3%) among female and man patients, respectively. For *Stenotrophomonas maltophilia*, *Burkholderia cepacea* complex and *Ochrobactrum* spp. the main age range was over 60 years (44.5%, 44.9% and 42.5%) while for *Elizabethkingia* spp. the main age range was 0-10 years of age with 42.5% of cases in the pre-COVID-19 period and over 60 years old with 43.9% in the COVID-19 period. Compared the two periods (2018-2019 versus 2020-2021) for *Stenotrophomonas maltophilia* the resistance rate to levofloxacin and sulfamethoxazole-trimethoprim was (3.9% vs 4.6%) and (4% vs 4.2%), respectively.

Conclusion: Our data point to a scenario wherein the presence of opportunistic pathogens may have been intensified by the covid-19 pandemic. Further studies should be carried out to clarify these findings.

A-290

Evaluation of a commercial turbidity monitoring system followed by Mass spectrometry to rapid identification of microorganisms from urine samples

J. M. Nurmberger, F. M. Inoue, A. T. Lobo, D. R. Ramadan, S. Tufik. *Associação Fundo de Incentivo a Pesquisa (Afip - Medicina Diagnóstica), São Paulo, Brazil*

Background: Urinary tract infections (UTIs) are one of the most common bacterial infections in worldwide and represent a worried public health problem, especially due to a significant rise in global antibiotic use in recent years. The purpose of the study was to evaluate the performance of Uro-Quick (UQ) system (Alifax) and the Vitek-MS system (bioMérieux) to identify bacteria isolates directly from urine samples.

Methods: We analyzed 352 clinical samples of urine. From automated screening by UQ system was used 500 µL of homogenized urine sample manually pipetted into a UQ vial containing 4 mL of broth. The inoculated vials were manually introduced into the Uro-Quick reading unit and incubated for 3 hours. The signals are processed by software which monitors the growth curves and calculates the microbial count as colony forming units (CFU/mL). Sample preparation to the mass spectrometry (Vitek-MS) was performed using an in-house protocol based on a centrifugation-wash (CW) method. In parallel with the Vitek-MS direct identification, all the urine samples were submitted to standard culture (SC) method on ChromID™CPS® plate by semi-quantitative technique using 1µL disposable loop, incubated at 35-37°C and the colony counting were read after 24 and 48h of incubation. Bacterial isolates from positive cultures were identified by Vitek®2 System, according to the manufacture's recommendations. Cultures with the presence of three or more microorganisms were considered negative.

Results: Out of 334 clinical samples, 261 (78.1%) were negative for the growth by UQ system and for the presence of microorganisms by SC, 78 (21.9%) samples were positive with growth as $\geq 10^4$ CFU/mL by UQ system, of which 32 samples were also positive with microorganism isolated by SC. Among the 78 microorganisms detected on the samples with growth curve by UQ system, 74 (94.8%) were identified to the species level by the mass spectrometry and 4 (5.2%) were not identified. The microorganisms were *Escherichia coli* (n=40), *Enterococcus faecalis* (n=12), *Klebsiella pneumoniae* (n=6), *Proteus mirabilis* (n=4), *Citrobacter koseri* (n=4), *Streptococcus agalactiae* (n=3), *Morganella morganii* (n=2), *Enterobacter cloacae* (n=1), *Citrobacter freundii* (n=1) and *Gardnerella vaginalis* (n=1). Among the 32 microorganisms detected by SC, 28 showed the same identification at species level when was compared with direct identification by Vitek-MS.

Conclusion: This study showed that both UQ and CW methods by Vitek-MS technology used together were able to provides a rapid result (< 4 hours) for bacterial identification directly from urine samples, that can be useful in clinical practices, especially in severe infectious cases like pyelonephritis. So, future studies should address with a larger number of samples to improve the sensibility of this test for different groups of microorganisms.

A-291

Deep Study of the Accuracy of Two Commercial Real-Time PCR Assays: A Retrospective Approach in Polish Population

J. Sánchez-Sánchez[1], M. Litwin[2], A. Jańska[2], A. Kuzan[3], &. Madej[4], K. Żmijewska[2], &. Fuławka[2]. *[1]Vitassay Healthcare SLU, Huesca, Spain, [2]Molecular Pathology Centre Cellgen, Wroclaw, Poland, [3]Molecular Pathology Centre Cellgen and Department of Biochemistry and Immunochemistry, Wroclaw Medical University, Wroclaw, Poland, [4]Molecular Pathology Centre Cellgen and Collegium Medicum, Jan Kochanowski University, Wroclaw and Kielce, Poland*

Background: According to the WHO, more than 6 million of positive cases were registered in Poland since January 2020. Due to the shortage of public testing sites, the Polish Ministry of Health, together with the National Fund, increased capacity by including private laboratories in the testing and tracing network. One of these key institutions has been the Molecular Pathology Center Cellgen (Wroclaw), with more than 50,000 tests performed. On the other hand, many real-time PCR assays have been developed since the pandemic was declared, and so the need of studying the accuracy and reliability of the kits marketed. This way, specificity and specificity of assays kits developed by Vitassay Healthcare for SARS-CoV-2 detection have been analyzed at Molecular Pathology Center Cellgen

Methods: A total of 710 nasopharyngeal samples, from men and women, collected between May 2020 to May 2022 at different collecting points in Wroclaw (Poland) were analyzed with Vitassay qPCR SARS-CoV-2 and Vitassay qPCR SARS-CoV-2 ORF1ab, E & N. Specimens' RNA was extracted using the ANDiS Viral RNA Auto Extraction & Purification Kit (3DMed). The results were compared to the ones initially obtained with virellaSARSCoV- 2 seqc real-time RT-PCR Kit 2.0 (CE-IVD, Gerbion). Furthermore, the comparisons were grouped depending on the Ct obtained with this referenced method: Ct < 25, Ct 25-30, Ct 30-35 and Ct > 35. Fifty positive samples were included in each group. In addition, 510 negative samples were assayed. The discrepancies and inconclusive results found were addressed by testing in triplicate to get unequivocal results as indicated in the instruction for use. Sensitivity and specificity were calculated using the software MetaDisc 1.4 (IC 95%).

Results: The data obtained with Vitassay assays were interpreted following the instructions of the kit and later compared to the initial characterization with the reference method. Initially, no discrepants results between the three assays were found in the positive samples grouped in Ct < 25, Ct 25-30, Ct 30-35. Within the group Ct >35, for the Vitassay qPCR SARS-CoV-2, 7 discrepancies were obtained (6 inconclusive results and 1 FN). For the triple multiplex assay of Vitassay, the discrepancies found were 11 (9 inconclusive data and 2 FN). After addressing the incongruent data, Vitassay qPCR SARS-CoV-2 reported 2 FN and 1 FN was obtained with SARS-CoV-2 ORF1ab, E & N were obtained. 1 sample for each kit were found inconclusive. Regarding negative samples, 1 FP with Vitassay qPCR SARS-CoV-2, and 2 FP with Vitassay qPCR SARS-CoV-2 ORF1ab, E & N. Global sensitivity and specificity values for Vitassay qPCR SARS-CoV-2 were 0.99 (0.96-0.99) and 0.99 (0.98-1), respectively. Vitassay qPCR SARS-CoV-2 ORF1ab, E & N, mean sensitivity was 0.99 (0.97-1) and specificity was 0.99 (0.98-1).

Conclusion: The examined kits are characterized by high sensitivity and specificity. Moreover, the unquestionable advantage of the kits produced by Vitassay is their lyophilized ready-to-use format. These features confer a great advantage for their use as a tool for accurate in vitro diagnosis of SARS-CoV-2 infection.

A-292

The Use of Third-Party Controls During Covid-19 Pandemic: an Alternative to the Lack of Other Respiratory Infections

J. Sánchez-Sánchez. *Vitassay Healthcare SLU, Huesca, Spain*

Background: Since SARS-CoV-2 spread widely around the world, previously well-established infectious patterns have changed. The introduction of a new respiratory pathogen, together with changes in human behaviour, such as restrictions on social interactions and the use of facemasks, led to a decrease in the circulation of influenza and respiratory syncytial viruses. This phenomenon, together with the collapse of the healthcare system, significantly decreased the availability of certain types of samples for use in clinical studies to evaluate the performance of in vitro diagnostic products. To overcome this issue, EQA programmes are an excellent alternative. This material has been designed to be used in any equipment, kit or following any specific protocol, as the aim is to mimic human specimens. Thus, the CE-IVD Vitassay qPCR FluA + FluB + RSV, an assay for the detection and differentiation of Flu A, Flu B and RSV, has been routinely tested with material from QCMD programmes containing different strains and serotypes of Influenza and RSV. **Methods:** Vitassay qPCR FluA + FluB + RSV (Vitassay Healthcare SLU, Huesca, Spain) is a commercial kit consisting in a ready-to-use lyophilized master mix. This kit has been blindly tested with controls from 4 QCMD panels including RSV, influenza A and B (2020-2022). Each panel consist of 10 specimens in transport media, which were handled following the programme indications. RNA was extracted using automatic extraction (MagDEA Dx SV kit on the 12gC or Vitassay Microorganism-NA Isolation kit on the KingFisher Flex Automatic platform). Amplification was performed on BioRad systems (CFX96 and CFX96 OPUS). Interpretation of the results was done following manufacturer's instructions. Sensitivity, specificity, and Likelihood Ratio have been calculated with a 95% confidence interval using the software MetaDiSC 1.4. **Results:** In total, 40 human-like samples from QCMD EQA programmes were analysed with Vitassay qPCR Flu A + Flu B + RSV during Covid-19 Pandemic. Vitassay reported 14 Influenza A positive samples, 11 influenza B and 12 RSV positive samples; all of them were correctly detected when compared the initial characterisation. For Flu A sensitivity and specificity were of 1 (0,76-1) and 1 (0.86-1), LR+ 52.2 (3.34-814.62), LR- 0.034 (0.002-0.51). For Flu B sensitivity and specificity were of 1 (0.71-1) and 1 (0.88-1), LR+ 57.5 (3.67-900.49), LR- 0.042 (0.003-0.63). For RSV sensitivity and specificity were of 1 (0,73-1) and 1 (0.87-1), LR+ 55.76 (3.56-872.35), LR- 0.039 (0.003-0.59). **Conclusions:** Third party controls are an excellent alternative to clinical samples, especially in extraordinary circumstances. In addition, they allow to introduce different strains and variants, such as the QCMD specimens tested. Among these, we could find a such as H1N1 and H3N2 Influenza A serotypes, Influenza B Yamagata and Victoria

and RSV A and B, which were properly detected by Vitassay multiplex. In addition, Vitassay extraction kit showed satisfactory results as all samples were properly detected when used in combination with Vitassay qPCR Flu A + Flu B + RSV.

A-293

Assessment of the Detection of Gastrointestinal Viruses Using Two Commercial Real-Time PCR Assays.

J. Sánchez-Sánchez[1], C. Fuentes[2], A. Arrebola[2], M. Costafreda[2], A. Bosch[2], R. M. Pintó[2], S. Guix[2]. *[1]Vitassay Healthcare SLU, Huesca, Spain, [2]Enteric Virus Laboratory, Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona /Research Institute of Nutrition and Food Safety, University of Barcelona (INSA-UB), Barcelona/Santa Coloma de Gramenet, Spain*

Background: Acute Gastroenteritis (AGE) are the second most common infectious diseases, only overtaken by Respiratory Tract Infections. Sign and symptoms of AGE illness include nausea, diarrhoea, vomits, fever and abdominal pain. In infants and children, gastroenteritis is one of the most important causes of morbidity and mortality all around the Globe. Since their identification in the 1970s, enteric viruses are considered a leading cause of gastroenteritis worldwide. Among these, rotaviruses (RV) are considered to be the main cause of AGE in young children, whereas noroviruses (NoV) affect people of all ages. In addition, sapoviruses (SaV), human astroviruses (hAstV), human adenoviruses (AdV) are also relevant.

In the recent years, molecular methods, such as real-time PCR, have been widely used for detection of viral genome of enteric viruses due to their high sensitivity and specificity, becoming the gold standard. The aim of this study is the comparison of different commercial real-time PCR assays for the detection of human enteric viruses.

Material and methods: Three hundred and eighteen clinical stool samples were selected from different groups collected between 2016 and 2019, including specimens from symptomatic adults and symptomatic and asymptomatic children. Samples were stored at -20ºC. The collection included a minimum of 25 samples positive for RV, 55 positives for AdV, 16 positives for AstV, 22 positives for SaV and 65 positives for NoV (31 for GI and 34 for GII). All specimens were extracted using the Maxwell®RSC Blood DNA Kit on the Maxwell RSC equipment (Promega). Samples were analysed using the following assays: RIDA®GENE Viral Stool Panel II, RIDA®GENE Sapovirus and RIDA®GENE Norovirus I & II (R-Biopharm), Vitassay qPCR kits for the detection of Rotavirus, Astrovirus, Norovirus GI, Norovirus GII, and Sapovirus. Results obtained with Vitassay were compared to R-Biopharm assays. Sensitivity and specificity were calculated using the software MetaDisc 1.4. Discrepant samples were tested in triplicate.

Results: After assessing the discrepant results, 29/114 samples were positive for RV in both RIDA®GENE and Vitassay kit. This latter reported 2 false negative (FN) and 4 false positive (FP) results when compared to RIDA®GENE. Sensitivity was 93.5% (78.6-99.2) and specificity was 95.2% (88.1-98.7). For AstV, 27/138 were positive in both kits. Vitassay qPCR reported 1 FN and 2 FP, obtaining sensitivity and specificity values of 96.4% (81.7-99.9) and 98.6% (94.9-99.8), respectively. Regarding SaV, Vitassay reported 100% (88.8%-100) of sensitivity and 97.8% (88.5-99.9) of specificity, as 1 FP was obtained. 30/89 samples tested for Norovirus GI were detected by Vitassay qPCR Norovirus GI and 25/89 RIDA®GENE Norovirus I & II (R-Biopharm). On the other hand, Vitassay qPCR Norovirus GII reported 31/81 positive samples for NoV I, whereas RIDA®GENE obtained 30 positive results. In both cases, sensitivity was of 100%, whereas specificity was over 92%.

Conclusions: Vitassay real-time PCR assays for the detection of different enteric viruses present high specificity and sensitivity, which is essential for a proper diagnosis and epidemiology assessment. In addition, Vitassay qPCR kit presents the advantage of being a ready-to-use lyophilised kit.

A-294

Duplicate Testing of Samples Suspected of SARS-CoV-2 Infection Could Prevent Misdiagnosis of Covid-19

J. Sánchez-Sánchez[1], G. Thapa[2], A. Mylona[3], R. Vuono[2]. *[1]Vitassay Healthcare SLU, Huesca, Spain, [2]Medway School of Pharmacy, University of Kent, Kent, United Kingdom, [3]Kent and Medway Medical School, Canterbury Christ Church University and University of Kent, Kent, United Kingdom*

Background: There is no doubt that the whole world has been shaken by the SARS-CoV-2 pandemic, which has affected both global health and the global economy. At the same time the importance of molecular methods for the accurate diagnosis of respiratory infections has been reinforced together with the need of testing methodologies that minimize the risk of misleading results. During the last three years of the pandemic, SARS-CoV-2 exhibited a tremendous adaptation capability through numerous mutations, leading to the appearance of the well-known Variants of Concern (VOCs) responsible of increased transmission rates. Therefore, the development of molecular methods capable of detecting all variants has become a challenge. To overcome this, potential genetic targets with low mutation rates have been identified, including *ORF1ab*, *N* gene, *S* gene and *E* gene. While Real-time PCR based detection assays of viral genomes are very accurate, testing naso/oropharyngeal samples using singlet analysis does not guarantee the error free detection of true positive results for SARS-CoV-2 infections. Furthermore, it has been documented that the probability of a false-negative testing results is influenced by a number of variables . Therefore, testing protocol adaptations, such as replicate testing, has been shown to efficiently reduce the probability of missing SARS-CoV-2 positive cases.

The pilot study presented here investigates the outcome of testing 10,014 nasopharyngeal samples collected from patients with a suspected SARS-CoV-2 infection using two separate detection kits. In this study the error rate of single vs duplicate testing is discussed.

Methods: 10,014 nasopharyngeal swabs, collected from three different U.K. hospital trusts and Kent based care homes, were tested at the North Kent Pathology Service (NKPS). Each swab collected was placed in sterile transport media and stored at 4ºC prior to viral genome isolation using the HigherPurity™ Viral RNA extraction kit. All collected specimens were processed within 24 hours from collection. Total RNA isolated was immediately analyzed using two commercial CE-IVD kits: Vitassay qPCR SARS-CoV-2 and Primerdesign™ LTD, Coronavirus (COVID-19) Genesig Real-Time PCR assay. All samples were tested in duplicate.

Results: Following the testing and subsequent analysis, 491 samples (4.9%) were detected positive for SARS-CoV-2 using both assay kits. Of these, 302 samples (61.5%) were reported as duplicate positive (on both replicate 1 and 2) whereas 189 samples (38.5%) were detected positive on either replicate 1 or 2. In particular, 85 (17.3%) were detected positive on replicate 1 and 104 (21.2%) were detected positive on replicate 2.

Conclusions: Approximately 19% of tested individuals were detected as SARS-CoV-2 positive based on a positive Real-time PCR result in one of the two reactions. This percentage represents the potential SARS-CoV-2 infections that can be missed when a single reaction Real-time PCR testing is employed for infection detection in the wider population, and equates to 940 individuals out of 100,000 potentially being falsely confirmed as SARS-CoV-2 negative The authors believe that in the presence of fast transmitting variants this is a significant number of false negative tests and should be taken into consideration when testing protocols are developed and disease modelling is employed.

A-295

Validation of Two New Real Time PCR Assays for the Detection and Differentiation of Plasmodium

P. Cisteró[1], E. Franco-Marín[2], B. Dehesa-García[2], B. García-Manrique[2], H. Alonso[3], G. Matambisso[4], H. Mbeve[4], N. Ndimande[4], A. Mayor[1]. *[1]Instituto Salud Global (ISGlobal), Barcelona, Spain, [2]CerTest Biotec, San Mateo de Gállego, Spain, [3]Department of Microbiology, Paediatrics, Radiology, and Public Health, Faculty of Medicine, University of Zaragoza, Zaragoza, Spain, [4]Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique*

Background:

Malaria (or paludism), the mosquito-borne parasitic disease caused by Plasmodium, is a major global public health threat among communicable diseases. The objective of this study was to determine the clinical performance of VIASURE *Malaria* Real Time PCR Detection Kit and VIASURE *Malaria differentiation* Real Time PCR Detection Kit using dried blood spot on filter paper samples.The aim of this retrospective study was to validate and compare the clinical sensitivity and specificity of the VIASURE assays with the reference method used in the laboratory.

Methods:

The Malaria assay, which detects the genus *Plasmodium* spp. and the differentiation assay, which distinguishes the main human pathogenic Plasmodium species: *P. falciparum*, *P. ovale*, *P. vivax*, *P. malariae* and *P. knowlesi*, were used in comparison with an 18S rRNA in-house malaria screening and differentiation assays. A total of 300 blood on filter paper collected from 2016 to 2019, from patients with clinical suspicion of malaria in the Manhiça-Magude district of southern Mozambique were analysed. The DNA extraction was carried out using the Chelex® 100 sodium method and the thermocycler employed was Applied Biosystems 7500 Real-Time PCR System.

Results:

A total of 77 samples were considered as *Plasmodium* spp.-positive by both assays, 1 false positive result and 15 false negative results for VIASURE were obtained and 207 samples showed to be negative for this target by both assays. Regarding species differentiation: VIASURE assay showed 58 true positive and 3 false negative results for *P. falciparum*, 2 true positive results for *P. malariae* and 1 true positive and 1 false negative result for *P. ovale*. After data analysis, the sensitivity and specificity values obtained were:

Conclusion:

This retrospective study demonstrated the good sensibility and specificity of both VIASURE molecular assays using this extraction method on dried blood spots.

Table 1. Summary of obtained results

VIASURE target	Sensitivity	Specificity
Plasmodium spp.	0.83 (0.74-0.9)	0.99 (0.97-1)
P. falciparum	0.95 (0.86-0.99)	1 (0.94-1)
P. malariae	1 (0.15-1)	1 (0.94-1)
P. ovale	-	0.98 (0.91-1)

A-296

Validation of a Real Time PCR Kit for the Detection and Differentiation of MRSA, MSSA and MRCoNS in Respiratory Clinical Samples

E. Franco-Marín[1], B. Dehesa-García[1], C. Escolar[1], B. García-Manrique[1], R. Cebollada[2], M. Monforte-Cirac[2], M. Millán-Lou[2], H. Alonso[1], C. Aspiroz-Sancho[2]. *[1]CerTest Biotec, San Mateo de Gállego, Spain, [2]Sección de Microbiología, Hospital Royo Villanova, Zaragoza, Spain*

Background:

Staphylococcus aureus infections are remarkable due to their high morbimortality. In addition, antibiotic resistance of these bacteria is a cause of concern since it worsens the patient prognosis and it is associated to higher morbidity, mortality and cost of treatment. The aim of this study was to assess the clinical performance of the assay VIASURE *Methicillin resistant Staphylococcus aureus* Real Time Detection Kit in clinical and epidemiological respiratory samples.

Methods:

Respiratory samples were collected from patients admitted to intesive care or ward of the Hospital Royo Villanova (Zaragoza, Spain). A total of 653 respiratory samples (both clinical and epidemiological) were employed: 189 nasal swab, 103 pharyngeal, 161 bronchoalveolar aspirate (BAS), 154 bronchoalveolar lavage (BAL) and 46 sputa samples previously characterised using culture methods. Results were compared with other commercial validated molecular assay (RIDA®GENE MRSA (R-biopharm)) presenting the same molecular targets as the assay under study. The characterisation performed by VIASURE is based on the combined results of the three targets S. aureus SA 442/coA, mecA/mecC and Sccmec-orfX junction and may report the following genotypes: methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *Staphylococcus aureus* (MSSA)), methicillin-resistant coagulase negative *Staphylococcus* (MRCoNS) and methicillin-susceptible *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus* (MSSA and MRCoNS).

Results:

VIASURE assay correctly reported MRSA/MSSA/MRCoNS genotyping results with sensitivity and specificity values over 90% in all sample matrixes (Table 1).

Conclusion:

The diagnostic performance of the assay under study demonstrated to be suitable tool for the molecular diagnosis of coagulase negative Staphylococci, S. aureus and their methicillin resistance causing mutation when compared with reference methods.

Sample type	Diagnosis	Overall agreement	TP	TN	FP	FN	Sensitivity	Specificity
Nasal	MRSA	1 (0.98-1)	15	174	0	0	1 (0.78-1)	1 (0.97-1)
	MSSA	1 (0.98-1)	17	172	0	0	1 (0.80-1)	1 (0.97-1)
	MSSA and MRCoNS	0.99 (0.97-0.99)	19	169	1	0	1 (0.82-1)	0.99 (0.96-1)
	MRCoNS	0.99 (0.97-0.99)	113	75	0	1	0.99 (0.95-1)	1 (0.95-1)
Pharyngeal	MRSA	1 (0.96-1)	9	94	0	0	1 (0.66-1)	1 (0.96-1)
	MSSA	1 (0.96-1)	8	95	0	0	1 (0.63-1)	1 (0.96-1)
	MSSA and MRCoNS	1 (0.96-1)	4	99	0	0	1 (0.39-1)	1 (0.96-1)
	MRCoNS	1 (0.96-1)	42	61	0	0	1 (0.91-1)	1 (0.94-1)
BAS	MRSA	1 (0.97-1)	1	160	0	0	1 (0.02-1)	1 (0.97-1)
	MSSA	1 (0.97-1)	13	148	0	0	1 (0.75-1)	1 (0.97-1)
	MSSA and MRCoNS	0.99 (0.96-0.99)	2	158	1	0	1 (0.15-1)	0.99 (0.96-1)
	MRCoNS	0.99 (0.96-0.99)	29	131	0	1	0.96 (0.82-0.99)	1 (0.97-1)
BAL	MRSA	0.99 (0.96-0.99)	4	149	1	0	1 (0.39-1)	0.99 (0.96-1)
	MSSA	0.98 (0.94-0.99)	12	139	2	1	0.92 (0.64-0.99)	0.98 (0.95-0.99)
	MSSA and MRCoNS	0.98 (0.95-0.99)	2	150	2	0	1 (0.15-1)	0.98 (0.95-0.99)
	MRCoNS	0.99 (0.96-0.99)	11	142	1	0	1 (0.71-1)	0.99 (0.96-1)
Sputum	MRSA	1 (0.92-1)	1	45	0	0	1 (0.02-1)	1 (0.92-1)
	MSSA	1 (0.92-1)	4	42	0	0	1 (0.39-1)	1 (0.91-1)
	MSSA and MRCoNS	1 (0.92-1)	3	43	0	0	1 (0.29-1)	1 (0.91-1)
	MRCoNS	1 (0.92-1)	6	40	0	0	1 (0.54-1)	1 (0.91-1)

A-297

Usefulness of Non-Skin Samples in the PCR Diagnosis of Monkeypox

S. Algarate[1], J. Bueno[1], M. J. Crusells[1], M. Ara[1], B. Dehesa-García[2], B. García-Manrique[3], H. Alonso[3], E. Alvarado[1], M. Ducons[1], S. Arnal[1], M. Riesgo[1], R. Benito[1]. *[1]Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain, [2]CerTest Biotec, Zaragoza, Spain, [3]CerTest Biotec, San Mateo de Gállego, Spain*

Background:

During 2022, human mpox cases were reported in several European countries. Until November 2022, in Spain, 7404 cases were detected, most of them linked to sexual intercourse. In that context, fast identification of cases and sources of infection were essential for an optimal management. Polymerase chain reaction (PCR) was the preferred laboratory test. Our objective was to evaluate the usefulness of sera and nasopharyngeal samples for MPXV detection, and its application in contact studies without skin or mucosal injuries.

Methods:

In the Hospital Clínico Universitario of Zaragoza (Spain), 106 samples from 50 patients, 38 male and 12 female, aged between 11 to 62 years, with skin lesions suspected MPXV infection were analysed. Samples were collected between May and September 2022 in inactivating universal viral transport medium (Biocomma). Samples were obtained between 1 and 19 days of evolution. To perform the real-time PCR, the VIASURE *Monkeypox Virus* Real Time PCR Detection Kit and the VIASURE 48 - Real Time PCR System were used. Nucleic acid extraction was performed with the automated nucleic acid extraction system MagLEAD12gC using the MagDEA®Dx SV reagents.

Results:

Table 1 shows the results expressed in Ct values. Skin, anogenital and serum samples were the most efficient for MPXV diagnosis. Nevertheless, these data are influenced by the sample collection date.

Conclusion:

Real-time PCR was positive in samples from all locations in 71,4% of the infected patients. Respiratory samples (pharyngeal and nasopharyngeal) were the ones that showed the highest number of false negative results, whereas there was only one

skin sample and one serum sample with a negative result. This study shows that sera samples can be used in the diagnosis of MPXV infection because of ease of collection, applicability to contacts without lesions and the option of serology testing for other STI agents (HBV, HCV, HIV, *Treponema pallidum*...).

Table 1: Results of MPXV PCR of infected patients

Sample type	No.	Days of evolution (mean)	Positive (%)	Mean Ct
Skin	14	2-9 (4.9)	13 (92.9)	26.0
Anogenital	23	1-7 (3.7)	22 (95.7)	23.7
Nasopharyngeal / Pharyngeal	15	2-10 (4.8)	11 (78.6)	31.3
Serum	18	1-9 (5.1)	17 (94.4)	34.6
Total	70	1-10 (4.5)	63 (90.0)	28.4

A-298

Performance Analysis of Three Mpox Real Time PCR Kits With Lesion Swab Clinical Samples

M. Peris[1], E. Franco-Marín[2], B. Dehesa-García[2], B. García-Manrique[2], H. Alonso[2]. *[1]Instituto de Investigaciones Sanitarias de Aragón (IISA), Zaragoza, Spain, [2]CerTest Biotec, San Mateo de Gállego, Spain*

Background:

The appearance of skin lesions can be a symptom of infection or reactivation of the HSV-1, HSV-2, VZV or *T. pallidum* or even monkeypox virus infection. Due to the contact-bound ease of spread of these pathogens, an accurate and fast diagnosis, such as PCR-based testing, is key to a good epidemiological management. The aim of this study was to determine the clinical performance of three qPCR assays.

Methods:

A total of 334 cutaneous swabs collected from patients attended at the Hospital Universitario Miguel Servet were analysed using the three assays under study: VIASURE *Herpes virus 1, Herpes virus 2 & Varicella Zoster* Virus Real Time PCR Detection Kit, VIASURE *Treponema pallidum* Real Time PCR Detection Kit and VIASURE *Mpox* Real Time PCR Detection Kit. Samples were collected between December 2021 and November 2022. These samples were requested through the Biobanco del Sistema de Salud de Aragón (BSSA) and have the approval of the Aragón Ethics Committee (PI22/409). DNA/RNA extraction was performed using the automated extraction method magLEAD® 12gC instrument with the MagDEA Dx SV kit and amplification was performed using the thermocycler CFX96™ Real-Time PCR Detection System (BioRad). The obtained results were compared with the reference assays RealStar® Orthopoxvirus PCR Kit 1.0 (Altona) and Allplex™ Genital ulcer assay (Seegene®).

Results:

After data analysis a very good overall agreement was obtained for all the studied targets.

Conclusion:

The assays under study demonstrated to be a good tool for differential analysis in skin lesions compatible with HSV-1, HSV-2, VZV, *T. pallidum* or mpox virus infection. In addition, the shared thermal protocol of the assays allowed for the simultaneous detection in the sample, creating a personalized panel option for the user, as opposed to the reference assays employed, which required two separate analyses, taking longer to reach the full assessment of the sample.

Table 1. Summary of obtained results

	Overall agreement	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
Herpes Simplex virus type 1	1 (0.98-1)	49	285	0	0	1 (0.92-1)	1 (0.98-1)	1 (0.92-1)	1 (0.98-1)
Herpes Simplex virus type 2	1 (0.98-1)	50	284	0	0	1 (0.92-1)	1 (0.98-1)	1 (0.92-1)	1 (0.98-1)
Varicella Zoster Virus	1 (0.98-1)	30	304	0	0	1 (0.88-1)	1 (0.98-1)	1 (0.88-1)	1 (0.98-1)
Treponema pallidum	1 (0.98-1)	35	299	0	0	1 (0.90-1)	1 (0.98-1)	1 (0.90-1)	1 (0.98-1)
Mpox virus	1 (0.98-1)	38	295	0	0	1 (0.90-1)	1 (0.98-1)	1 (0.90-1)	1 (0.98-1)

A-299

Clinical Performance Validation of 7 qPCR Kits for the Specific Diagnosis of 5 Enteric Viruses in Clinical Stool Samples

M. Costafreda[1], A. Bosch[1], R. M. Pintó[1], B. Dehesa-García[2], B. García-Manrique[2], H. Alonso[2], S. Guix[1]. *[1]Grupo de Virus Entéricos, Departamento de Genética, Microbiología y Estadística, Facultad de Biología, Universidad de Barcelona, Barcelona, Spain, [2]CerTest Biotec, San Mateo de Gállego, Spain*

Background:

Among the most prevalent enteric viruses associated with gastroenteritis are norovirus (NoV), rotavirus (RV), astrovirus (AstV), sapovirus (SaV) and adenovirus (AdV). The diagnosis of infections associated with gastroenteritis viruses is mainly based on the detection of the genome of these viruses by molecular methods, mainly qPCR and RTqPCR reactions. The aim of this study was to validate the diagnostic performance of 7 qPCR diagnostic kits of the "VIASURE Real Time PCR Detection Kits" series with respect to commercial reference kits used for the diagnosis of these pathogens.

Methods:

A total of 318 clinical stool samples were used, including: 213 stool samples from children from sporadic cases of gastroenteritis collected between 2016-2018; 94 samples from outbreaks of gastroenteritis caused by NoV between 2017-2019; and 11 samples from healthy children with asymptomatic enteric virus infections collected between 2017-2018. Seven VIASURE kits were used for the specific detection of enteric viruses. At the same time, samples were also tested with the following commercial kits used as reference methods: RIDA®GENE Viral Stool Panel II (rotavirus, adenovirus 40/41 and astrovirus) and RIDA®GENE Sapovirus, RIDA®GENE Norovirus I & II. In the case of AdV, the positivity of the samples was confirmed using the qPCR protocol published by Heim et al. (2003).

Results:

The results obtained are summarized in Table 1.

Conclusion:

The results obtained show a high sensitivity and specificity of the VIASURE kits for the detection of rotavirus, adenovirus, sapovirus, astrovirus and norovirus GI and GII (in the latter case using both the monoplex and multiplex product). In addition, it should be noted that all these kits share the same thermal protocol, thus allowing their joint use for the simultaneous diagnosis of these 5 enteric viruses.

Table 1. Summary of obtained results

Virus	Number of analysed samples	Sensitivity	Specificity
Rotavirus	114	90.9% (81.1-100.7)	93.8% (88.6-99.1)
Astrovirus	138	96.4% (89.6-103.3)	99.1% (97.3-100.9)
Sapovirus	77	100% (100-100)	97.9% (93.9-102.0)
Adenovirus	115	100% (100-100)	96.7% (92.3-101.2)
Norovirus GI	89 (Monoplex analysis)	100% (100-100)	92.1% (85.4-98.7)
Norovirus GII	81 (Monoplex analysis)	100% (100-100)	98.0% (94.2-101.8)
Norovirus GI	92 (Multiplex analysis)	100% (100-100)	93.9% (88.2-99.7)
Norovirus GII	92 (Multiplex analysis)	96.7% (90.2-103.1)	100% (100-100)

A-300

Evaluation of Different Storage Conditions of Nasopharyngeal Swabs for the Detection of Influenza A, Influenza B and RSV

M. Martínez-Santolaria[1], C. Sota-Diez[2], B. García-Manrique[2], E. Machetti-Mareca[2]. *[1]Facultad de Ciencias, Universidad de Zaragoza, Zaragoza, Spain, [2]CerTest Biotec, San Mateo de Gállego, Spain*

Background:

Every year, Influenza A (FluA), Influenza B (FluB) and Respiratory Syncytial Virus (RSV) affect our communities and increase the number of patients in our health systems. Molecular techniques, such as real-time PCR have become essential, not only for the diagnostic but also for the epidemiological studies. High amounts of samples or retrospective analysis need the use of samples stored at different conditions. For this reason, a stability study of nasopharyngeal swabs (NP) in Viral Transport Medium (VTM) for FluA, FluB and RSV was performed.

Methods:

Twenty-eight negative FluA, FluB and RSV NP samples in VTM (Vircell, Spain) were used in this study. All samples were mixed into one pool and divided into 7 aliquots. One of them served as a negative control. The others were enriched (each) with different concentrations of viral cultures from ATCC (VR-95PQ™, VR1804PQ™, VR26PQ™) to obtain two positive samples for each pathogen. Samples were stored under specific conditions of time and temperature before being processed: 1 and 2 days stored at 25ºC, 3 and 6 days stored at 4ºC, and 8, 30 and 180 days stored at -20ºC. Each condition was extracted in triplicate with the MagDEA Dx SV kit, using the magLEAD® 12gC instrument (Precision System Science Co.) and analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Certest Biotec S.L) in triplicate on CFX96™ Real-Time PCR Detection System (Bio-Rad). The results were compared with the data obtained on the first day (0 hours).

Results:

All positive samples were detected in all conditions and matrices. No amplifications were observed in negative samples. There were no major differences compared with the data obtained on the first day. However, a Cq delay of around 1 unit was observed in all pathogens when samples were stored 2 days at 25ºC. For this reason, this storage condition would not be recommended.

Conclusion:

NP samples in VTM for the detection of FluA, FluB and RSV can be stored at 25°C for up to 1 day, 4°C for up to 6 days, or frozen at -20°C for up to 180 days.

A-301

Analytical and clinical performance of the molecular method for Monkeypox virus detection

J. d. Silva, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Monkeypox (MPX) is a zoonose caused by the Monkeypox virus (MPXV) belonging to the Orthopoxvirus genus of the Poxviridae family. Currently, MPXV is responsible for more than 84,000 infections worldwide, resulting in major social impacts. Although the reservoir is unknown, the main hosts are small rodents, typical of the tropical forests of West and Central Africa. The transmission occurs by interactions of infected animal-to-person (direct contact with the blood, bodily fluids, or cutaneous/mucosal lesions) and person-to-person (close contact with respiratory secretions, skin lesions, or recently contaminated objects). After infection, there is an incubation period of approximately 1-2 weeks (asymptomatic and non-transmissible), followed by the development of skin rashes over the body, lymphadenopathy, and systemic symptoms (fever, malaise, headache, myalgia, and fatigue). The rapid and accurate diagnosis of MPX is extremely important for combating the virus and also for epidemiological tracking. In this sense, molecular methods have been shown essential for the accurate detection and characterization of MPXV. Therefore, this study aims to describe the analytical and clinical validation of real-time polymerase chain reaction (qPCR) assay for MPXV detection. **Methods**: The qPCR assay used in this validation was based on recommendations published by the Center for Disease Control and Prevention (CDC). The primer/probe sequence was commercially acquired and included the analysis of a MPXV-specific genomic region (CADT ID APNKY-AD; Thermo Fisher, USA). Assay performance was evaluated using inactivated viral isolates and positive samples with known MPXV positive results. The analysis parameters included: (i) assay verification (performance evaluation of probes and reagents); (ii) Analytical sensitivity; (iii) Limit of detection (LOD); (iv) Analytical specificity (interference study with Herpes simplex virus and Varicella-Zoster virus); and (v) clinical validation (10 positive skin rashes samples for the MPXV). **Results**: The assay demonstrated good performance visualized by the qPCR amplification curves (low cycle threshold (Ct) value and high fluorescence level) with an efficiency >92%. Analytical sensitivity and LOD tests were 100% and 50 copies/µL, respectively, considering a 95% confidence interval. The regression equations obtained show satisfactory reaction conditions with a positive correlation between the variables (coefficient of determination (r2) = 0.99). The assays indicated no cross-reactions with closely related viruses, demonstrating 100% of specificity. Finally, clinical validation showed 100% agreement with the expected results for all samples evaluated. **Conclusion**: qPCR can play an important role in the rapid and accurate diagnosis of several viruses. The analytical and clinical validation provided data indicating high sensitivity and specificity for MPX detection. This study allowed the implementation of a sensitive, specific, and accurate molecular test for viral agent identification and allow prompt surveillance action by health such as epidemiological monitoring and control measures of these infections.

A-302

Agreement assessment between molecular and rapid tests in respiratory viruses detection

L. A. Martins, R. S. Domingues, J. d. Silva, D. A. Zauli, A. B. de Lima. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Diseases caused by respiratory viruses are the most frequent diseases in humans and have a significant impact on morbidity and mortality worldwide. Due to their transmission nature, these viruses are able to widespread dissemination. The pandemic caused by COVID-19 highlights this potential, however, other respiratory viruses also continue to circulate such as Respiratory Syncytial Virus (RSV) and Influenza (FLU). Efficient diagnostic tools are necessary, especially in endemic regions. Although molecular tests are gold standards, rapid tests (RTs) represent a great alternative once they are more accessible, faster, easier to manage, and can be performed at the point of care. Therefore, this work aimed to evaluate the analytical performance of RTs compared to molecular tests in the detection of viral antigens of RSV or FLU in swab samples. **Methods:** Swab samples in saline solution (n=60) were tested by qPCR molecular assay Multiplex Alinity m Resp-4-Plex Assay (Abbott Molecular) and RTs immunofluorescence-based (ECO-F RSV ag and ECO-F Influenza A/B - ECO Diagnostica). For the molecular assay, samples were prepared according to the manufacturer's instructions. For RTs, samples were submitted to the extraction buffer, incubated for 10 or 15 minutes respectively, and analyzed by ECO Reader F200 equipment, according to the manufacturer's instructions. It uses a cut-off value ≥ 1.00 for positive samples and < 1.00 for negative samples. **Results:** All negative samples (RSV=20; FLU=20) in molecular test were concordant with RTs results. The number of positive samples detected by molecular test was 4 and 16 for FLU and RSV respectively, while RTs detected a lower number of positive samples (FLU=2; RSV=10). Both RTs achieved a specificity of 100%; sensibility was 62,50% for RSV and 50% for FLU. Using Cohen's Kappa index to assess the agreement between the compared tests, it was obtained the expected and observed values of 0.83 and 0.53 for RSV and 0.92 and 0.78 for FLU, which represents an index of 0.649 and 0.625 respectively. These results indicate substantial agreement between the tests, although the sensitivity of the molecular assay was superior. **Conclusion:** Molecular tests can be used to confirm the results of RTs, once they are the gold standard for diagnosis. However, they have a high operational cost, which makes RTs a good alternative to help in the screening of these respiratory diseases, especially in resource-limited settings, allowing effective treatment and management for the patient.

A-303

Validation of a quantitative and qualitative molecular test for the detection of Epstein-Barr virus by Real-time qPCR

C. P. Mendonça, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: The Epstein-Barr virus (EBV, HHV-4) is a human virus of the Herpesviridae family, mainly associated with infectious mononucleosis in adolescence, but it can also be related to other tumors. About 90% of the world's population has been infected by this virus, although the vast majority of these people are asymptomatic. In immunocompromised individuals, such as transplant recipients and HIV-infected patients, high EBV replication is a major predisposing factor to the development of a wide range of B-cell lymphoproliferative disorders such as Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's and non-Hodgkin lymphoma. Early detection of EBV is crucial for the effective management of patients on immunosuppressive therapy after transplantation and immunoproliferative disease. EBV-related diseases, such as in suspected cases of chronic fatigue syndrome and after transplants can be identified by EBV-DNA quantification. Molecular assays, such as Real Time qPCR, have high sensitivity and specificity and are a useful tool for the early diagnosis of EBV infection. This assay differentiates healthy carriers, with low levels of viral load, from ill carriers with a high rate of viral replication. In this way, this work aimed to validate and verify the performance of the alinity m EBV assay. **Methods:** To evaluate the molecular detection of the EBV virus, we used the automated EBV alinity m assay (Abbott). Were evaluated 141 samples from the Molecular Genetics routine of the Pardini Group. Of those, 91 samples (63 plasma and 28 cerebrospinal fluid) were previously tested by in-house PCR for qualitative EBV detection and 50 samples (plasma) were previously tested for quantitative EBV detection by Real-Time PCR (Seegene platform). At the end of the tests, the previous qualitative and quantitative results were compared with the Alinity m EBV test results. **Results**: The range of Alinity m EBV assay detection was < 20 IU/mL or < 1.30 log(IU/mL) to 200,000,000 IU/ml or 8.30 log(IU/ml). For qualitative analysis, the positive agreement was 100% in both plasma (7/7) and cerebrospinal fluid (6/6) samples, with quantification variation from 2.07 to 4.60 Log(IU/mL) and <1 .30 to 6.30 Log(IU/mL), respectively. The negative agreement was 89% (50/56) for plasma samples, with 6 discordant samples between 1.13 to 3.39 Log(IU/mL) and 91% for cerebrospinal fluid samples (21/23), with two disagreements (1.69 and 2.23 Log(IU/mL), considering the results below the detection range of the assay (<20 IU/mL or < 1.30 Log (IU/mL)) as negative because they did not clinical relevance. In quantitative analysis, the negative agreement between comparative methodology (Seegene platform) was 81.0% (33/42), considering as discordant only the samples with a quantifiable detected result. Discordant samples detected by Alinity - and not detected by Seegene platform - presented quantification between 21-791 IU/mL, indicating greater sensitivity of the analyzed methodology. Positive agreement was 100% (5/5). **Conclusion:** The Alinity m EBV Assay demonstrated good performance for molecular detection of EBV. This test can be used as an important tool for clinicians to identify patients with infection, especially in immunosuppressed cases that require a fast, accurate, and reliable diagnosis.

A-304

A RT-PCR assay for detection and quantification of Hepatitis D virus (HDV)

J. d. Silva, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Hepatitis D virus (HDV), also called Delta Hepatitis, is associated with the presence of the Hepatitis B virus (HBV) and is responsible for the infection and inflammation of liver cells. It is estimated that 15-20 million people worldwide are affected by HDV. In Brazil, Hepatitis D has high prevalence rates in the Amazon Basin. HDV is an enveloped virus with a 36 nm average diameter. The HDV virion involves a negative-sense, single-stranded circular RNA molecule associated with an inner-core delta antigen surrounded by envelope glycoproteins. HDV-infected individuals may not show signs or symptoms of the disease. When present, the most common are: tiredness, dizziness, nausea and/or vomiting, fever, abdominal pain, and yellow skin/eyes. Chronic Hepatitis D is considered the most severe form of chronic viral hepatitis, with more rapid progression to cirrhosis and an increased risk for decompensation, hepatocellular carcinoma (HCC), and death. Molecularly, determining the concentration of virus circulating in the blood of HDV patients is extremely important for diagnosis. The Quantitative Polymerase Chain Reaction (qPCR) is a molecular technique capable of detecting the viral genome with high sensitivity and specificity, ensuring the safety of the result. Therefore, this study aims to describe the analytical validation of a one-step RT-qPCR assay for HDV detection. **Methods:** Assay performance was evaluated using commercial quantified positive control to HDV (1000, 100, 10, 1, and 0.5 IU/µL), and the parameters of analysis included: standardization assays, efficiency, and detection limit. For specificity evaluation, positive samples for Epstein-Barr Virus (EBV), Herpes Simplex Virus (HSV), Cytomegalovirus (CMV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV) were used. RNA isolation was performed with the MagNA Pure 24 Total NA Isolation Kit at the automated platform MagNA Pure 24 System (Roche Diagnostics, Basel, Switzerland), according to the manufacturer's instructions. Altona RealStar HDV RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) was used to directly amplify RNA samples on the qPCR instrument, according to the manufacturer's instructions. **Results:** The standardization assays indicated good qPCR amplification curves and high fluorescence levels between the controls evaluated. The assay demonstrated an average efficiency of 95%, with a coefficient of determination (r2) of 0.99. The detection limit of the test was 1 IU/µL for the HDV target (95% confidence interval). No cross-reactions with closely related viruses were observed (15 HDV-negative human plasma samples), indicating 100% specificity. The experiments performed to evaluate the precision demonstrated optimal repeatability and reproducibility. **Conclusion:** The HDV diagnostic is required to ensure sensitive, specific and accurate identification of the viral agent and thus prompt surveillance actions such as epidemiological monitoring and control measures of infection. The RT-qPCR assay described here provides a reliable, highly specific, and sensitive method for the detection and quantification of HDV. Nevertheless, the assay needs to be evaluated in conjunction with a clinical and epidemiological context.

A-305

Evaluation of the analytical performance of rapid tests to detect Arboviruses antibodies intended for point-of-care screening

R. S. Domingues, L. A. Martins, D. A. Zauli, A. B. de Lima, J. d. Silva. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Arboviruses are recognized as a group of RNA viruses that can transmit diseases by different sucking arthropods vectors, especially *Aedes Aegypti,* a mosquito that can transmit several viral diseases including Dengue (DENV) and Chikungunya (CHIKV). The re-emergence and spread of these diseases are considered a concern in global health and Latin American countries, such as Brazil. Therefore, it's necessary to offer efficient, simpler, and faster diagnosis tools for accurate case identification and help promote adequate treatment. Rapid tests (RTs) can be used as one of these tools for qualitative detection and it's important mainly in point-of-care (POC) testing places. Thus, this work aimed to evaluate RTs to identify antibodies (IgG and IgM) of DENV and CHIKV. **Methods:** Serum samples were previously selected as positive, negative, or inconclusive results based on ELISA method or RTs confirmation. It was used the immunofluorescence-based ECO Diagnostica RTs kits (ECO-F Dengue IgG/IgM and ECO-F ChikV IgG/IgM). The samples were diluted in proper buffer and incubated for 15 minutes. The results were read in ECO Reader F200 equipment that uses the Cut-Off value for results. Cut-Off values >1.00 are considered positives, while Cut-off values <1.00 are considered negatives. **Results:** The results are presented in Tabela 1. **Conclusion:** Both RTs showed specificity that surpasses 88%. The sensibility obtained was 57,14% and 33,3% for DENV and CHIKV tests respectively. All samples that obtained inconclusive results by other methodologies showed negative results in the RTs assessed. The NPV was 64% and 86,96% for DENV and CHIKV tests respectively. Although RTs can be used to help in the initial screening of diseases caused by arboviruses, it is necessary to carry out molecular tests for confirmation, especially in negative cases.

Table 1: Summary of the analytical performance of the kits evaluated.

RTs evaluated	*Samples*	*Negative (Another metodologies)*	*Negative (RTs)*	*Positive (Another metodologies)*	*Positive (RTs)*	*Specificity*	*Sensibility*	*Negative predictive value (NPV)*
ECO-F Dengue IgG/IgM	78	36	32	42	24	88,89%	57,14%	64,00%
ECO-F ChikV IgG/IgM	49	40	40	9	3	100%	33,33%	86,96%

A-306

Standardization of automated methodology for qualitative molecular detection of HIV-1 in dried blood spots (DBS)

C. P. Mendonça, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Human Immunodeficiency Virus (HIV) is the etiological agent of acquired immunodeficiency syndrome (AIDS) with more than 35 million people infected worldwide. Typically, after seroconversion, individuals enter a clinically stable and relatively asymptomatic phase that can last for years. Without antiretroviral treatment,

individuals usually progress to AIDS, which is characterized by a decrease in CD4+ cells in the immune system, which increases the susceptibility to opportunistic infections and eventual death. Historically, HIV tests have relied on the antibody response that patients produce due to the virus infection. Although these antibodies are ineffective in combating the virus, they have been found in most chronically infected patients. In newborns, nucleic acid amplification tests can diagnose HIV infection during the first 18 months of a baby's life, while their blood still contains maternal antibodies and can result in a false-positive interpretation of serological tests. the availability of a diagnostic test capable of detecting HIV infection that offers flexibility, stability and ease of collection is of great importance. In this sense, the detection of HIV-1 in dried blood spots (DBS) represent a good strategy, with simple collection of sample and provides evidence of current infection using nucleic acid amplification technologies such as polymerase chain reaction (PCR). In this way, this work aimed to evaluate a qualitative test for detetion of HIV 1 in DBS. **Methods:** Were selected 103 whole blood samples. The samples were tested from the qualitative HIV test (PCR In house) from the Pardini Group's Genetics sector. With the aid of a pipette, approximately 70 µl of blood from each sample were dispensed in duplicate, within the circle outlined on the DBS filter paper. DBS was previously identified with the sample data. The DBS with the sample was placed in a tray on a flat surface at room temperature (between 18 and 25 °C) for at least 4 hours protected from direct light to dry. After drying, the pre-extraction of the sample was performed and later the samples were placed in the equipment on the cobas® 4800, using the COBAS HIV 1 kit (Roche diagnostics), (qualitative test) for extraction. After the extraction step was complete, the plate was transferred to the cobas z 480 analyzer for detection. All steps were performed according to the manufacturer's instructions. At the end of the tests, the results were compared between the in-house methodology and the cobas® HIV-1 qualitative test. **Results:** The test cobas® HIV-1 qualitative test showed 100% agreement with the in-house methodology for 90/90 negative and 13/13 positive samples. When comparing the test matrix (whole blood or DBS), no significant differences were observed in relation to test sensitivity and specificity. In addition, test automation provided a substantial reduction in test execution time. **Conclusion:** The availability of tests that use DBS as a matrix offer important advantages, especially for children and the elderly, due to greater stability, ease of sample collection and transport. The qualitative cobas® HIV-1 test in DBS has been shown to be a good choice for detecting HIV-1 in infected patients.

A-307

Spread dynamic of SARS-CoV-2 variants and related alterations in transmission rates: a national scale study

J. d. Silva[1], A. B. de Lima[1], L. B. Alvim[1], F. S. Malta[1], C. P. Mendonça[1], P. L. Fonseca[2], R. P. de Souza[2], R. S. de Aguiar[2], D. A. Zauli[1]. *[1]Instituto Hermes Pardini, Vespasiano, Brazil, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Background: In late 2019, the world was surprised by the new coronavirus (SARS-CoV-2), responsible for the COVID-19 pandemic and the ability to develop and share knowledge about SARS-CoV-2 was essential in combat of the pandemic. Genomic surveillance has played a fundamental role in identifying new mutations, detecting emerging variants, and monitoring viral evolution. Although next-generation sequencing (NGS) remains the gold standard for the identification of new variants, this technology is still restricted due to the high cost. Thus, the use of accessible methods for SARS-CoV-2 monitoring is necessary. This study aimed to collect and share monitoring data of COVID-19 in Brazil and to evaluate the dynamics of SARS-CoV-2 variants during the two periods of changes in the epidemiological scenario (second and third pandemic waves) and the transmissibility rates among circulating VOCs. **Methods**: The study evaluated 27,003 samples from all federative units in Brazil between April/2021 and January/2022. Specific primers and probes were used for the mutations K417T/N, L452R, E484K/Q, N501Y, P681R (TaqMan SARS-CoV-2 Mutation Panel), and the SGTF (S-gene target failure) by the TaqPath COVID-19 CE-IVD RT-PCR Kit according to the frequencies of the Gamma, Delta, and Omicron VOCs in the evaluated time. Samples showing an unexpected mutation profile in RT-qPCR genotyping were submitted to NGS. Data for viral targets of about 227,661 positive samples were collected to assess the period of predominance of each VOC as an inference of transmissibility potential. **Results**: Samples were collected in all 27 Brazilian federative units. The replacement of the Gamma by Delta variant occurred in about 5 months, between June and September 2021, when the Delta variant reached 100% of infections. The transition of predominance among the Delta to Omicron variant was only 2 months, demonstrated by the progressive increase of Omicron in November (3.4%), December (67.5%), and January (97%), accompanied by the consequent growth of COVID-19 positive cases in the country. These results reinforce the higher transmissibility of Omicron compared to other variants identified in Brazil. The adopted strategy was able to identify the Gamma, Delta, and Omicron VOCs as well as the presence of other variants in 99.2% of the samples. Only 0.8% of the samples were not characterized and it was submitted to NGS. **Conclusion**: We described a methodology that was able to effectively follow the fast establishment of Omicron VOC related to its higher transmission rates and genomic mutations that explain the highest peak of COVID-19 in our country. This study comprises the largest national-scale of genomic surveillance of SARS-CoV-2, providing information about the virus spread in Brazil.

A-308

The molecular diagnosis of Varicella Zoster virus (VZV): a validation of a Real-Time PCR (qPCR) assay for the qualitative detection

C. P. Mendonça, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Varicella zoster virus (VZV) is the etiological agent of chicken pox and herpes zoster. After the primary infection, which usually occurs in childhood, VZV remains latent in sensory nerve roots for life. When reactivation occurs, especially in immunocompromised patients, the virus migrates to the skin and causes painful rashes that can evolute to a dermatome. Even after the end of the viral acute infection, some individuals may experience continued pain. Furthermore, VZV reactivation can lead to a wide spectrum of clinical manifestations such as self-limited radicular pain and central nervous system involvement. During acute illness, 90% of patients experience pain and 12% have symptoms similar to influenza infection (influenza-like). Immunocompromised patients may have an unfavorable course with disseminated infection, pneumonitis, hepatitis, and encephalitis. The cerebrospinal fluid polymerase chain reaction (PCR) test is indicated for suspected involvement of the central nervous system and complications of ocular or periocular lesions. Compared to other diagnostic methods, real-time PCR has great advantages as its high throughput, efficiency, and sensitivity. Therefore, the detection of VZV by real-time PCR presents great importance for the diagnosis and monitoring of the disease due to its precision and agility**.** Thus, we intended to validate a qualitative method for VZV detection by real-time PCR. **Methods:** We evaluated the performance of the inventoried TaqMan assay (Vi06439647_s1) (Thermo Fisher Scientific). For this validation, we used 64 samples - whole blood (n=31), cerebrospinal fluid (n=32), vaginal lesion scraping (n=1) - from the Genetics sector of the Pardini Group, previously tested by in-house PCR for the qualitative detection of VZV. Samples were extracted using the MagNA Pure 24 System platform (Roche Diagnostics) with the MagNA Pure 24 Total NA Isolation Kit, according to the manufacturer's instructions. All assays were performed using Taqman Universal Master Mix (Thermo Fisher Scientific). The inventoried B-actin Assay (assay ID: Hs01060665_g1) (Thermo Fisher Scientific) was used as endogenous control. Assay verification tests, evaluation of reaction efficiency, and determination of the detection limit (LOD) were performed using a synthetic control of known concentrations and a pool of negative samples (healthy volunteers). At the end of the tests, were made a comparison of the previous results with the results obtained in the ThermoFisher Real-Time PCR Assay. **Results:** The Inventoriad Taqman Assay (VZV) had a limit of detection of 10 copies/µL and the Cq>35 was defined as a cut-off to consider a sample as positive. Whole blood samples had higher Cts than cerebrospinal fluid samples, indicating lower viremia. Whitin all evaluated samples, 11 samples had positive results detected by in-house PCR (9 from whole blood and 2 from cerebrospinal fluid). Of these, 10 had a result detected in Real-time PCR and the agreement with the positive results between compared methodologies was 91.0%. The divergent sample was whole blood and can be related to DNA degradation. The 47 negative samples evaluated were concordant in both methods. **Conclusion:** The inventoried TaqMan Assay with Taqman Universal Master Mix protocol evaluated was sensitive, fast, and applicable as a tool for the qualitative detection of VZV.

A-309

Challeging Diagnostic of Neurosyphilis: Use of CXCL13 Concentration in Cerebrospinal Fluid

R. d. Carvalho[1], I. d. Rangel[1], N. B. Bacarov[2], M. M. Soane[2], V. Herbst[3], F. d. Ferry[1]. *[1]Hospital Universitário Gaffrée e Guinle, Rio de Janeiro, Brazil, [2]EUROIMMUN Brazil, São Caetano do Sul, Brazil, [3]EUROIMMUN AG, Lubeck, Germany*

Background: Previous retrospective has been demonstrated the concentration of chemokine ligand CXCL13 in cerebrospinal fluid (CSF-CXCL13) is a promising biomarker in the diagnosis of neurosyphilis (NS) and monitoring of therapeutic efficacy. The goal of this study was to measure CXCL13 concentrations in CSF of NS suspected patients and compare with patients with syphilis non-NS and patients with treated syphilis. **Methods**: One hundred and eleven NS-suspected participants from Rio de Janeiro (Brazil) were investigated for the detection of IgG antibodies against

Treponema pallidum, using ELISA and Indirect Immunofluorescence tests (EUROIMMUN, Lübeck, Germany). CSF-CXCL13 concentration was prospectively performed by ELISA (EUROIMMUN, Lübeck, Germany) assay in all participants at baseline and in participants diagnosed with NS in follow-up visits at 3, 6, and 12 months after therapy. **Results**: CSF-CXCL13 concentrations were significantly higher in patients with established NS presenting a median concentration of 207.4 pg/mL (IQR 69.3 - 1515.5 pg/mL) than in patients with syphilis non-NS with a median of 1.0 pg/mL (IQR 0.1 - 11.0 pg/mL, P<0.001) or patients with treated syphilis with a median of 0.1 pg/mL (IQR 0.1 - 7.1 pg/mL, P < 0.001). The CSF-CXCL13 concentrations decreased after 3, 6 and 12 months of therapy compared to baseline in all cases of NS. The added concentration of CSF-CXCL13 above 62.8 pg/mL plus CSF-FTA-ABS reactivity agreed with diagnosis of NS in 88.9% of People Living with HIV (PLWHs) and plus CSF-TPHA reactivity agreed with the diagnosis of NS in 100% of HIV-uninfected patients. **Conclusion**: The study suggests that the clinical use of CSF-CXCL13 is useful for supplementary biomarker for NS and as monitoring the effectiveness of NS therapy, especially in PLWHs with nonreactive CSF-VDRL, excluded other neurologic diseases.

Pediatric and Maternal Fetal Medicine

A-310

Infections Associated With Infertility and Success of In Vitro Fertilization

N. B. Bacarov[1], A. V. Nunes[2], R. Dias[1], C. Resende[1], L. Garcia[1], C. Barbosa[3], M. Soane[1], D. Christofolini[2]. *[1]EUROIMMUN Brazil, São Caetano do Sul, Brazil, [2]Centro Universitário Faculdade de Medicina do ABC (FMABC), Santo André, Brazil, [3]Centro Universitário Faculdade de Medicina do ABC (FMABC), São Paulo, Brazil*

Background: Preimplantation genetic analysis aims selecting the best embryo to transfer increasing the chance of a successful pregnancy. However, failure in the implantation of euploid embryos is frequent in the practice of assisted human reproduction, observed in approximately 30 to 50% of transfers, with no cause found in most cases. Previous or active infectious may be associated with infertility, but this topic still lacks deeper studies. Our aim was to analyze the occurrence of sexually transmitted infections in women who underwent the transfer of euploid embryos with different gestational outcomes.

Methods: A prospective cohort study was carried out for which 36 plasma (EDTA) samples and 17 endocervical smears (Digene HC2 DNA Collection Device) were collected from patients who would be submitted to assisted reproduction cycles (using euploid blastocysts) shortly after the collections, at a private assisted reproduction center. The collections were performed on the 3rd day after the start of luteal phase support with progesterone (clinic protocol). After collection, the samples were placed in a freezer at -80°C until the time of analysis. Women with signs of cervicitis at the time of collection, deep endometriosis, patients over 40 years of age, cases of severe male factor, chronic pelvic pain, patients who would transfer more than one embryo, transfer of aneuploid embryos or with chromosomal mosaicism were also excluded. Samples were submitted to IIFT (EUROIMMUN) to diagnostic specific anti-IgG antibodies against *Chlamydia trachomatis* (MIF)*, Neisseria gonorrhoeae*, *Herpes simplex virus* 1 and 2, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Treponema pallidum*. Active infection was verified by Microrray technic using EUROArray STI 11 kit (EUROIMMUN) to detect *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Herpes simplex virus* 1 and 2, *Haemophilus ducreyi*, *Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum*, *Ureaplasma urealyticum, Treponema pallidum* and *Trichomonas vaginalis*.

Results: From the 36 plasma samples analyzed for IgG antibodies presence, the following were seropositive: 30 anti-herpes simplex 1 and 2, 3 anti-Treponema pallidum, 10 anti-Chlamydia MIF and 32 anti-*Mycoplasma hominis* and anti-*Ureaplasma urealyticum*. From the 16 endocervical smears samples analyzed, *Ureaplasma parvum* was detected in 3 women, who failed to get pregnant. No other infectious agent was detected in the samples. For the statistical analysis of the descriptive results, Fisher's exact test was used, the qualitative variables were presented by absolute and relative frequency, the confidence interval level adopted was 95%. The p value was calculated using the statistical program Stata version 14.0, and no statistic difference was observed between the group got pregnant through IVF and the group didn't.

Conclusion: Although, a high prevalence of IST specific antibodies anti-IgG in this cohort and 3 patients with active infection was found, a statistical significant difference was no obtained between groups who have succeed on IVF and not, against to the bibliography. In another hand all the patients with active infection of *Ureaplasma parvum* didn´t have success on IVF which suggests that infection increase the unsuccess IVF. On this context, a larger sampling will clarify the hypotheses of associations of STIs as a cause of failed IVF.

A-312

Segregation in Two Families of the PCDH19*G*ene Deletion Associated With Infantile Epileptic Encephalopathy of X-Linked Inheritance and Expression in Female Carriers

M. BELLIDO DIAZ[1], T. De haro Romero[2], T. Gonzalez[2], T. de Haro[2]. *[1]HOSPITAL UNIVERSITARIO VIRGEN DE LAS NIEVES, Granada, Spain, [2]HOSPITAL UNIVERSITARIO CLINICO SAN CECILIO, Granada, Spain*

Background: Developmental and epileptic encephalopathy-9 (DEE9) is an X-linked disorder characterized by seizure onset in infancy and mild to severe intellectual impairment. Autistic and psychiatric features have been reported in some individuals. The disorder affects heterozygous females only; transmitting males are unaffected. We described the segregation in two unrelated families where the proband was clinically affected as a female and another family where the proband was clinically unaffected as a male.

Methods: Array-CGH analysis was performed by a CGXTM HD v1,1 4-plex array 180 k (PerkinElmer), with an average resolution of 40 kb in the backbone and 20 kb in the regions of interest.

Results: In the first family a deletion of 809.93 Kilobases was detected in the chromosomal region Xq22.1, which includes the *PCDH19* gene, in a 1 year old patient with very early onset seizures, 8 months old, inherited from an asymptomatic father. In the second family, a deletion that includes the entire *PCDH19* gene was detected in a 5-year-old male patient referred to genetics for autism spectrum disorder (ASD) and not symptomatic for epilepsy; this deletion was inherited from a mother who had episodes of epilepsy in childhood.

Conclusion: The aCGH technique is currently the technique of choice to determine the presence of deletions/duplications in genes of interest with high reliability in patients with neurodevelopmental disorders such as epilepsy or ASD. The probands of these two families have been studied for different clinical pathology and in both the deletion of the complete *PCDH19* gene has been detected.

The *PCDH19* gene is expressed in developing human and mouse central nervous system, including the hippocampus and cortex, suggesting a role in cognitive function. Malfunction of the protocadherin19 protein is the cause of epileptic seizures in early childhood, the seizures are mostly focal and cause serial seizures (many seizures that cluster together over a few days). It is a type of epilepsy that usually begins in early childhood. Onset may occur spontaneously or in association with fever. Mutations/deletions of the P*CDH19* gene are described to cause Early Childhood Epileptic Encephalopathy, with high but incomplete penetrance. DEE9 affects only females heterozygous for the deletion and is characterised by childhood onset of seizures/epilepsy, usually with mild/severe intellectual disability, with males being healthy carriers. Although the cause is not known specifically, it is thought to be related to X-chromosome silencing that occurs in females heterozygous for this mutation. It causes an autosomal dominant X-linked disorder. Some cases of males with a mosaic *PCDH19* mutation/deletion have been found to be affected and it has been suggested that the presence of both the normal gene and the mutated/deleted gene is necessary to develop for this epileptic encephalopathy.

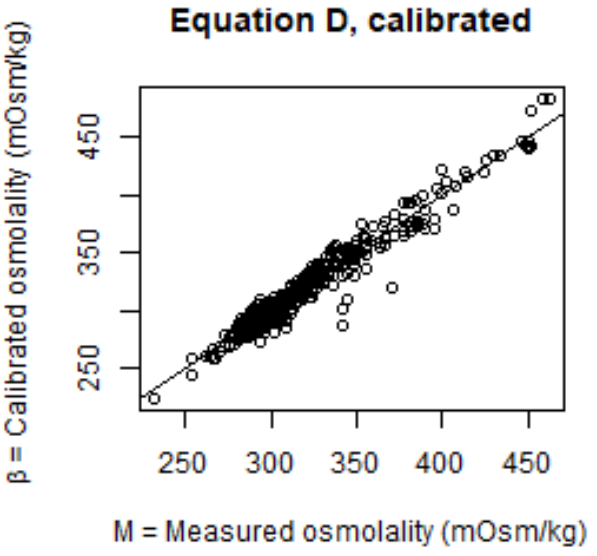
A-314

Comparison of calibrated calculated osmolality to measured osmolality by different equations: a study using pediatric specimens.

U. C. Garg[1], A. Wiebold[1], M. Knoll[1], R. McDonough[1], D. F. Stickle[2], A. M. Ferguson[1]. *[1]Children's Mercy Hospital, Kansas City, MO, [2]Jefferson University Hospital, Philadelphia, PA*

Background: American Diabetes Association recommends using serum osmolality to aid in differentiating between hyperosmolar hyperglycemia state (HHS) and Diabetic Ketoacidosis (DKA). Unfortunately, measured osmolality is unavailable in many small hospitals. Calculated osmolality is used commonly to evaluate the gap between calculated and measured osmolality. Using pediatric samples, we investigated whether calculated osmolality when calibrated against measured osmolality might serve as a surrogate for the measured osmolality. **Methods**: Primary data were 441 samples having measured osmolality along with coincident measurements of Na, K, BUN and glucose. Calculated osmolality (α) was obtained using six equations from the literature: A: α=2*Na+(Glu/18); B: α=2*Na+(Glu/18)+(BUN/2.8); C: α=2*(Na+K)+(Glu/18)+(BUN/2.8); D: α=1.86*Na+(Glu/18)+(BUN/2.8)+9; E: α=1.

86*(Na+K)+(Glu/18)+(BUN/2.8)+10; F: α=1.89*Na+1.38*K+(1.08*Glu/18)+(1.03*BUN/2.8)+7.45. For each equation, α was correlated with measured osmolality (M) by linear regression: α=slope*M+intercept. Calibrated values (β) were obtained as follows: β=(α-intercept)/slope. This procedure produces a linear regression for β vs. M having slope=1 and intercept=0. The best calculated osmolality equation was the one for which the standard deviation (sd) of the distribution of errors for the calibrated data (the distribution of e=(β-M) across all points) was least. **Results**: Measured osmolality (M) ranged from 232-462 mOsm/kg. The best relationship between β and M was for equation D. For D, calculated osmolality α=0.77*M+49.2 mOsm/kg; r2=0.945. Calibrated β vs. M is shown in **Figure** (r2=0.945). Errors (e) for β were normally distributed: e=0±8.9(1sd) mOsmol/kg. Thus, the 95% confidence interval (±2sd) for calibrated calculated osmolality using D was ±17.8 mOsm/kg. In our database, 18 specimens (4.1%) were outside of this boundary. **Conclusions**: Calculated osmolality when calibrated to measured osmolality may be reasonable to report in pediatric hospitals when measured osmolality is not available, so long as physicians are fully informed and in agreement with what is being reported.



A-315

Pregnant Women Showed Lower Incidence Rates of False-positive Treponemal Antibody Screening Results When Compared with Nonpregnant Women and Men at an Appalachian Academic Medical Center

J. Yang, D. Tacker. *West Virginia University, MORGANTOWN, WV*

Background: Screening for syphilis infection is recommended for all pregnant women and any adult or adolescent with increased risk for infection. Many clinical labs have adopted the reverse syphilis serology testing algorithm, that employs an automated and high-throughput treponemal antibody test as the initial screen followed by a non-treponemal antibody test to confirm all reactive samples. For specimens with discordant results, a second treponemal antibody test is used as the adjudicator. Syphilis serological tests can have false-positive results, causing discordant results between screening test and confirmatory test and complicating result interpretation. It is controversial from the literature whether pregnancy is a cause of false-positive results. This study is to determine the incidence rate of false-positive treponemal screening results among pregnant and nonpregnant patients, and to assess whether false-positivity is associated with pregnancy and age.

Methods: This is a retrospective study of sequential results from two treponemal antibody screening tests, including BioPlex 2200 Syphilis Total immunoassay between May 2020 and Jan 2022, and Alinity i Syphilis TP assay between Feb 2022 and Nov 2022. For each screening test, the incidence rates of false-positive results were calculated based on the reverse algorithm criteria and compared among pregnant women, nonpregnant women and men. The age differences between patients with false-positive results and patients with nonreactive results were also evaluated.

Results: Overall, 0.32% of 9,575 samples tested by Alinity assay showed false-positive results not confirmed by RPR or TP-PA. This is significantly lower ($P < 0.01$) than 0.60% of 14,983 samples observed for BioPlex assay. However, the lower incidence rate of false-positive results for Alinity assay relative to the Bioplex assay is only observed in pregnant women (0.18% vs. 0.44%, $P < 0.01$) and nonpregnant women (0.32% vs. 0.71%, $P = 0.06$), but not in men (0.68% vs. 0.63%, $P = 0.81$). For both assays, male patients with false-positive results had an older median age than those with nonreactive results, and female patients showed the opposite trend, although statistical significance was only observed in nonpregnant women for Bioplex assay (median [IQR], 25 [19-35] vs. 30 [22-43], $P = 0.047$). For both assays, pregnant women had lower incidence rates of false-positive results than nonpregnant women, although the differences did not reach statistical significance without age adjustment (Bioplex assay: 0.44% vs. 0.71%, $P = 0.07$; Alinity assay: 0.18% vs. 0.32%, $P = 0.26$). When age is adjusted, binary logistic regression analysis showed that pregnant women had significantly lower incidence rates of false-positive results relative to nonpregnant women for both assays (BioPlex assay: $P = 0.049$; Alinity assay: $P < 0.01$).

Conclusion: Although the incidence rates of false-positive results in BioPlex and Alinity treponemal antibody screening tests were overall within a low range, a significantly lower rate was observed for Alinity assay in pregnant women but not in men. For both assays, pregnant women showed a significantly lower incidence rate of false-positive results in comparison with nonpregnant women after age adjustment.

A-316

Suboptimal Compliance to Postpartum Glucose Monitoring in a High-risk Pregnancy Population - a Single Center Study.

L. Olayinka, K. Fox, K. M. Aagaard, S. Devaraj. *Baylor College of Medicine/Texas Childrens Hospital, Houston, TX*

Background Gestational diabetes mellitus (GDM) is a predictive factor in developing type 2 diabetes mellitus (T2DM) in affected mothers and their offspring. Its prevalence is increasing proportionally with the obesity epidemic, affecting 10% of pregnant women and 50% of which are likely to develop T2DM. Current guidelines by the American Diabetes Association and the American College of Obstetricians and Gynecologists recommend screening at 4-12 weeks with a 2-hour postpartum glucose tolerance test (OGTT) in patients with a history of GDM. However, several studies have reported suboptimal test ordering and completion rates attributable to the busy postpartum period and provider role confusion between primary care physicians and obstetricians. Due to the complex maternal-fetal cases encountered at our institution, the aim of this study is to evaluate the ordering and test completion rates amongst care providers and patients to ensure compliance with stipulated guidelines. **Method** We conducted a retrospective audit of women diagnosed with GDM who delivered at our institution from Jan 2019 - Oct 2022. We abstracted data from electronic health records. Gestational diabetes was defined by one or more of the following: abnormal 3hr OGTT or 1-hour OGTT of >/= 200mg/dL at >/= 24 weeks. We excluded patients with pre-existing diabetes defined by diagnosis code or by an abnormal 1st trimester 3hr GTT or HbA1C and patients who did not return for a postpartum visit. We compared the proportions of patients with postpartum glucose monitoring orders (glucose tolerance tests or Hemoglobin A1C) to those without orders. Factors such as race, GDM management, provider type and insurance carrier were assessed as potential barriers to non-compliance. **Results** We identified 356 patients who were diagnosed with GDM during the study period. Of these, 158 patients were excluded who had pre-existing diabetes or did not return for postpartum care. The mean (SD) maternal age was 33.2 (6.0) years. Seventy-four percent (147/198) of patients had a postpartum glucose tolerance test order (Group A), and 26% (51/198) had no laboratory order for glucose monitoring (Group B). The proportion of patients with a completed test order was 24% (35/147), of which four results were abnormal (indicated for diabetes or pre-diabetes). There was no difference in parity, ethnicity, insurance type, diabetes management and provider type between the two groups. Poor compliance was observed in patients with glucose tolerance laboratory orders, while patients with an HbA1c order were 100% compliant. **Conclusion** Overall, this report demonstrates poor patient compliance with postpartum glucose tolerance testing. Although HbA1C is not recommended for postpartum glucose monitoring, patients were more likely to complete this test.

A-318

Pseudo-hypertriglyceridemia in a 2-year-old male with global developmental delay, myopathy and adrenal hypoplasia

X. R. Fu, C. P. Williamson, K. P. Bosfield. *University of Tennessee Health Science Center, Memphis, TN*

Background Pseudo-hypertriglyceridemia is an overestimation of serum triglyceride levels due to laboratory assays that measure free glycerol concentrations instead of triglycerides directly. Consequently, conditions presenting with elevated levels of endogenous or exogenous free glycerol, such as glycerol kinase deficiency, result in an overestimation of serum triglycerides. Glycerol kinase deficiency (GKD) is caused by pathogenic variants of the *GK* gene on chromosome Xp21. Three forms of GKD are recognized, characterized as infantile, juvenile, and adult forms. While the juvenile and adult forms are recognized as isolated GKD, the infantile form manifests itself as part of the Xp21.3 contiguous gene deletion syndrome, also known as complex GKD. This syndrome is composed of genes associated with Duchenne muscular dystrophy (*DMD*), X-linked congenital adrenal hypoplasia (*NR0B1*), and intellectual disability (*IL1RAPL1*). GKD is characterized biochemically by hyperglycerolaemia and glyceroluria. **Case Description** A 2-year-old male presented to the genetics clinic with a history of global developmental delay (gross motor and speech), axial hypotonia, poor head control and inability to sit unassisted or walk. Significant findings on examination included a startled appearance, absent eyebrows and temporal thinning, high forehead, frontal bossing, axial hypotonia and peripheral hypertonia. Laboratory findings included elevated creatine kinase (CK) of 14809 units/L (normal 27-160 units/L), aspartate aminotransferase (AST) of 307 (normal 20-60 units/L), alanine aminotransferase (ALT) 265 (normal 15-45 units/L), Adrenocorticotropic Hormone (ACTH) 156 (normal 6-48 pg/mL), and elevated triglycerides at 683 (normal 44-157 mg/dL). There was also a family history of developmental delay and intellectual disability in patient's mother and younger sister. **Method** Triglycerides are commonly measured indirectly with different enzyme reagents. The Triglycerides in this boy were measured by an automated chemistry analyzer based on a colorimetric serial enzymatic reaction including lipase, glycerol kinase and L-α-glycerol-phosphate oxidase[1]. Urinary organic acids profile including glycerol was extracted from acidified urine (pH 1-2) using diethyl ether. Organic extracts are then evaporated to dryness under nitrogen and trimethysilyl derivatized by the addition of N, O-bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchorosilane (BSTFA/TMCS) and analyzed by gas chromatography-mass spectrometry (GC-MS). **Results** A comprehensive work-up including qualitative urinary organic acid analysis and chromosomal microarray was carried out. Urinary organic acids analysis revealed very abnormal accumulation of glycerol. Chromosomal microarray results showed a 4.2 Mb deletion of Xp21.3p21.1 (29296579-33551038) including complete copies of *GK, DMD*, and *NR0B1* genes as well as multiple exons of *IL1RAPL1*. This confirmed his GKD as part of the Xp21 continuous gene deletion syndrome. Elevated triglycerides were then recognized as pseudo-hypertriglyceridemia after the diagnosis. Mild glyceroluria is also observed in the younger sister of this patient. **Discussion and Conclusion** This case highlights the importance recognizing pseudo-hypertriglyceridemia and diagnostic challenges. Earlier identification through urine organic acid analysis could have been made. The combination of clinical presentations and increased glycerol should cause suspicion for GKD. Female carriers of this X-linked disorder need to be evaluated for intellectual disability, as seen in this patient's mother and younger sister.

A-320

Validation of a Multi-Analyte Immunoassay for Distinguishing Bacterial Versus Viral Infections in a Pediatric Cohort

A. Chokkalla[1], E. Tam[2], R. Liang[2], A. Cruz[1], S. Devaraj[1]. *[1]Baylor College of Medicine/Texas Children's Hospital, Houston, TX, [2]Texas Children's Hospital, Houston, TX*

Background: Clinical presentation of viral and bacterial infections or co-infections overlaps significantly. Pathogen identification is the gold standard for appropriate treatment. Recently, FDA approved a multivariate index test called MeMed-BV on the MedKey analyzer that distinguishes viral and bacterial infections based on a score derived from the differential expression of 3 host proteins. Here, we sought to validate the MeMed-BV immunoassay on the MedKey analyzer in our pediatric hospital, following guidelines from the Clinical and Laboratory Standards Institute. **Methods**: The analytical performance of MeMed-BV was evaluated by precision (intra- and inter-assay), accuracy (sensitivity and specificity) and interference studies. A retrospective cohort study (n=60) was conducted to assess the diagnostic accuracy using plasma samples from pediatric patients with acute febrile illness who visited the emergency department of the Texas Children's Hospital, Houston, TX, for whom a C-reactive protein test was requested. The following inclusion criteria were employed: 1) age ≤18 years during the clinical encounter; 2) peak fever >37.5 °C with the onset of symptoms such as cough, sore throat, abdominal pain or nausea and vomiting; 3) duration of symptoms ≤7 days; and 4) a positive nucleic acid amplification test (for virus) or abnormal blood culture (for bacteria). Patients with 1) active malignancy, 2) congenital immunodeficiency, 3) active immunosuppressive or immunomodulatory therapy and 4) human immunodeficiency virus or hepatitis B/C virus infection were excluded. Clinical performance was evaluated by blinded adjudication from our emergency room physicians. **Results**: MeMed-BV showed acceptable intra- and inter-assay precision with a range of <3 score units in both the high-score bacterial and the low-score viral samples. Accuracy studies revealed a sensitivity of 94% and specificity of 88% for identifying bacterial infections or co-infections. Our MeMed-BV results showed an excellent agreement (R=0.998) with the manufacturer's laboratory data and compared well with ELISA studies. Gross hemolysis and icterus did not affect the assay, but gross lipemia showed a considerable bias in samples with a moderate likelihood of viral infection. Importantly, the MeMed-BV score performed better than routinely measured infection-related biomarkers like white blood cell counts, procalcitonin and C-reactive protein in classifying bacterial infections. Furthermore, it is important to note that the score but not individual levels of the biomarkers, should be used to discriminate between bacterial and viral infections. **Conclusion**: MeMed-BV score on the MedKey analyzer demonstrated acceptable analytical performance and is reliable for distinguishing viral and bacterial infections or co-infections in pediatric patients. Future studies are warranted to examine the clinical utility, especially with respect to reducing the need for blood cultures and reducing the time to treatment for the patient.

A-322

Implementation of Three Age- and Sex-Dependent Equations to Estimate Glomerular Filtration Rates (eGFR) at a Pediatric Institution

A. Ferguson, A. Wiebold, D. Weidemann, B. Warady, U. Garg. *Children's Mercy, Kansas City, MO*

Background: The National Kidney Foundation estimates that 37 million people in the United States are affected by kidney disease, although 90% of them are unaware of it. While kidney disease is less common in children and adolescents, early diagnosis and treatment is important due to the significant long-term impact it can have on growth, cognitive development, and cardiovascular disease. Unlike adults, the estimated glomerular filtration rate (eGFR) for pediatric patients is not routinely reported by clinical laboratories. The process of estimating GFR is cumbersome and time-consuming for clinicians, requiring data extraction to use estimating equations with variable accuracy. The Chronic Kidney Disease in Children (CKiD) Study has developed and published new, more precise, estimating equations for use in children and young adults that utilize a sex- and age-dependent constant (*k*) in conjunction with height and serum creatinine and/or serum cystatin-C. In collaboration with Nephrology and Laboratory Information Technology, we implemented three eGFR equations into our laboratory information system (LIS) and currently report the results in the patient's electronic medical record (EMR).

Methods: The three equations that were implemented were named eGFR-creatinine (eGFR= *k* x (height/sCr) with height in m and serum creatinine in mg/dL), eGFR-cystatin (eGFR= *k* x (1/cysC) with serum cystatin-C in mg/L), and eGFR-average, which reports the average of the creatinine and cystatin-C estimating equations when both are ordered. The *k* constant is dependent on the patient's age and sex. An eGFR value of >90 mL/min|1.73m^2 is considered normal. When an eGFR is ordered, the system identifies the most recent creatinine or cystatin-C result. If one has not been resulted in the last 2 hours, the system reflexes an order for a basic metabolic panel or a cystatin-C. For the eGFR-creatinine equation, the system looks back up to 3 months for a height and uses the most current value. Concordance and discordance were determined in patients who had all three equations ordered and compared as to whether all three values gave a result above or below 90 mL/min|1.73 m^2.

Results: The eGFR-creatinine equation went live in June 2022. As of January 2023, 287 eGFR-creatinine values have been resulted. The cystatin-C assay, eGFR-cystatin equation, and e-GFR average equations went live in November 2022. As of January 2023, 114 eGFR-averages and 167 eGFR-cystatins have been resulted. When both equations were ordered and the eGFR-average was calculated, all three values were concordant 69 times (61%) and discordant 45 times (39%). In 26 instances, the eGFR-creatine value was discrepant compared with the other two equations and in 19 instances, the eGFR-cystatin value was discrepant. The differences between the eGFR equations ranged from 1.1-41.9%, however 65% of the values varied by less than 10% and 88% varied by less than 20%.

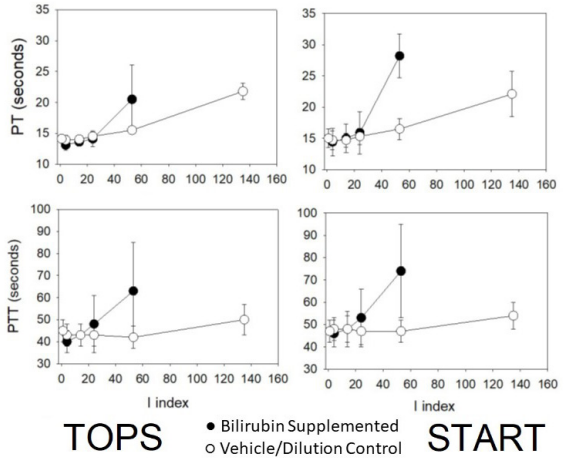
Conclusion: Implementation of pediatric eGFR equations into the LIS and reporting of eGFR in the EMR will allow more accurate assessment of pediatric patients' kidney function and improved clinician and patient awareness of impaired kidney function. Availability of this information will allow an earlier diagnosis and treatment of chronic kidney disease.

A-323

Pre-analytic interference in PT and PTT measurement using mechanical vs. optical clot detection.

D. A. Maciak[1], D. J. Dietzen[2]. *[1]St. Louis Children's Hospital, St. Louis, MO, [2]Washington University School of Medicine, St. Louis, MO*

Background: Mechanical clot-detection is commonly thought to be less susceptible to optical pre-analytic interference. Pediatric specimens are particularly prone to be hemolyzed and/or icteric. Our hospital laboratory approached an impending change in coagulation instrumentation from mechanical (STAGO) to optical (IL) with some trepidation. We, therefore, performed a detailed comparison of plasma samples containing a broad range of hemoglobin, bilirubin, and triglyceride using IL reagents (RecombiPlasTin2G, SynthASil) on the TOPS 550 and a manual method on the STart 4. **Methods**: 7 independent pools (5 normal, 2 prolonged) of residual citrated plasma were supplemented with hemoglobin, bilirubin, or triglyceride up to 1000 mg/dL, 150 mg/dL, or 750 mg/dL, respectively, or with equal volumes of vehicle as control. Supplementation was confirmed and quantified by measuring HIL indices on a Roche Cobas 6000. PT and PTT were performed in duplicate on each experimental and control sample on the TOPS and STart systems. Imprecision of the two systems was monitored at two levels throughout the study. **Results**: Imprecision (n = 20) of the TOPS PT and PTT assays ranged from 1.4 - 3.1% (CV). Imprecision (n = 20) of the STart PT and PTT assays ranged from 1.9 - 18.3% (CV). PT and PTT on both platforms were not impacted by hemolysis or lipemia up to an H index of 1000 and an L index of 500, respectively. As shown in the Figure, PT and PTT in normal pools on both platforms were significantly prolonged by the presence of bilirubin above an I-index of 30. Results were similar in the prolonged pools. Data points are the means (± SD) of 10 determinations across the 5 normal plasma pools for experimental (●) and control specimens (○). **Conclusion**: Mechanical clot detection did not show superior resistance to pre-analytic interference vs. an optical clot detection method.



A-324

Incorporating the screening for congenital cytomegalovirus into a newborn genetic testing performed on saliva swab: an intersection between human genetics and infectious diseases

J. Arancibia, A. Coqueiro, I. Sgardioli, A. Lamas, P. Mesquita, R. Benício, N. Pessoa, A. Andrade, G. Martins, R. Jácomo, L. Nery, G. Barra. *Sabin diagnóstico e saúde, Brasília, Brazil*

Background: Newborn genetic testing is a powerful tool for improving the future healthcare of infants because of its potential to screening for a wide range of disorders with a single test. The assay can be performed in non-invasive sample such as saliva swabs. Hundreds of genes associated to disorders with significant morbidity and mortality (if undiagnosed) are analyzed. Despite the knowledge that infectious disorders such as congenital cytomegalovirus virus (cCMV) infection also has potential to cause serious illness to newborns (10-15% of apparently asymptomatic infants with cCMV may develop hearing loss) most of the newborn genetic testing does not test the saliva for cCMV. This validation aims to compare two laboratory-developed PCR-based CMV tests in saliva samples to add the cCMV screening into our newborn genetic testing. **Methods:** Saliva swabs (OCR-100, DNA Genotek) from infants of our NGS-based newborn genetic testing were enrolled (n=171). Parent signed informed consent agreeing with the used of the left-over specimens. Infants age ranged from 0 to 10 years. Saliva DNA was extracted by using QIAsymphony and DSP DNA mini kit (Qiagen). CMV was tested by two independent qPCR assays performed on LightCycler 480II using LightCycler Multiplex DNA Master (Roche). Each assays target two separated regions of the CMV genome (UL55+UL123 and UL34+UL80.5). A third assay targeting human *CPOX* gene was used as process control. The assays limits of detection were calculated by applying probit regression to the qualitative results returned from a serial dilution (1:10) of a positive pool (2,310,000 UI/mL) into a negative pool of samples. The accuracies for CMV detection were estimated by the total, positive and negative agreement between the compared sets of assays. The assay with lower limit of detection were chosen and its precision was evaluated by testing a subset of 26 saliva swabs (6 positives and 20 negatives) during 3 distinct days. For accuracy and precision experiments, Cq values >35 (~750UI/mL) was considered as not detected. **Results:** The limits of detections were 40.7 UI/mL (95%CI:19.2-62.27 UI/mL) for UL55+UL123 and 184 UI/mL (95%CI:64-304 UI/mL) for UL34+UL80.5. CMV was detected in 8 and not detected in 163 saliva swabs by either UL55+UL123 and UL34+UL80.5 resulting in total, positive and negative agreements of 100% (95%CI:97-100%), 100% (95%CI:67-100%) and 100% (95%CI:97-100%), respectively. The median (min-max) Cq values for UL55+UL123 were 30.75 (21.39-34.42) and for UL34+UL80.5 were 30.81 (20.53-34.1). In the assay precision evaluation, UL55+UL123 returned the expected result in 26/26, 26/26 and 25/26 samples on day 1, 2 and 3, respectively. The exception was a borderline positive sample that reach the Cq cut-off value (>35) on day 3 evaluation. **Conclusion:** The UL55+UL123 assay had lower limit of detection for CMV compared UL34+UL80.5 and was chosen to be used in our cCMV screening. Both assays showed complete agreement when applied to saliva swabs samples. UL55+UL123 presented acceptable precisions for positive and negative results. The cCMV screening by qPCR is now performed in parallel to NGS in our newborn genetic testing. cCMV test is only performed for newborns with less than 21 days of life, as recommend by specific guidelines.

A-326

Argininosuccinic acid - is it routinely detectable in the urine of healthy individuals?

K. M. Karger[1], G. Ogilvie[2], M. Y. Tsai[1]. *[1]University of Minnesota, Minneapolis, MN, [2]MHealth Fairview, Minneapolis, MN*

Background: Argininosuccinic aciduria is a genetic disorder that results in elevated levels of argininosuccinic acid (ASA) in urine and blood. While there is seemingly a consensus within the clinical literature that any detectable amount of ASA in blood/plasma is diagnostic for argininosuccinic aciduria, yet there is conflict as to whether ASA in urine can be found in unaffected individuals. Some literature states that ASA should not be found in the urine of a healthy individual, while other literature reports low levels of ASA in the urine of healthy individuals. Additionally, many laboratories do not have a normal range for ASA within urine, which implies that any level of ASA found within urine is indicative of argininosuccinic aciduria. This conflict may in part be due to differences in the methods used to determine ASA levels. Newer liquid chromatography tandem mass spectrometry (LCMSMS) methods have been shown to be more sensitive than ion-exchange chromatography (IEC) based amino acid methods for detection of ASA. Additionally, the detection of ASA via IEC requires the heating and acidification of the sample to convert ASA into a single peak for quantitation. Heating and acidification is not routinely done for urine amino acid analysis with IEC; with routine IEC urine amino acid analysis, ASA migrates as three small peaks that may go unnoticed. In our clinical laboratory, which utilizes the less sensitive IEC method, our procedure describes the presence of any ASA in urine as diagnostic for argininosuccinic aciduria. Given the conflicting literature, we set forth to determine whether low levels can be detected in unaffected individuals with an IEC method, and if so, establish a normal range of ASA levels in urine with IEC.

Methods: Twenty waste urine samples from patients with no prior diagnoses of argininosuccinic aciduria were evaluated. ASA levels within the samples were determined via high performance ion exchange chromatography for separation of compounds followed by derivatization with ninhydrin for spectrophotometric detection

with a Hitachi Amino Acid analyzer. The samples were acidified and heated, which converts ASA into a single anhydride peak for accurate quantitation, and the results were normalized to urine creatinine.

Results: ASA was detected in all twenty urine samples, with an average ASA concentration of 27.2 µmol/g Cr and a range of 11.3-47.7 µmol/g Cr.

Conclusion: Given these findings, our clinical laboratory procedure has been updated to include the information that ASA can be present in the urine of healthy individuals up to a concentration of 50 µmol/g Cr. Other clinical laboratories that analyze urine to identify argininosuccinic aciduria should be aware of the misinformation in the literature that claims that ASA is not present in the urine of healthy individuals.

A-327

Pre-analytical and analytical modifications to improve the quality of pediatric lead tests

J. Nassif, J. Liebreich, K. Granger. *Association of Public Health Laboratories, Silver Spring, MD*

Background:

The recent decrease in the CDC Blood Lead Reference Value (BLRV) to 3.5 ug/dL has highlighted the need for systematic changes that some clinicians and laboratories need to implement to assure the quality of reported pediatric lead results. Building upon the recommendations of the Blood Lead Reference Value Workgroup, APHL has developed specific recommendations and resources designed to assist medical professionals and laboratory scientists in minimizing the likelihood of contamination when collecting and testing blood specimens. Also included are analytical practices that can improve precision, accuracy, and sensitivity of pediatric lead assays.

Methods:

Evaluation of existing protocols and practices to identify potential sources of contamination and opportunities for process improvement.

Results:

APHL will provide practical recommendations to systematically improve point of care and fixed laboratory testing to improve confidence in reported pediatric lead concentrations. These improved measurements will improve environmental health surveillance and inform public health intervention and patient care.

Conclusion:

Rigorous pre-analytical practices including education, updated specimen collection guidance, pre-screening of collection materials, improvement of point of care technology and refinement of spectroscopic analytical methods result in less ambient contamination, improved sensitivity, precision and accuracy of pediatric lead testing. Clinician office practices, public health and commercial clinical laboratories will all benefit from implementing these strategies.

A-328

Urine Toxicology Screening: Tetrahydrocannabinol Positivity Patterns at an U.S. Urban Tertiary Care Children's Hospital

D. M. Unsihuay Vila[1], I. Poventud-Fuentes[2], K. Osterhoudt[3], K. Patel[2]. *[1]University of Pennsylvania, Philadephia, PA, [2]Children's Hospital of Philadelphia, Philadelphia, PA, [3]Children's Hospital of Philadelphia, Philadephia, PA*

Background: Given the recent legalization of recreational and medical marijuana in different states, there is an increasing exposure of children and teenagers to marijuana products which has led to an increase in health care visits and hospitalizations. Our drug testing algorithm uses an immunoassay test for initial urinary drug screening (UDS) followed by an automatic confirmatory test only when a positive UDS result is obtained. The immunoassay test is performed in-house and detects the major metabolites of delta9- tetrahydrocannabinol (THC), the main psychoactive component of marijuana. In contrast, the liquid chromatography-tandem mass spectrometry (LC-MS/MS) confirmatory test is more specific towards THC and is carried out in an external lab. In light of the ongoing trend of marijuana exposure cases and the fact that immunoassay cross-reactivity with non-cannabinoid compounds is uncommon, we aim to assess the necessity of automatic confirmatory tests in our patient population and evaluate its impact on patient management.

Methods: We conducted a retrospective study to assess the trends in cannabinoid hospital-related encounters by age, gender, and incidence with other drugs, in our population among the main campus and satellites (November 2021 - October 2022). The UDS tests that were positive for cannabinoids were further evaluated to assess their agreement with the confirmatory testing.

Results: Cannabinoid positive tests account for 62.0% (504/813) of all positive UDS tests in all our locations and 84% (425/504) of the cases did not test positive for other drug classes. Among this population, 92.7% (467/504) are in the adolescent age (≥13 years), whereas 7.3% (37/504) were children (<13 years). Female population comprises 56.0% (217/504) of the cases whereas male, 43% (271/504) of the cases. The true positive rate for the immunoassay test was 99.6% (465/467) and 94.6% (35/37) for adolescents and children, respectively. Clinical decisions were made after a positive immunoassay result and before the confirmatory test as this is a send out test and takes a few days to receive the result.

Conclusion: Our evidence-based approach demonstrates unnecessary confirmatory testing for the adolescent group in our population due to a high positivity rate (99.6%). Discontinuing automatic confirmatory testing for this group will eliminate workload and reduce healthcare costs. On the other hand, automatic confirmatory testing in children will continue as these exposures are mainly considered unintentional and require more investigation.

A-329

Preliminary Clinical Method Comparison of a Near-Patient Platform for Low Blood Volume Testing of aFXa in Pediatric Patients on Heparin Therapy

A. Aljudi[1], W. Lam[1], R. Sista[2], V. Pamula[2], B. Rogers[1]. *[1]Children's Healthcare of Atlanta, Atlanta, GA, [2]Baebies, Inc, Durham, NC*

Background: Up to 15% of hospitalized children receive heparin therapy and require frequent monitoring for Anti-Factor Xa activity (aFXa) and activated partial thromboplastin time (aPTT) to achieve hemostatic balance. Current heparin tests require large blood volumes, which can lead to iatrogenic anemia with frequent sample collection. We performed a small clinical method comparison of a rapid, near-patient platform for simultaneous whole blood testing of aFXa from 50 µL of sample against the clinical standard test. The aPTT assay is in development on the same digital microfluidic (DMF) platform. **Methods**: Four DMF instruments were placed in our laboratory. Preliminary precision of the DMF aFXa assay was obtained using commercial controls (n=19 for aFXa at 0.98 U/mL; n=20 for aFXa at 0.42 U/mL). We obtained whole blood or plasma from residual aFXa samples from pediatric patients. Venous samples were collected from patients for routine testing in the core laboratory, and 150 µL of whole blood was saved for aFXa method comparison testing. Patients were either on low molecular weight heparin or unfractionated heparin. We performed a method comparison study to determine assay equivalence to in-house clinical standard tests using plasma for aFXa on the Stago STA R MAX to both plasma and whole blood on the DMF platform. **Results**: For precision, we observed an assay coefficient of variation (CV) of 4.68% and 6.6% for aFXa at 0.98 U/mL and 0.42 U/mL, respectively. The aFXa assay correlated well (R^2=0.94 for whole blood (n=9) and R^2=0.97 for plasma (n=10)); additional studies are needed to demonstrate aPTT assay equivalence. **Conclusions**: We demonstrated good correlation of the DMF aFXa assay to the clinical standard test. The low blood volume requirement make these tests suitable for use in heparin monitoring where frequent testing is necessary. A large scale clinical method comparison is planned to ensure clinical utility.

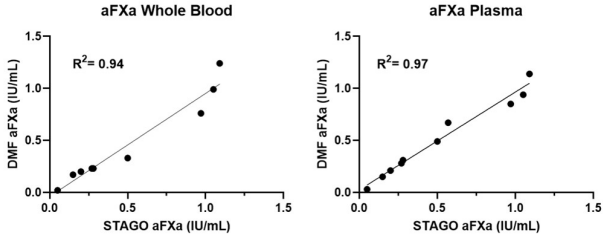


Figure. Method comparison for the aFXa assay whole blood (n=9; left) and plasma (n=10; right) samples run on the digital microfluidic platform and the Stago STA R MAX with either whole blood or plasma samples.

A-330

Trending Treatment of Transplant-associated Thrombotic Microangiopathy (TA-TMA): Validation and Performance of Soluble C5b-9 on the Dynex DS2 Platform

R. J. Wilson[1], L. M. Johnson[2], M. Bhandari[2], J. A. Dickerson[2]. *[1]University of Washington, Seattle, WA, [2]Seattle Children's Hospital, Seattle, WA*

Background: The complement system membrane attack complex is comprised of the C5b fragment along with C6, C7, C8 and C9 polymers that form a pore to disrupt a membrane during complement activation. Inactive complexes that are not membrane-bound combine with Protein S to form soluble C5b-9 (sC5b-9). Elevated concentrations of sC5b-9 are associated with risk of development of hematopoietic stem cell transplant-associated thrombotic microangiopathy (TA-TMA), which can be treated by eculizumab therapy. TA-TMA is a complex syndrome of abnormal cell activation, microvascular hemolytic anemia, and complement dysregulation which can lead to multiple organ dysfunction and death. Therefore, recognition of this syndrome is critical in the context of not only preventing poor outcomes in patients but also promoting stewardship of an expensive, yet effective therapy. Our laboratory evaluated the performance of the Quidel Microvue sC5b-9 Plus enzyme immunoassay on the Dynex-DS2 platform for clinical use.

Methods: EDTA plasma samples were collected from remnant CBC samples ordered for bone marrow transplant (BMT) patients and other patients not at risk for TA-TMA. Validation studies included method correlation, inter- and intraday precision, assay linearity, hemolysis interference, and other experiments. Clinical accuracy was assessed by performing a retrospective review of sC5b-9 results from BMT pediatric patients at risk for TA-TMA.

Results: Intraday precision was 6.7%, 7.1% for the manufacturer low- and high-quality control materials, respectively and 9.9% for an in-house prepared plasma pool. Inter-day precision was 9.8% and 5.3% for manufacturer low- and high-quality control material, and 12.8% for an in-house plasma pool over a period of at least five days. The linearity of the assay was verified with a lower limit of quantification of 80 ng/mL and upper limit of qualification of 1600 ng/mL. Our method comparison indicated good correlation with send-out results, with an average percent bias of -10% and Pearson's r2 value of 0.986. The assay showed no interference up to 100 mg/dL of hemolysis. Retrospective analysis of a few patient results demonstrated that weekly monitoring of sC5b-9 concentrations detected rapid increases in patients at risk of TA-TMA, which prompted treatment with eculizumab therapy. Given the analytical performance, at least 2- to 3-fold changes in sC5b-9 concentrations are clinically meaningful when tracking trends in patients at risk for TA-TMA.

Conclusion: Our results indicate the Quidel Microvue sC5b-9 Plus enzyme immunoassay on the Dynex-DS2 platform had acceptable analytical and clinical performance for monitoring trends in patients at risk for TA-TMA.

A-331

Prenatal Maternal First Trimester Screening and Establishing the Multiple of Medians of Double Marker Tests in Tertiary Care Hospital in India

B. Das[1], P. M. Pal[1], N. S. Mehtalia[1], V. Joshi[1], M. P. Gade[1], N. Pawar[2]. *[1]Kokilaben Dhirubhai Ambani Hospital and Medical Research Institute, Mumbai, India, [2]Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute, Mumbai, India*

Background: Down's syndrome, Edwards syndrome, and Patau syndrome (trisomy 21, 18, 13 respectively) in a fetus can be screened by prenatal testing using the maternal serum. Prenatal testing can provide an accurate assessment of the patient's risk of carrying an affected fetus. Double marker testing which includes the levels of free β- Human Chorionic Gonadotropin and Pregnancy Associated Plasma Protein A in the maternal serum along with the measurement of Crown Rump Length and Nuchal Translucency helps in determining the risk of Trisomy 21. Prenatal testing prevents the need for invasive methods.

Methods: This is a study conducted in Kokilaben Dhirubhai Ambani Hospital and Research Centre, Mumbai, Maharashtra, India from January 2012 to December 2018 for 628 pregnant women. The data for 1st trimester was divided into 3 groups: 11 to 11.6 weeks(86 patients), 12 to 12.6 weeks (421 patients), and 13 to 13.6 weeks (121 patients) based on their gestational age. This study included the determination of levels of double markers like free β- Human Chorionic Gonadotropin and Pregnancy Associated Plasma Protein A using maternal serum along with the ultrasound studies which included the Crown Rump Length and Nuchal Translucency measurements. Additional factors like a history of chromosomal abnormalities, smoking habits, insulin-dependent diabetes, and other dichotomous markers like the presence of nasal bone, abnormal ductal flow, and tricuspid regurgitation are also considered in calculating the risk. The concentration of biochemical parameters was expressed in Multiple of Median (MOM) with respect to maternal age. The risk assessment was analyzed using SSDW 5 and SSDW 6 software and the cut-off was set at 1:250 for age as well as trisomy 21, 18, and 13.

Results: The total number of positive patients for trisomy 21 and trisomy 13/18 were represented in percentage which turned out to be 2.86% and 1.11% respectively in association with the gestational age, dichotomous markers, double marker test, and ultrasound studies. The MOM for β-HCG, PAPPA, and NT were compared in SSDW5 and SSDW6. The mean β-HCG MOM negative and positive patients (trisomy 21) were found to be 1.34 ± 0.945 and 2.74 ± 1.685 respectively indicating the levels increase above normal. The mean PAPPA MOM negative and positive patients (trisomy 21) were found to be 1.47 ± 0.9125 and 0.77 ± 7667 respectively indicating the levels decrease below the normal. The NT mom for negative and positive patients was 0.91 ± 1879 and 1.88 ± 1.6508 respectively which suggests that the thickness of nuchal translucency increases with the increased risk for trisomy 21.

Conclusion:18 out of 628 patients tested positive for trisomy 21 which was assessed using double marker testing and ultrasound studies. The levels of free β-HCG were seen to be increased and the levels of PAPPA were decreased in the patients who showed higher risks. Prenatal testing provides an advantage for early detection of this disorder.

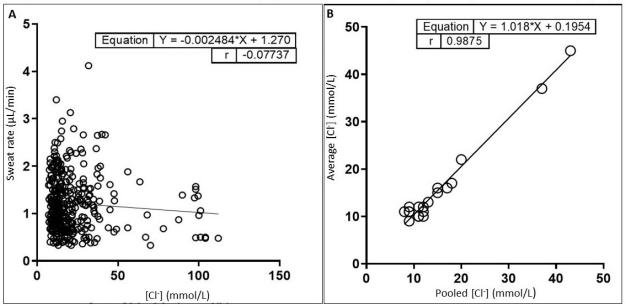
A-332

Sweat rate does not influence sweat chloride concentration

C. Omosule[1], S. Roper[2]. *[1]Washington University School of Medicine, St. Louis, MO, [2]Washtington University School of Medicine, St. Louis, MO*

Introduction: The recommendations for sweat chloride ([Cl^-]) testing from the Clinical and Laboratory Standards Institute (CLSI C34-A4 2019) endorse a minimum sweat rate of 1g/m^2/min to validate efficient sweat stimulation. For labs that use macroduct ® collection devices, this rate corresponds to a sweat volume ≥15µL collected within a 30-minute period. Bilateral collection and testing is also advised to improve the odds of obtaining a sufficient specimen from at least 1 site. When both sites fail to meet the minimum rate, the test is invalid (QNS) and should be repeated, potentially delaying Cystic Fibrosis (CF) diagnosis. In this study, we evaluate the premise for the minimum sweat rate by investigating the relationship between sweat rate and [Cl-]. In a sub-analysis, residual sweat available after clinical testing is used to examine the effect of specimen pooling on [Cl^-]. This study has the potential to inform best practices for future sweat testing guidelines, decrease QNS rates, and reduce time to CF diagnosis. **Methods**: Thirteen months of data from sweat tests performed at St. Louis Children's Hospital were reviewed. All collections were performed using macroduct devices following CLSI 34-A4 guidelines (results from QNS specimens were not reported clinically). Pearson correlation was calculated to examine the relationship between sweat rate and [Cl-] (n=354). Deming regression was applied to evaluate the effect of specimen pooling, versus results from individual sites, in 19 collections with residual volume after clinical testing. **Results:** Figure 1 **Conclusion:** Negligible correlation between rate and [Cl^-] across a wide range of values suggests the current minimum volume for macroduct collections (15µL) may be overly stringent. Reporting of [Cl-] in specimens with ≥10µL sweat may decrease QNS rates without compromising accuracy. Preliminary data suggests pooling of bilateral collections may be a feasible option to achieve the minimum volume required for testing (10µL).

Figure 1A: Correlation of sweat rate vs. sweat [Cl-], 1B: Regression analysis of pooled specimens vs. average [Cl^-]



A-333

Unusually Low Glycated Albumin Results Suggest Unexpected Interference in Study Samples from Pregnant Women

J. Powers Carson[1], A. Shin[2], K. Kabytaev[2]. *[1]Washington University in Saint Louis, Saint Louis, MO, [2]University of Missouri, Columbia, MO*

Background: Glycated albumin (GA) is an alternate marker of glucose control that is recommended in situations when hemoglobin A1c is unreliable. One of us (JPC) has examined its use in pregnant women with diabetes since 2019 and regularly observed GA values of 8.0 – 19.4%, with occasional outliers. However, in a recently recruited cohort of 149 women with and without gestational diabetes, 58% of the samples showed GA results <8.0%, despite no apparent physiological differences. Here we further investigate a subset of these samples using a liquid chromatography-mass spectrometry method (LC-MS/MS). **Methods:** Glycated serum proteins (GSP) were measured using the GlycoGap kit (Diazyme Labs, Poway, CA), an enzymatic method, and subsequently converted to percent glycated albumin (GA%) using the equation provided by the manufacturer.The LC-MS/MS assay was performed according to a previously published protocol. The samples were first reduced and alkylated, then spiked with a blend of two isotope-labeled standards and digested using Glu-C enzyme. The resulting digests were analyzed using the QTRAP 6500+ (AB Sciex, Foster City, CA) coupled with a LC system (Shimadzu, Japan) in MRM mode to provide values representing the percentage of glycated to non-glycated Lys-525 in albumin (MSGA%). Samples from a previously collected cohort (A, n=78) of pregnant women with and without diabetes, with GA% range of 8.5-24.2%, were analyzed using both methods, and a standard curve (MSGA% vs GA%) was established. Thirty-eight samples with GA <8.0% (cohort B) were then analyzed using the LC-MS/MS method, and the standard curve was used to generate the expected enzymatic GA% results. **Results:** The comparison of the LC-MS/MS and Diazyme methods resulted in a straight-line equation (r^2=0.84, slope=1.81, y-intercept=0.79). For the cohort B samples analyzed by LC-MS/MS, the median MSGA% result was 5.89% (range: 4.32-8.95%). Based on the equation derived from the method comparison (MSGA% vs GA%), the expected median value with the Diazyme enzymatic method would have been 11.48% (range: 8.63-17.02%). These GA% values are well within the typically expected range. **Conclusion:** LC-MS/MS results and GA% calculated from a method comparison curve were within the expected range while original enzymatic results were not, suggesting the presence of an interfering substance. Our hypothesis is that prenatal supplements may lead to elevated ascorbic acid, which is known to cause interference in certain enzymatic assays. We are continuing to investigate this possibility.

A-334

Investigation on Use of NHANES Data to Develop Continuous Pediatric Reference Intervals - Pilot Study with Hemoglobin

A. N. Lyle, L. J. Akinbami, T. Chen, F. Pokuah, B. K. Kit, H. W. Vesper. *Centers for Disease Control and Prevention, Atlanta, GA*

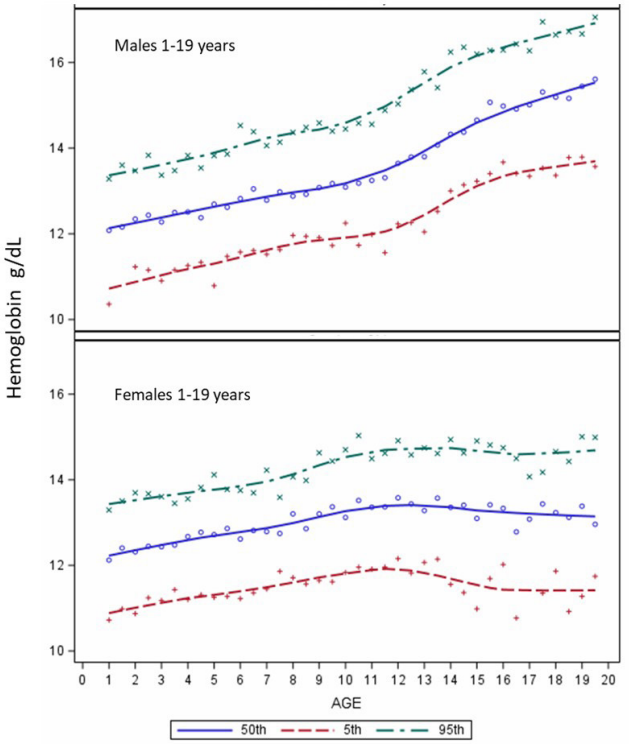
Background: Accurate Pediatric Reference Intervals (PRIs) are critical for assessing health, diagnosing disease, and establishing treatment efficacy. A recent literature review established that current PRIs are inconsistent, and partitioning reference intervals into arbitrary age groups may conceal the underlying biochemistry of child development. To overcome these challenges, continuous PRIs derived using specimens collected with standardized procedures from a well-defined population were proposed. The National Health and Nutrition Examination Survey (NHANES) meets these criteria and could be used to provide accurate PRIs for public health and clinical community use. However, no U.S. studies have described developing continuous PRIs for biomarkers. This study investigates a potential approach to develop continuous hemoglobin PRIs with NHANES data.

Methods: Total hemoglobin values for children and adolescents 1-19 years from NHANES 2011-2018 were assessed. To exclude potentially abnormal hemoglobin values, individuals with diabetes, anemia, BMI &gt95th percentile, serum cotinine &gt10 ng/L, C-reactive protein &gt10 mg/dL, and self-reported tobacco use, or poor health were excluded. Hemoglobin values at the 5th, 50th, and 95th percentiles were estimated at 6-month increments of age for males and females using survey weights. Continuous curves over the age range were then generated using smoothing methods.

Results: The initial sample size was 16,315, of which 3,183 had missing hemoglobin values. After applying exclusions (n=3,311), the final sample size was 9,821, of which 51.8% were males and 48.2% were females. Males had higher hemoglobin levels with a steeper slope in the curve over the age range and an increase in slope with adolescence, whereas hemoglobin levels in females peaked around age 12 before plateauing.

Conclusion: This investigation suggests that cross-sectional NHANES data can be used to develop continuous hemoglobin PRIs that reflect the physiologic changes commonly obscured by age group binning. More detailed assessments of appropriate sample exclusions and smoothing methods are underway.

Figure. Hemoglobin values for 5th, 50th, and 95th percentiles by year of age and sex, NHANES 2011-2018



A-335

Pediatric Cortisol Reference Intervals (RI): Impact of Age-Specific RI on the Rate of Abnormal "Low" Cortisol Results

B. D. Andress, D. R. Block, N. A. Baumann. *Mayo Clinic, Rochester, MN*

Background: Serum cortisol, measured by automated immunoassay, is a rapid test used for the initial assessment of adrenal dysfunction. Physiologic serum cortisol concentrations are influenced by many factors including diurnal variation, stress, and patient age. In the absence of established pediatric reference intervals (RIs), our laboratory has utilized adult RIs to flag pediatric cortisol results as abnormally high or low in the electronic health record. However, pediatric cortisol RIs recently defined by the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) are considerably lower than the adult RIs currently in use. This could cause pediatric cortisol results to be incorrectly flagged as "low" when they are within the age-specific RI and lead to unnecessary follow-up testing. The aim of this study was to determine the impact of implementing CALIPER pediatric cortisol RIs on the rate of "low" result flagging for pediatric patients. **Methods**: AM cortisol results for patients age <18 years reported between 10/2012- 09/2022 were obtained from the Mayo Clinic data repository. These results contained values measured by both Beckman Access (n=3,818) and Roche Cobas (n=625) immunoassays. Results classified as abnormal "low" by current adult RIs (Beckman: 7-25mcg/dL, Roche: 4.8-20mcg/dL) were compared to results which would flag low according to the appropriate method- and age-specific RIs defined by CALIPER (Beckman: 1-<13yrs 2.19-12.8mcg/dL, 13-<16yrs 3.03-17.1mcg/dL, 16-<19yrs 3.78-19.4mcg/dL; Roche: <1yr 0.58-17.2mcg/dL, 1-<12yrs 2.39-14.9mcg/dL, 12-<19yrs 3.63-17.4mcg/dL). The proportion of results flagging low by adult versus pediatric RI were compared by chi-squared test, with p<0.05 considered significant. **Results**: For Beckman, the percentages of pediatric patient cortisol results flagging low using the adult RI versus (vs.) the pediatric age-specific RI were: 35% vs. 7% for 1-12 years of age (p<0.001, n=1,866), 22% vs. 5% for 13-15 years (p<0.001, n=966), and 16% vs. 5% for 16-18 years (p<0.001, n=986). For Roche, the same analysis yielded: 52% vs. 9% for 0-1 years (p=0.001, n=23), 14% vs. 8% for 1-11

years (p=0.03, n=238), and 9% vs. 6% for 12-18 years (p=0.16, n=364). **Conclusion**: The implementation of pediatric RIs for serum cortisol will have a significant impact on the number of "low" result flags for patient results on both Beckman and Roche platforms. Statistically significant decreases in the percentage of low result flags were observed on both platforms for all age ranges except for the age bracket of 12-18 years by Roche. Furthermore, pediatric RIs appeared to produce more consistent rates of abnormal results across methods and age ranges, with all groups flagging low at a rate of 5-9%, whereas the same age ranges with adult RIs flagged at rates ranging from 9-52%. Lowering the RIs for pediatric cortisol to more appropriate ranges as identified by CALIPER will decrease the number of "low" flag results. This has the potential to decrease unnecessary follow-up, including repeat testing and further diagnostic procedures such as ACTH stimulation testing. Implementation of pediatric RIs has potential benefits regarding stress and cost for patients and parents, alarm fatigue for physicians, and utilization of laboratory resources.

A-336

Comparison between the immune profile of pregnant and postpartum women with and without COVID-19 by flow cytometry

M. L. Machado[1], A. T. de Souza[2], S. E. Silva[3], A. L. Fonseca[4], J. D. Camargo[5], A. J. Vale[4], E. P. Azevedo[6], G. H. Oliveira[7], A. D. Luchessi[8], G. B. Junior[9], V. N. Silbiger[8], R. N. Cobucci[1]. *[1]Post-Graduate Program in Sciences Applied to Women's Health, Maternidade Escola Januário Cicco (MEJC/EBSERH), Federal University of Rio Grande do Norte, Natal, Brazil, [2]Department of Pharmacy, Health Sciences Center, Federal University of Rio Grande do Norte, Natal, Brazil, [3]Biosciences Center, Federal University of Rio Grande do Norte, Natal, Brazil, [4]Department of Medicine, Health Sciences Center, Federal University of Rio Grande do Norte, Natal, Brazil, [5]Maternidade Escola Januário Cicco (MEJC/EBSERH), Federal University of Rio Grande do Norte, Natal, Brazil, [6]Biotechnology Graduate Program, Potiguar University, Natal, Brazil, [7]Flow Cytometry Laboratory, Hemocentro do Rio Grande do Norte Dalton Cunha (Hemonorte), Natal, Brazil, [8]Department of Clinical and Toxicological Analyses, Federal University of Rio Grande do Norte, Natal, Brazil, [9]Laboratory of Immunology, Department of Clinical and Toxicological Analysis, Center for Health Sciences, Universidade Federal do Rio Grande do Norte, Natal, Brazil*

Background: The impacts caused by COVID-19 on pregnant and postpartum women are not well characterized yet. It is well known that, due to pregnancy, the maternal organism undergoes immunological adaptations, which may increase the risk of complications of viral diseases. These adaptations, in addition to the inflammatory disorder caused by the SARS-Cov-2, may contribute to severe outcomes as a result of COVID-19 infection. In this study the peripheral levels of total T lymphocytes, CD4+ T lymphocytes, CD8+ T lymphocytes, B lymphocytes and natural killer cells in pregnant and postpartum women with positive and negative PCR for SARS-CoV-2 were determined. **Methods**: A cross-sectional study was performed using peripheral blood samples from pregnant and postpartum women who underwent PCR for the diagnosis of COVID-19 between May 2021 and March 2022. Samples were collected in a public maternity hospital in Northeast of Brazil right on admission for delivery as well as in the immediate puerperium. Blood was collected in tubes containing EDTA for flow cytometry, using the activated cell fluorescence analyzer (FACScan) and the Cell Quest software. The total of T lymphocyte subsets (CD3+), CD3+/CD4+ (ThL) and CD3+/CD8+ (TcL) was determined according to published protocols. B lymphocytes were identified by expression of CD19 (CD19+) and NK and NKT cells were identified by the CD16+/56+ and CD3+/CD16-56+ phenotype, respectively. Lymphocytes were identified by high CD45 expression and low side scatter using the following 3-color monoclonal antibody combinations: fluorescein isothiocyanate (FITC), phycoerythrin (PE) and chlorophyll pyridine protein (PerCP). **Results**: A total of 99 women with a mean age 29 (± 7) years old were used in this study, of which 31 (31.3%) were pregnant and 68 (69.4%) were postpartum women. The diagnosis of COVID-19 was confirmed by PCR in 78 (78.8%) of the participants and in 21 (21.2%) it was discarded after negative PCR. Pregnant and postpartum women infected with SARS-CoV-2 had higher levels of cytotoxic T cells, median (ME) = 574.2; interquartile range (IIQ) = 382.4, when compared with uninfected patients, ME = 404.7; IIQ = 363.5); p = 0.032. Pregnant women with COVID-19 had a lower mean CD3+ count (1,351.1 ± 337.7) compared to postpartum women with the disease (1,605.6 ± 538.6); t (53.6) = -2.45, p = 0.009. Likewise, lower CD16+/56+ (113.3; IIQ = 172.0) and CD3+/CD16-56+ (52.1; IQ = 53.4) medians were found in pregnant women with COVID-19, when compared with the group of postpartum women without infection (191.2; IIQ = 192.5); U = 396.0, p = 0.043 and (70.8; IIQ = 91.6); U = 376.5, p = 0.025, respectively. **Conclusion**: Pregnant and postpartum women with COVID-19 had significantly higher levels of cytotoxic T cells in peripheral blood. These results may be useful to understand what the SARS-CoV-2 infection causes in the immune system of pregnant and postpartum women. On the other hand, observational studies with larger samples and bias control are required.

A-337

Study of the relationship between the variant dup 4q26 and Autism Spectrum Disorder

B. B. Perez[1], M. V. dos Santos[1], H. R. Oliveira Jr[1], D. R. Ramadan[1], S. Tufik[1], M. C. Feres[2]. *[1]Associação Fundo de Incentivo a Pesquisa - Afip, Sao Paulo, Brazil, [2]Associação Fundo de Incentivo - Afip, Sao Paluo, Brazil*

Background: Autistic Spectrum Disorder (ASD) refers to a neuronal condition, caused by a disorder in neurodevelopment. With hypersensitivity, repetitive behaviors and other symptoms, this condition has both genetic and environmental causes. The etiology is still not fully known, but it is known that there are chromosomal abnormalities (2%), monogenic syndromes (5%), microduplications and microdeletions (10%), environmental (3%), multifactorial and epigenetic (80%). In 2004, many authors studying the first genome wide screening for chromosomal regions involved in classical autism reported over 354 genetic markers for this disorder. In this context, techniques emerged for identifying these genes for the elucidation and diagnosis of ASD, among others - the Array. Revolutionary high-resolution technique capable of investigating thousands of chromosomal regions, detecting losses and add in a single exam. With this method it is possible to detect CNVs and SNPs (variations in the number of copies and polymorphism of a nucleotide), uniparental disomy and loss of heterozygosity. Objective: With this technique, the authors of this study aimed to associate a dup 4q26 variant found with ASD and analyze its classification as a variant of uncertain meaning. Method: Genetic data from CGH-ARRAY tests performed from June/2018 to December/2022 were analyzed in a large laboratory in São Paulo- Brazil. 149 patients with variants of uncertain and/or pathogenic significance were selected. Of these, 38 cases were under investigation for ASD, characterized by the diagnostic hypothesis. The authors found 49 variants. Of these, 6.12% were detected on chromosome 1, 4.08% on chromosome 2, 4.08% on chromosome 3, 14.28% on chromosome 4, 4.08% on chromosome 5, 6.12% on chromosome 7, 2.04% on chromosome 8, 6.12% on chromosome 9, 6.12% on chromosome 10, 4.08% on chromosome 11, 2.04% on chromosome 13, 4.08% on chromosome 15, 6.12% on chromosome 16, 6.12% on chromosome 18, 2.04% on chromosome 20, 12.24% on X chromosome and 10.02% on Y chromosome. Results: The study showed that chromosome 4 had the highest number of detected variants. Therefore, we carefully analyzed all cases and 2 patients, who did not have consanguinity, had the 4q26 duplication, classified as VOUS (unclear meaning). This duplication is associated with genes:TRAM1L1, LINC01378, NDST3, SNHG8, SNORA24, PRSS12, CEP170P1, LOC729218, LOC101929741, METTL14, SEC24D, SYNPO2, MYOZ2, LOC101929762, USP53, C4orf3, FABP2, LINC01061, GTF2IP12, LOC645513, PDE5A, LINC01365, LOC100996694. Of these genes, only the PRSS12 gene correlated with autism. PRSS12 (Serine Protease 12) encodes a protein secreted by neuronal cells, located in the synaptic cleft and is associated with cognitive impairment. Conclusion: The two cases studied had global delay in development with a suggestion, after medical evaluation, of ASD. The findings in the genetic exam did not show other alterations that justify the cognitive delay and the hypothesis of autism only the duplication of the PRSS12 gene. Thus, it is possible that this variant is associated with the observed phenotype and that it could be studied and reclassified as a probably pathogenic variant.

A-338

Tsh reference range in the first trimester in a iodine sufficient population of pregnant women attended by the public health system in curitiba South of Brazil

R. F. Paula[1], P. C. Felchner[2], T. M. Telles[3], C. O. Júnior[4], K. D. Valdati[5], T. M. Panizza[5], T. P. Moraes[4], G. B. Carlos[1]. *[1]Siemens Healthineers, São Paulo, Brazil, [2]Pontifical Catholic University -PR, Curitiba, Brazil, [3]Federal University of Paraná -UFPR, Curitiba, Brazil, [4]Complexo Hospital das Clinicas Complex / Federal University of Paraná, Curitiba, Brazil, [5]Municipal Laboratory - SMS, Curitiba, Brazil*

Background: Thyroid diseases are common among women of reproductive age, and the serum concentration of thyroid-stimulating hormone (TSH) is the most used index to assess thyroid function during pregnancy. Changes in thyroid function during pregnancy can have a clinical impact on a woman's health and pregnancy outcome, as well as interfere with fetal health and child development. An adequate thyroid function is essential for normal intrauterine development. When comparing the levels of TSH between pregnant and non-pregnant women, TSH levels are lower in pregnan-

cy, mainly in the first trimester, with gradual increase during pregnancy. Although some guidelines propose fixed TSH reference values in pregnancy according to the gestational trimester, the main recommendation is to carry out a study of their local population due to the significant variation in TSH reference range levels in different populations and different assays. Therefore, it is necessary to determine values of a reference interval for TSH for the local population of pregnant women. This study aims to determine the normal reference range of TSH in the first trimester of gestation in the public health system pregnant women from Curitiba. **Methods:** This is an ongoing prospective cohort study, with the population sample from the pregnant women using the public health system, with age >18 years old in Curitiba, South of Brazil. 383 pregnant women were invited to participate. After evaluate the exclusion criteria (gestational age more than 13 weeks, twin pregnancy, thyroid disease, iodine ingestion, identification of the samples in the laboratory), 225 pregnant women had the TSH measured by chemiluminescent immunoassay on Atellica IM Analyzer (Siemens Healthineers), as well as the TPO-ab presence, FreeT4 and Total T4. The 2.5, 50 end 97,5 TSH percentiles were estimated for the normal pregnant population. Results: The mean gestational age was 8,6 weeks (standard deviation 2.33). From 225 pregnant women, 22 (9.8%) had TPO-ab positive. The 2.5 and 97.5 percentiles of TSH were 0.12 and 5.28 µIU/mL after using de exclusion criteria. The populational iodine status was sufficient. In the TPO-ab positive population was found a higher level of TSH. The proportion of TPO-ab positive was significantly higher in the population with TSH >2.5 µIU/mL. **Conclusions**: This preliminary data shows the TSH reference range with levels higher than other regions, reinforcing to do not use fixed values of TSH from other populations to evaluate the thyroid function in pregnancy. Comparing to the laboratory reference for non-pregnant adult (0.48 to 5.60 µIU/mL), the recommended reduction of 0.5 µIU/mL on upper limit and 0.4 µIU/mL on the lower limit found similar values to our findings.

A-339

Automated flow of a non-invasive fetal gender determination lab-developed molecular test

T. S. Xavier Dias, V. G. da Silva, M. C. Santos, V. P. Batistutti, P. S. Osorio, D. Moratori, J. E. Levi. *DASA, Barueri, Brazil*

Background: The discovery of cell-free fetal DNA in maternal circulation during pregnancy fostered the introduction of non-invasive tests. Fetal gender determination may be determined at early pregnancy by real-time PCR, identifying the presence or absence of Y chromosome markers in maternal plasma. Dasa has been offering this test for more than 10 years. In 2022, the assay was adapted to an automated flow.

Methods: The Flow Solution platform, powered by Roche®, was adapted to the in-house test, allowing the processing of 94 samples. Whole blood is obtained from pregnant women from the 8th week of pregnancy onwards. Each maternal plasma sample is extracted once and the DNA eluate submitted to real-time amplification targeting the DYS14 gene from the Y chromosome, in triplicate. The human RNAseP gene is co-amplified as an internal control. Full process takes 5 hours, with the possibility of overlapping a new routine every 2 hours, with a daily capacity of 12 routines (1,128 samples/day).

Results: In 2022, 129,849 fetal gender determination results were released. Of these, 49% of the results indicated male and 51% female fetuses.A few samples displayed indeterminate results, showing amplification curves but with cycle threshold values that are above the cutoff. Other samples do not reach internal quality metrics and the analysis is repeated from the plasma or a new collection is requested. Results that, after the second collection, remain indeterminate, are forwarded with the patient's clinical history to the medical board. In contact with the patient, possible causes for inconclusive results are explained. In 2022, for every 10,000 tests there were 8 were medical contacts.The automated methodology developed and implemented, allowed a high processing volume, with 129,849 tests released in 2022, an increase of 33% compared to 2021 when the method had manual steps. The Total Service Time (TST) from collection to results release in 2022 was approximately 5 days, a 20% reduction compared to 2021, and the repetition rate of 2.43% in 2022 was 0.9% lower than 2021. Recollections also diminished from 1% in 2021 falling to 0.8% in 2022.

Conclusion: A new automated flow was successfully implemented in 2022 improving the productivity and quality of the service, achieving a high accuracy and leading to a significant reduction in errors and repetitions while reducing the turnaround time and recollections.

A-340

Cftr modulator therapy increases serum/plasma concentrations of vitamin a in people with cystic fibrosis

H. A. Nelson[1], I. DeBiase[1], K. Balogun[2], C. M. McDonald[3], F. Asfour[4], T. Yuzyuk[1]. *[1]University of Utah / ARUP Laboratories, Salt Lake City, UT, [2]Montefiore Medical Center, Bronx, NY, [3]Primary Children's Hospital, Salt Lake City, UT, [4]University of Utah, Salt Lake City, UT*

Introduction: Pancreatic insufficiency (PI) is common in people with Cystic Fibrosis (PwCF), contributing to malabsorption of fat and fat-soluble vitamins. Thus, PI PwCF are prescribed pancreatic enzyme replacement therapy (PERT) and supplementation with fat-soluble vitamins. The circulating vitamin levels are monitored to guide dietary therapy. Currently, there are multiple cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapies available that have shown significant improvements in pulmonary function, anthropometric parameters, and life expectancy in PwCF, but the effects on serum/plasma fat-soluble vitamin levels remain unknown. The goal of this study was to determine how modulator therapy affects serum/plasma vitamin A and E levels in individuals with CF. **Methods:** The analysis included 1,139 serum/plasma samples from 157 children and adolescents with CF: 74 males, 83 females, between the ages of 2 - 17 years. Most individuals were homozygous or compound heterozygous for the F508del variant (n=81 and n=72, respectively). 141 individuals (90%) were diagnosed with PI (fecal elastase <200 µg/g stool). 76 individuals (48%) received different CFTR modulator therapies for at least 1 month. Vitamin A (retinol and retinyl palmitate) and Vitamin E (α-tocopherol and γ-tocopherol) were measured using a high-performance liquid chromatography assay. Data analyses were performed in Prism software (La Jolla, CA), and statistical significance was defined as $p < 0.05$. The study was approved by the IRB. **Results:** Overall, vitamin deficiency was rarely observed in PwCF prescribed PERT and vitamin supplements; <3% of them had low concentrations of Vitamin A and/or E. The proportion of individuals with Vitamin A and/or E above the reference range was larger; 10.9% and 30.8% for Vitamin A and E, respectively, most likely reflecting supplementation. There was no correlation of plasma vitamin A or E results with sex, weight-for-age, height-for-age, or BMI-for-age. However, pancreatic status affected vitamin levels in individuals not taking modulators: samples from PI PwCF (n=367) had significantly lower Vitamin A and E than those from PS PwCF (n=71). In contrast, we have observed higher concentrations of Vitamin A retinol in individuals on modulators: 0.49±0.13 mg/L on modulators *vs* 0.43±0.12 mg/L without modulators. Pairwise analysis of samples from 76 individuals before and after modulator therapy confirmed this finding, showing an average 16% increase in serum/plasma retinol concentration ($p<0.0001$). No changes in retinol were observed between different measurements in PwCF who were not taking modulators (n=82). Vitamin E levels were not impacted by modulator therapy. **Conclusions:** Advances in clinical care improved fat-soluble vitamin status in people with CF. However, even when supplemented and on PERT, PI PwCF not on modulators tend to have lower fat-soluble vitamin concentrations than PS PwCF. CFTR modulator therapy increases serum/plasma concentrations of vitamin A. Thus, individuals receiving modulators and vitamin supplementation should be monitored to avoid Vitamin A toxicity. This is especially true for PS PwCF who already have higher baseline vitamin levels.

A-341

Development and validation of an ICP-MS method to quantify lead in dried blood spots

R. A. Muldrow, C. Torres, K. Kaiwusaier, S. Martir, D. N. Greene. *LetsGetChecked, Monrovia, CA*

Introduction: The CDC estimates that ~500,000 children in the US between ages 1-5 have unsafe blood lead concentrations (> 3.5 ug/dL). Lead toxicity can affect all bodily systems and can be detrimental to children's developing brains and nervous systems. Since lead exposure does not always induce obvious and immediate symptoms, screening is an essential tool to public health. Recently, there was a point of care device recalled that was used to measure blood lead across the country. The demise of this method left a gap for lead screening nationally. Dried blood spot (DBS) sampling is an excellent alternative to traditional collections as it is cost effective, less contamination prone, less invasive, and is amenable to non-standard collection locations. The objective of this study was to develop an accurate and precise DBS lead method with the intention of improving access to screening in underserved populations.

Methods: Lead was detected using an Agilent7800 ICP-MS. DBS were punched (6mm) and lead was extracted into a water based diluent containing EDTA, ammonium pyrrolidine dithiocarbamate, tetramethylammonium hydroxide, Triton X-100, ethanol and iridium. All DBS were sampled from Whatman903 protein saver cards using

a 6mm punch. A 6-point calibration curve was made using custom UTAK whole blood lead controls and a diluent blank (target concentrations: 0, 1, 7, 14, 60 and 100 ug/dL). Exact concentrations of the liquid calibrators were determined using a previously validated lead whole blood method. Once the whole blood calibration concentrations were determined, 40 uL of each calibrator was spotted and allowed to dry for at least 24 hours before a 6mm spot was punched and extracted. Precision was determined using three concentrations of UTAK Whole Blood Lead QC (40 uL/spot; 6mm punch). Each level was run in replicates of five for five days. Linearity was confirmed by running five levels of DBS calibration standards for five days. Accuracy was evaluated using Wisconsin DBS Event Proficiency Testing (PT) Samples and results were compared to the peer means. For the method comparison, venous whole blood samples were collected into Royal Blue EDTA metal free vacutainer tubes; DBS samples were spotted directly from the finger (n=20). Additional comparison samples (n=10) were derived from the Pennsylvania whole blood PT program by spotting 40 uL.

Results: The interday precision for the low, mid, and high QC material was 5.0%, 7.5%, and 3.0% at concentrations of 6.8, 20.7, and 35.8 ug/dL, respectively. Lead concentrations from 1.0-100 ug/dL were within the allowable nonlinearity of 1 ug/dL or 5.0% (y=1.01x-1.6;R=0.999). Wisconsin DBS PT results (n=12; range 0.0-38 ug/dL) were accurate within 10% of the peer mean and within acceptability criteria. Comparison of paired whole blood to DBS samples had an average bias of 4.68% (0.31 ug/dL; Y = 1.027x + 0.13(R = 0.999); range 0.1-77.7ug/dL).

Conclusions: We developed and validated an accurate and precise lead DBS method using matrix-matched calibrators. This method supports that DBS collection may be an adequate sample to screen for lead exposure and could supplement the current need for additional ways to screen underserved and/or remote populations.

A-343

Provider Performed Microscopy Program Makeover: One Institution's Experience

A. M. Glomb, S. Guzman, N. R. Wadhwani, B. Suh-Lailam. *Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL*

Background: Provider Performed Microscopy (PPM) is a subset of moderate complexity tests performed by physicians and midlevel practitioners. Regulatory bodies require PPM to meet specific accreditation standards. Many institutions face challenges with engaging providers to complete required training and ongoing assessments. Not immune to these challenges, we set out to improve PPM compliance. We reviewed guidelines to identify potential gaps in our PPM program. The existing PPM program practitioners were not aware of specific regulations and current learning systems were not robust enough to meet the regulations. The Point of Care Testing team (POCT) set out to establish a root cause for non-compliance, identify and collaborate with practitioners, and develop a sustainable training and competency system. Quality improvement must establish sustainable solutions to ensure compliance. **Method:** POCT focused efforts on improving practitioner training, competency, and collaboration with practitioners. Over a period of 2 months, we identified and engaged key stakeholders in each department to review historical data from training, competency completion, and result audits revealing gaps. We clearly communicated the standards and how the current program was not meeting standards such as a lack of formalized training and competency documentation, observation of result documentation, and proper reporting of results. By identifying gaps in training, we utilized stakeholders to develop simple processes to enable compliance such as creation of a tip sheet to guide the resulting process, development of a specific training checklist requiring both provider and POC technical consultant (TC) signatures. In collaboration, a system was created to identify practitioner availability for direct observation of the specific elements of assessment. We leveraged our clinical consultant (CC) to assist in the conversations on a peer level with providers to bolster the importance of compliance. Every practitioner was re-trained by our TC along with the CC. With sustainability in mind, POCT collaborated with administrative assistants and department administrators to be notified of new practitioners. Ongoing monitoring involves POCT completing periodic check-ins with the departments as a second layer of assurance. POCT maintains the records of training and competency expiry dates and assigns critical thinking assessments. **Results:** After 2 months, significant improvements were observed. Completed trainings improved from 50% to 100%, competency from 40% to 85%, and proper result documentation by practitioners from 25% to 70%. A 6-month review showed training was maintained at 100%, competency maintained at 85%, and result documentation improved to 90%. Sustainable improvements were observed at 1 year where training and result documentation achieved 100% compliance and competency completion reached 95%. **Conclusion:** Sustainable quality improvement with PPM compliance was achieved by collaborating with practitioners, understanding obstacles to meeting standards, and developing simple solutions specific to practitioner workflows. In doing so, we were able to develop a robust yet succinct system for initiating and maintaining compliance with PPM regulatory standards.

A-344

Don't Get Rejected: Reduce Specimen rejection in Phlebotomy

A. M. Glomb, B. Suh-Lailam. *Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL*

Background: Pre-analytical specimen rejection plagues laboratories, patient care decisions, and satisfaction. Rejection data used for quality improvement varies and often excludes pediatric-only populations. With an outdated benchmark of 2.2% total rejections, our phlebotomy department was maintaining a baseline rejection rate of 1.39%. We assessed only tests where a result was not able to be given for reasons of: Quantity Not Sufficient (QNS), Clotted, Hemolyzed, improperly collected or handled, and unlabeled or mislabeled. While our department exceeded current benchmarks, continued improvements were desired to provide the best care and experience. We collaborated with the Vascular access team (VAT) at our institution to improve our processes. **Methods**: Specimen rejection was defined as any sample(s) collected for analysis not performed due to Quantity Not Sufficient (QNS), Clot, Hemolysis, improper collection/handling, and unlabeled/mislabeled specimens. Over the next 3 months, samples obtained by outpatient and inpatient phlebotomist draws were reviewed for pre-analytical errors. VAT and phlebotomy began meeting monthly and developed a 1-day shadow experience for new phlebotomy hires and a mechanism to identify DVA patients. In collaboration with Information Management, coping comfort plans (CCP) were implemented in patient medical record which documented patient preferences and staff observations for potentially painful procedures. The CCP paved updates tip sheets, purchases of transilluminators, and stock heat packs in phlebotomy stations. Additional aids were purchased for patient comfort in outpatient areas. A communication center installed and allowed for an area to write down room numbers of DVAs and to display current rejection rates. Improved staff communication regarding rejected samples was publicly posted on the communication center by phlebotomist name. These characterized type and number of rejections as well as a stoplight color coding to depict acceptable, at risk, and unacceptable errors by phlebotomists monthly. Goals were posted monthly in the communication center. 18 months was allotted for implementation of the final goal of ≤0.5% total rejections, allowing for quarterly reports, changes, and PDSA cycles. **Results**: Initial staff surveys discovered 71% of staff were unaware of their rejected samples, 57% of staff were unaware this was a monitored metric, 100% of staff agreed importance. During quarter one of data reporting, it was assumed that all rejections would fall ≤1.25% monthly in Q2-Q3, ≤1.00% in Q4, and by Q2 of the following fiscal year to ≤0.50%. The average rejection rates were: Q1: 1.05%, Q2: 1.0%, Q3: 0.69%, Q4: 0.70%. In year 2 rejection rates were: Q1: 0.5%, Q2: 0.3%. Post-implementation survey conducted resulting in 100% of staff were aware of their rejected samples, aware of the metric, and agreed it was important. Staff additionally suggested improvements and have noted to have higher morale and trust within their group by partnering in this project to improve inpatient workflows. Average rejection rates were reviewed at 1- and 2-year post implementation: 0.05% and 0.42% respectively. **Conclusion**: Rejection not only causes waste but a risk to patients as results and treatment can be delayed. By changing configurations in workflow, tools, communication and medical records, phlebotomy staff can be the key drivers of change<!--EndFragment-->

Point-of-Care Testing

A-347

Performance Evaluation of Standard Q COVID/FLU Ag Combo for Detection of SARS-CoV-2 and Influenza A and B Antigens from the Patients with Suspected Respiratory Infections

S. Kim[1], W. Choi[2]. *[1]Gyeongsang National University College of Medicine, Changwon, Korea, Republic of, [2]Department of Nursing Science, Kyungsung University, Busan, Korea, Republic of*

Background: COVID-19 and influenza are highly contagious respiratory diseases and are sometimes fatal, making them a major concern worldwide. Hence, rapid and accurate detection and differentiation are required as preventive measures to control the further spread of these diseases.

Methods: We evaluated performance of the Standard Q COVID/FLU Ag Combo test (Q Ag combo test) (SD Biosensor, Korea) for detection of SARS-CoV-2, influenza A,

or influenza B using a single point-of-care device compared with real-time PCR. A total of 408 individuals: 55 positives of SARS-CoV-2, 90 influenza A, 68 influenza B, and 195 negatives from all viruses investigated.

Results: The Q Ag combo test demonstrated a high sensitivity of up to 92.73% and specificity of up to 99.49% for the detection of SARS-CoV-2. Similarly, a sensitivity of 92.22% and specificity of 100% for influenza A, and a sensitivity of 91.18%, and a specificity of 99.49% for influenza B were observed. When the days from symptom onset (DSO) were restricted to 0<DSO≤6, the sensitivity of the Q Ag combo test to detect SARS-CoV-2 was up to 100%, and when the Ct value of *RdRp* is ≤ 20, the sensitivity to detect SARS-CoV-2 was up to 93.10%.

Conclusion: The Q Ag combo might be useful to detect and differentiate SARS-CoV-2, and influenza A/B efficiently and rapidly.

A-349

Septec: a microfluidic platform for the rapid diagnosis of bloodstream infection diagnosis and associated sepsis management

H. McArdle[1], A. Cassedy[1], K. Adamson[1], D. Burke[2], A. Farghaly Mohammed[2], N. McEvoy[3], M. Smyth[2], G. Curley[2]. *[1]Novus Diagnostics, Dublin, Ireland, [2]Beaumont Hospital, Dublin, Ireland, [3]RCSI, Dublin, Ireland*

Background: Sepsis is a life-threatening disorder resulting from a dysregulated host response to infection. Globally, sepsis is responsible for 11 million deaths annually and is associated with high mortality and morbidity. Sepsis can arise from any infection by microbes or viruses; however, the most common cause is bloodstream infection (BSI) via bacterial pathogens. Rapid identification of organisms responsible for BSI may reduce time until the administration of suitable antibiotics and employment of rapid diagnostics at patient bedside further strengthens their value as informed clinical decisions can be made and acted upon within shorter timeframes, potentially enhancing patient outcomes and preventing progression to more critical sepsis or septic shock. This study reports on the analytical and clinical performance of the SepTec microfluidic assay, an *in-vitro* diagnostic device capable of rapid detection of bloodborne pathogens of gram-positive, gram-negative and fungal origin from whole blood within 15 minutes.

Methods: Analytical performance of the SepTec assay was studied as follows: limit of detection (LoD), reproducibility and impact of interfering substances. Sterile and infected blood samples were obtained either through the generation of contrived samples or through clinical collaboration. Clinical performance was determined retrospectively and prospectively on a total of 146 patients. For both the retrospective and prospective clinical studies, results were compared against blood culture.

Results: The LoD of the SepTec assay for S. Aureus, E. coli and C. albicans from contrived blood samples was 10 CFU/mL for each pathogen. CVs were calculated at 4% for organism detection in whole blood samples. From retrospective and prospective analysis, the SepTec assay showed 100% sensitivity and >96% specificity.

Conclusion: The SepTec assay can detect microbes from blood and differentiate between gram-positive, gram-negative or fungal pathogens with high sensitivity and specificity. Turnaround time for the test is estimated at 15minutes, rendering it with the potential to be a significantly more rapid approach than the gold-standard blood culture, or many molecular approaches. It is hoped that this work with yield a point-of-care diagnostic platform which will enhance sepsis management, overall improving patient outcomes.

A-350

Myth Debunked: Same UrineAliquot is not Suitable for hCG Test AfterMicroscopic Analysis

X. Li. *Emory University, Atlanta, GA*

Background:

It has been imparted in the laboratories that the suspending fluid, Lamina, from Iris IQ 200 Analyzer system is prone to interfere with urine human chorionic gonadotropin (hCG) test, especially on a small volume urine specimen. The myth is that the sample is diluted or contaminated by Lamina, which gives falsely negative result for hCG test. An add-on urine pregnancy test was ordered for a specimen, which was completed urinalysis and microscopic analysis. This is the only urine aliquot available, and it is approximately 2mL. This order raised many concerns among laboratory technicians regarding the accuracy of the result. However, no documents or articles were found to support the suspicions. In this study, A serial of experiments was completed to address this concern.

Methods:

Known pregnant patients were confirmed their pregnancies with serum hCG tests prior to collecting the urine specimens. The experiment was conducted with 4 positive patient urines. A series of samples ran on DXI 800 system and the point-of-care (POC) Alere hCG combo cassettes. Then the same specimen performed urinalysis and microscopic analysis on Iris IQ 200 Analyzer. To evaluate the Lamina impact, the sample concentrations were verified with point-of-care (POC) Alere hCG combo cassettes and later DXI 800 system. Meantime, the significance of sample size was also evaluated in the study.

Results:

The final concentrations of the samples were at <5, 5, 10, 15, 20, 25, 30, 40, 50, and 60 mIU/mL. The cutoff for Alere hCG combo cassettes was verified at 20 mIU/mL for urine, the assay gives the accurate result at and above that value. The results are consistent between immunoassay and POCT prior to and after microscopic analysis. Therefore, no significant interferences were identified with the desired sample volume, 3mL, or short sample volume, 2mL. The volume usage by iQ 200 Analyzer was less than 500 uL.

Conclusion:

In this study, Lamina doesn't cause any interference with hCG values using Alere hCG combo cassettes despite the short sample volume. However, variations between POC urinary hCG devices may contribute to different outcomes. Therefore, a separate validation is highly recommended if a different POC urinary hCG device is involved.

A-351

Reliability of POCT AccuChek Inform II® Performance Assessed with The Laboratory Method. A practical example for a System with Fourteen Facilities Monitored for Nine Years.

V. M. Genta[1], R. Mao[1], C. Aston[1], F. Alferes[1], S. Shumate[2], L. Wyer[2]. *[1]Sentara Virginia Beach General Hospital, Virginia Beach, VA, [2]Sentara Healthcare, Norfolk, VA*

Background: Reliability is acceptable performance over time and by location. The reliability of AccuChek Inform II® glucose meters was assessed in fourteen Sentara Healthcare (SH) facilities for nine years by comparison with the laboratory method. **Methods**: Nine hundred and twenty-one patient specimens collected in parallel by fingerstick and by phlebotomy in lithium heparin tubes (Becton Dickinson) were assayed with the AccuChek Inform II glucose meter (Roche Diagnostics) and cobas c-501® (Roche Diagnostics). The data were stored in RALS® (Abbott Diagnostics) and electronically transferred to Minitab® (Version 19, Minitab Inc.) statistical software. The data, in the interval 30-600 mg/dL, were analyzed with orthogonal, ordinary least squares (OLS), weighted least squares polynomial (WLSP) and locally weighted scatter plot smoother (lowess) regression models. The aptness of the OLS model was evaluated by comparison with the orthogonal model and that of the WLSP model was evaluated with the standardized deleted residuals (Std.Del.Res) diagnostics. The equality of the regression lines, as obtained for each year and for each location, was assessed with the F statistical test. The linearity of the relationship of the two methods was assessed with the pure error test. The absolute and relative differences between the paired values as obtained with the two methods were evaluated using CLIA's acceptance limits (target value ± 6 mg/dl or 10%, greater). **Results**: The regression lines obtained with the OLS model (Inform II = 3+0.97 c-501) and the orthogonal model (Inform II = 2+0.97 c-501) were very similar and the differences were not clinically significant. The standardized residuals for both models showed an increase in variance for increasing levels of the values as obtained with the c-501 method. Consequently, a WLSP model was employed. The analysis of the Std.Del.Res. for the WLSP model showed a quasi-normal distribution with twelve possible outliers (Std.Del.Res.>|3|) and no influential observations (Leverage < 0.5). The test for equality of regression lines did not show probabilistically significant differences for either year (P=0.93) or facility (P=0.46). The pure error test showed no probabilistically significant lack-of-fit (P=0.07). The lowess of the Std.Del.Res. plotted by the fitted value showed a quasi-linear relationship between 30 and 500 mg/dL. For values between 30 and 100 mg/dL the majority of the differences between methods was within ± 6 mg/dL. Similarly, for values between 101 and 600 mg/dL the majority of the relative differences were within ±10%. **Comments**: The reliability of the POCT method has been demonstrated. The POCT and the laboratory methods demonstrated a linear, similar and within the CLIA's acceptance limits of total error performance in fourteen SH facilities for nine years. The application of regression models to a large data base was possible due to the availability of Minitab statistical software. Additionally, these results demonstrated the organizational reliability of the SH POCT protocols for QC, QA, acceptance of new reagents and instruments, designed by the POCT leadership and performed by the POCT personnel and nurses. This reliable performance could not have been possible without the POCT team dedication to quality.

A-352

Investigation of the Immunochromatographic Method using 150 nm Gold Colloids (1)-Comparison of different types of gold colloids

A. Hirano, M. Asahi, M. Otsuka, R. Kubota. *Graduate Course of Health and Social Services, Saitama Prefectural University, Koshigaya, Japan*

Background:

Gold colloids, which have been extensively used in immunochromatography, are typically 40 nm in particle size. Recently, 150 nm gold colloidal nanoparticles, which are prepared by gold coating a silica nucleus up to a diameter of 150 nm, have attracted considerable attention. These nanoparticles enable high sensitivity in immunochromatography. The purpose of this study is to compare the antibodies labeled with 40 and 150 nm gold colloids using the physisorption and covalent bonding methods, which are applied to the immunochromatography method developed in our laboratory.

Methods:

1) For the physisorption method, 40 nm gold colloids (gold colloid AuH2, Morinaga Institute of Biochemistry) and 150 nm gold nanoshells (nanoComposix) were used to physically adsorb the antibody. The covalent gold conjugation kit (nanoComposix) was used to prepare 40 nm gold colloid-labeled antibodies by covalent bonding, and the high sensitivity gold conjugation kit was used to perform covalent bonding of the antibody and gold colloid after buffer displacement of the antibody.2) Anti-human albumin monoclonal antibody (abcam) was applied as the solid-phase antibody onto a nitrocellulose membrane (HF075, Merck Millipore) and dried. A human serum albumin sample (Fujifilm Wako Pure Chemicals) was diluted to a concentration of 1 mg/mL using phosphate-buffered saline (PBS). The sample was mixed with the gold colloid-labeled antibodies and a blocking solution (EZBlock Chemi, ATTO). Subsequently, 90 µL of the mixture was added dropwise and allowed to expand for 15 min before analysis.

Results:

1) Comparison of detection linesA red line was detected in the judgment zone for the 40 nm gold colloid, whereas a black line was detected in the judgment zone for the 150 nm gold colloid. The background of the detection area for the physisorption method was higher than that for the covalent bonding method.

2) Comparison of detection sensitivitiesUsing the physisorption method, the 40 nm gold colloid detected up to 10 ng/mL. In contrast, using the covalent bonding method, the 40 nm gold colloid detected up to 100 ng/mL. Using the physisorption method, the 150 nm gold colloid detected up to 3 ng/mL when tested at 100, 10, and 3 ng/mL. In contrast, using the covalent bonding method, the 150 nm gold colloid detected up to 3 ng/mL.

Conclusion:

Currently, 40 nm gold colloids are extensively used as a labeling material in immunochromatography. However, the use of 150 nm gold colloids, which have better detection sensitivity, may increase sensitivity and lead to more accurate detection.

A-353

Investigation of the Immunochromatographic Method using 150 nm Gold Colloids (2)-Comparison with the ALP-based chromogenic detection method

M. Asahi, A. Hirano, M. Otsuka, R. Kubota. *Graduate Course of Health and Social Services, Saitama Prefectural University, Koshigaya, Japan*

Background:

Currently, standard test kits based on the immunochromatography principle use 40 nm gold colloids as the labeling material. However, some kits use enzymes such as horseradish peroxidase and alkaline phosphatase (ALP) to improve sensitivity. Among the various labeling substances, 150 nm gold colloidal nanoparticles are thought to enable higher sensitivity in immunochromatography than the commonly used 40 nm gold colloids. This study aims to compare a detection method using 150 nm gold colloidal nanoparticles as a labeling material with a colorimetric detection method using ALP-labeled antibodies, which was previously studied in our laboratory.

Methods:

1) Detection method using 150 nm gold colloidal nanoparticlesThe BioReady high sensitivity gold conjugation kit for lateral flow (nanoComposix) was used to prepare gold colloid-labeled antibodies. Solid-phase antibodies, such as anti-human albumin monoclonal antibody (abcam) or anti-human troponin I monoclonal antibody (Merck Millipore), were applied onto a nitrocellulose membrane (HF075, Merck Millipore) and then transferred onto a test plate. The sample (human serum albumin or troponin I standard) was diluted with phosphate-buffered saline (PBS), and the gold colloid-labeled antibody and blocking solution (EzBlock Chemi, ATTO) were added and mixed. The mixture was added dropwise onto the test plate and incubated for 15 min. Subsequently, the results were recorded.

2) Chromogenic detection using ALP-conjugated antibodiesThis method was performed according to a method previously investigated in our laboratory. An anti-human albumin polyclonal antibody (DAKO) was applied onto a nitrocellulose membrane and then transferred onto a test plate. The membrane was mixed with the PBS-diluted sample, ALP-labeled anti-human albumin monoclonal antibody, and blocking solution. The mixture was added dropwise onto the test plate and incubated for 5 min. Subsequently, a drop of the chromogenic reagent was added directly onto the test plate, and the results were recorded after 10 min.

Results:

Based on the immunochromatography results using 150 nm gold colloidal nanoparticles, black lines were detected in the judgment zone at albumin concentrations of 100, 10, and 3 ng/mL. Moreover, albumin concentrations of up to 3 ng/mL were detectable. Subsequently, the colorimetric detection results using ALP-labeled antibodies indicated that albumin concentrations of up to 100 ng/mL were detectable, with the exception of the 10 ng/mL concentration. Similarly, when troponin I was analyzed using 150 nm gold colloidal nanoparticles, black lines were detected in the judgment zone at troponin I concentrations of 30, 10, and 5 pg/mL.

Conclusion:

In this study, the application of troponin I in point of care testing (POCT) was evaluated, indicating that troponin I was detectable at concentrations of up to 5 pg/mL. Because the detection sensitivity of commercially available immunochromatographic kits for troponin I is approximately 30 pg/mL, the developed method is six times more sensitive. Moreover, the developed method was demonstrated to be more sensitive than the chromogenic method using ALP-labeled antibodies.

A-355

Eyescore, a point of care smartphone app for early dry eye diagnosis and management

S. Zhang, J. Echegoyen. *Westview Eye Institute, San Diego, CA*

Current diagnosis of dry eye disease (DED) has significant challenges with limited accuracy and poor correlation of clinical symptoms. The goal of this study is to develop a smartphone App, namely “EyeScore”, to provide a point of care (POC) and digital solution for early diagnosis of DED. The authors tested the hypothesis of measuring eye blink rate and partial blink count as early clinical biomarkers for the calculation of so called “eye healthiness score”, which allows a convenient, rapid eye exam at home with low resource settings. EyeScore uses an iPhone as an imaging and sensing device for in-App recordings of eyelid movements. The use of facial landmark recognition and eye aspect ratio (EAR) enabled comprehensive digital analysis of video frames for determination of the eye opening/closed states. The smartphone videos from 10 DED patients and 10 healthy controls were tested to optimize EAR derived thresholds for accurate measurements of blink rates and partial blink counts. The authors formulated a clinically relevant algorithm for the calculation of “eye healthiness score”. This 10-point scale score can be easily measured anytime with a non-invasive manner and remotely shared with the patient’s eye doctor. As a result, both patients and doctors can monitor the eye conditions over time. Our results showed that EyeScore confirmed the diagnosis of all 10 DED patients. Importantly, Eyescore also identified three individuals with “pre-DED” conditions from 10 healthy controls, demonstrating its potential clinical values for early diagnosis and management of DED.

Materials and Methods

An Apple MacBook Pro installed with iOS software (iOS 16.1.2) and Xcode 14.0 was used for coding and debugging. Visionkit SDK from Apple Developer was adopted to extract coordinate data from six eye facial landmarks. EAR values were calculated based on the previously published formula, with a dynamic EAR developed as a threshold for the measurement of blink rate and half blink count. For eye healthiness score, a 10-point formula was formulated (0-3, normal; 4-5, Pre-DED; 6-10, DED) with several contributing factors including blink rate, half blink count, eye discomfort and demographic characteristics.

Results

A total of 20 iPhone videos from DED patients and healthy controls were analyzed and evaluated with EyeScore App. All DED patients obtained scores above 7, confirming their DED diagnosis. On the other hand, three controls obtained scores of 4 or 5, suggesting they were in the Pre-DED state with no noticeable clinical DED symptoms. All other controls obtain scores blow 3 with normal eye conditions. All tests were performed with the consents from the test participants.

Conclusions

Smart phone-based platform offers great potentials for point of care diagnostic methods. A POC enabled EyeScore App has been successfully developed for early diagnosis of DED. In the future, the authors plan to test EyeScore with a large-scale sample set of DED patients and controls to further validate the accuracy and predictability of eye healthiness score for its broader clinical applications.

A-357

Performance of Blood Urea Nitrogen on the epoc Analyzer

M. Dilks[1], L. M. Evans[1], B. M. Goldsmith[2]. *[1]Thomas Jefferson University Hospital, Philadelphia, PA, [2]Thomas Jefferson University, Philadelphia, PA*

Background:

The epoc Blood Analysis System (Siemens, Tarrytown, NY) performs blood gases, electrolytes, and metabolites using a Blood Gas Electrolyte and Metabolite (BGEM) Test Card panel on 92 uL of whole blood. The BGEM Test Card uses potentiometric sensors to measure sodium, potassium, chloride, ionized calcium, pH, pCO2; amperometric sensors to measure pO2, glucose, creatinine, and lactate; and a conductometric sensor to measure hematocrit. Results are available in 3-10 minutes, depending upon the time of sample injection. TJUH implemented the epoc in its ICUs in 2012 to provide rapid, whole blood Point of Care (POC) results, then expanded to other locations (Emergency Department, Outpatient Radiology, Cardiothoracic Surgery). Siemens recently added a potentiometric sensor for measurement of Blood Urea Nitrogen (BUN) to its BGEM card. We evaluated this card for possible future use.

Methods:

Precision and method comparison studies were performed at our Center City location. Inter- and intra-precision studies were performed on two levels of controls (Eurotrol, Elizabethtown, KY). Remnant whole blood was collected from 30 patients. Comparison studies were performed on the Piccolo Xpress (Abbott, Princeton, NJ), which provides a whole blood BUN measurement using a coupled-enzyme reaction.

Results:

Intra-assay precision for epoc BUN (n=20, 2 devices) was 2.8%CV and 6.5%CV for Levels 1 and 3, respectively. Inter-assay precision for epoc BUN (n=20, 2 devices over 10 days) was 2.4%CV and 7.1%CV for Levels 1 and 3, respectively. Method comparison for BUN (n=30) was: mean=42, median=29, r^2=0.9889, y=0.8840x-3.6416.

Conclusion:

The epoc BUN studies showed comparable results when compared to the Piccolo Xpress. Previous studies showed good correlation between whole blood on the Piccolo Xpress and plasma on the Roche Cobas c702 which is also a coupled-enzyme reaction; therefore the test is considered acceptable for implementation at our Center City sites where epoc is currently in use.

A-359

Performance of Measured TCO2 on the epoc Analyzer

L. M. Evans[1], M. Dilks[1], B. M. Goldsmith[2]. *[1]Thomas Jefferson University Hospital, Philadelphia, PA, [2]Thomas Jefferson University, Philadelphia, PA*

Background:

The epoc Blood Analysis System (Siemens, Tarrytown, NY) performs blood gases, electrolytes, and metabolites using a Blood Gas, Electrolyte and Metabolite (BGEM) Test Card on 92uL of whole blood. The BGEM Test Card uses potentiometric sensors to measure sodium, potassium, chloride, ionized calcium, pH, pCO2; amperometric sensors to measure pO2, glucose, creatinine, and lactate; and a conductometric sensor to measure hematocrit. Results are available in 3-10 minutes, depending upon the time of sample injection. TJUH implemented epoc in its ICUs in 2012 to provide rapid, whole blood Point of Care (POC) results and has expanded to other locations (Emergency Department, Outpatient Radiology, Cardiothoracic Surgery). While previous epoc cards provided calculated TCO2 (cTCO2), Siemens recently added measured TCO2 (mTCO2; combination of potentiometric sensors and calculation) to its BGEM card. We evaluated this card for future use.

Methods: Precision and method comparison studies were performed at our Center City location. Inter- and intra-precision studies were performed on two levels of controls (Eurotrol, Elizabethtown, KY). Whole blood was collected in heparinized syringes from 20 patients. Comparison studies were performed on the ABL800 (Radiometer, Brea, CA). We simultaneously compared epoc mTCO2 against epoc cTCO2 and against the cTCO2 on the ABL800.

Results:

Intra-assay precision for epoc mTCO2 (n=20, 2 devices) was 0.8%CV and 2.3%CV for Levels 1 and 3, respectively. Inter-assay precision for epoc mTCO2 (n=20, 2 devices over 10 days) was 1.1%CV and 3.3%CV for Levels 1 and 3, respectively. Method comparison for epoc mTCO2 (n=20) was: mean=22.9, median=22.1, r^2= 0.9174, y=0.9751x+1.2925. In comparison, method comparison for epoc cTCO2 (n=20) was: mean=24.7, median=23.9, r^2= 0.9165 y=0.9855x+2.8559.

Conclusion:

The epoc measured TCO2 studies show better correlation to the Radiometer ABL800 than the epoc calculated cTCO2, and it is considered acceptable for implementation at our Center City sites where epoc is currently in use.

A-360

Reproducible Graphene Sensor with Desiccated Biology

S. Aithal, S. Gupta, A. Kumar, R. Kumawat, K. Duong, N. Ho, R. Liu, J. Warden, S. Le, D. H. Ho. *Hememics Biotechnologies Inc, Gaithersburg, MD*

Background: Graphene, a two-dimensional material composed of carbon atoms, has garnered significant attention for its potential application in biosensors due to its unique electrical properties. Despite its promising attributes, however, the non-reproducible nature of graphene biosensors has hindered their widespread commercialization. This issue arises from the difficulty in obtaining a clean graphene layer due to: photoresist such as PMMA residues that effect the performance of graphene; etching process which affect the physical and chemical properties of graphene; variation in the coating of bio-receptors on to graphene. All these issues affect reliable and accurate sensor performance. These challenges have made it difficult for graphene biosensors to reach their full potential as commercial products and have limited their widespread adoption. Hememics has developed a unique process for synthesizing and fabricating graphene-based biosensors. This represents a major breakthrough in the commercialization of graphene-based biosensors and will likely lead to widespread adoption of these devices in the medical and biotechnology industries. **Methods**: HemChip™ contains multiple sensor areas that are coated with ultra-sensitive graphene. Using Hememics' unique proprietary process, the graphene layer is functionalized with bioreceptors such as aptamers and stabilized with HemSol™ to allow room temperature storage. The relative difficulty of stabilizing aptamers on a hydrophobic graphene substrate has been overcome by using HemSol™ to secure its structure during dry storage. Each sensor on the biochip allows independent functionalization with a bioreceptor. These sensors coated with bioreceptors are freeze-dried and stored in a sealed pouch at room temperature until use. **Results**: We have tested over 2,000 samples using our modified process. The functionality of the graphene-based biosensor is evaluated by measuring the distribution of Dirac points. The results show that the distribution follows a Gaussian distribution with a median of 80 mV and a very narrow spread of approximately 35 mV. This indicates that the majority of the Dirac points are tightly clustered around the median value of 80mV. Furthermore, one standard deviation of the Dirac points fall within the range of 50mV to 120mV. In contrast, the standard published process of producing graphene-based biosensor shows a much wider distribution of Dirac points, ranging from 250-1500mV. **Conclusion**: Hememics has developed a production process for making graphene-based biosensors with desiccated detection biology that deliver repeatable performance data. The results of the Dirac point distribution demonstrate the high precision and accuracy for the functionalized biosensors. This combined with the ability to mass produce the biosensor, make graphene-based biosensors an attractive molecular and immuno-based detection tool. Additionally, the results suggest that the Hememics process can be adapted to produce functional graphene-based biosensors for wide ranging bio detection applications.

A-361

Implementation of a Fit-for-purpose Point of Care Quality Assurance Program

W. J. Dimech, L. Cabuang, G. Vincini. *National Serology Reference Laboratory, Australia, Fitzroy, Australia*

Background:

All individuals should have equitable access to accurate and timely testing for infectious diseases. Point of care testing (PoCT) has been developed to support populations having limited access to laboratory-based testing. Unlike laboratory-based testing, PoCT is often performed by non-laboratory staff and outside the jurisdiction of regulatory frameworks. Under these conditions, quality assurance (QA) of PoCT is often lacking; inaccurate testing can go undetected, leading to poor patient outcomes. A fit-

for-purpose QA program was developed to monitor the quality of PoCT for infectious disease testing, particularly in low-middle income countries and in testing outside the laboratory regulatory environment.

Methods:

In collaboration with the World Health Organization (WHO), the National Serology Reference Laboratory (NRL) reviewed the barriers to participation in QA programs experienced by PoCT sites. Using this information, NRL developed a QA model specifically designed for PoCT for infectious disease. In collaboration with Foundation for Innovative and New Diagnostics (FIND, Geneva, Switzerland), Kirby Institute (Sydney, Australia) and Flinders University (Adelaide, Australia), NRL implemented the PoCT QA model across Africa, Asia, and remote regional Australia. The model consists of competency panels (CP - one positive and one negative sample) and external quality assessment (EQA) panels. The EQA panels contain five samples. Whereas the test site knows the reactivity of the CP samples, the EQA samples are tested blinded. Both panels use inactivated samples that are stable at ambient temperature. The model uses streamlined logistics channels, with data collection and analysis using smartphone technology and QR codes. NRL designed a method for randomizing the identity of EQA sample vials so test sites can test QA panels ad hoc, and not be limited to specific test event dates. A range of support material such as pictorial instructions for use, virtual training events and on-line videos were produced to support test sites.

Results:

More than 100 test sites from 14 countries have participated in the NRL PoCT QA programs. The model is designed so data is collected by test sites scanning QR codes and entering results directly into a data entry screen. The NRL PoCT QA program removed barriers to participation by offering sample types that are inactivated and stable at ambient temperature for extended periods of time, removing the need for dry ice shipping. The program is cost effective making it accessible to low- and middle-income countries. Data is collected using novel smartphone technology, facilitating results to be entered into a central database for immediate analysis. Testing of the QA samples can be done at any time, avoiding set test events.

Conclusion:

Implementation of a fit-for-purpose PoCT QA for infectious disease testing removes the barriers to participation and facilitates the monitoring of testing over time. Any deficiencies in testing can be detected and addressed rapidly, ensuring that accurate test results are reported, and the population protected.

A-363

Evaluating the Performance of Emerging STI Point of Care Assays Using Novel, Room Temperature Stable CT/NG/TV/MG Positive Swab Quality Control Materials

S. Rivers, P. Zhelev, M. Thakur, A. Alagic, J. Li, C. Randall, M. Luscher, K. Hughes. *Microbix Biosystems Inc, Mississauga, ON, Canada*

Background:

The emergence of Point of Care tests that detect Sexually Transmitted Infections (STIs) is critical for screening and diagnostic programs in low-resource settings. Point of Care tests that are designed to simultaneously detect *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae (*NG), *Trichomonas vaginalis* (TV), and *Mycoplasma genitalium* (MG) promote rapid diagnosis and early treatment of the most common etiological agents causing STIs. Along with the roll-out and use of these tests, there is a growing need for complex multi-analyte quality control materials that are stable at room temperature and challenge test performance by identifying result deviations, lot-to-lot variability, and routine laboratory workflow errors. Microbix developed a whole-workflow multiplex quality control desiccated on a Copan FLOQSwab® that is stable at 2-30°C and contains inactivated whole-genome target pathogens. The objective of this study is to evaluate sample performance with multiple Point of Care diagnostic platforms that are currently in development and commercial IVD tests that are routinely used in the laboratory.

Methods:

Microbix designed an inactivated STI whole-workflow control that contains whole-genome CT, NG, TV, MG, and human cells to satisfy sample adequacy control requirements. The formulation is desiccated on a Copan FLOQSwab® to mimic patient specimen formats and ensure sample compatibility with all assay workflows and elution buffers. Sample performance was evaluated with Cepheid Xpert® CT/NG assay, Cepheid Xpert® TV assay, Abbott Alinity m STI assay, BD MAX™ CT/GC/TV assay, Seegene Novaplex™ CT/NG/TV/MG assay, EliTechGroup STI PLUS ELITe MGB®Kit, and commercial Point of Care assays currently in development.

Results:

The STI positive swab formulation demonstrated acceptable performance when tested with various nucleic acid amplification tests that detect CT, NG, TV, and MG targets. The formulations also behaved as useful quality management tools by highlighting design limitations for commercial Point of Care assays that are currently in development.

Conclusion:

CT/NG/TV/MG whole-genome multiplex formulation desiccated on a Copan FLOQSwab® is an advantageous prospective quality control material that is stable at room temperature and supports the clinical use and accuracy of emerging STI assays, including the most challenging Point of Care test formats. The formulations showed acceptable performance on multiple platforms, demonstrating excellent commutability. Additionally, the dry swab format offers great practicality for monitoring assay performance in low-resource settings.

A-364

The Utility of the Highly Sensitive Detection System for Immunochromatographic Assay by Addition of MPC polymer

T. Nakahara[1], M. Matsuda[2], S. Uesugi[3], K. Murakami[1], K. Kobayashi[2], H. Kataoka[1], E. Harata[2], K. Tohyama[1]. *[1]Kawasaki University of Medical Welfare, Okayama, Japan, [2]NOF CORPORATION, Tokyo, Japan, [3]Kawasaki Medical School Hospital, Okayama, Japan*

Background: Immunochromatographic assay (ICA) is frequently used as a point-of-care testing (POCT) in the field of infectious diseases, but it has a problem of low detection sensitivity. Many interfering substances in ICA test materials have been reported to case a decrease in detection sensitivity. For the purpose of increasing the sensitivity of ICA, bovine serum albumin or a polymer with phosphorylcholine groups (MPC polymer) has been used as an additive to a sample diluent. In this study, we evaluated the usability of the sensitizing effect for the addition of MPC polymer with a serum-based HBs antigen test kit.

Methods: A 2-fold dilution series of a commercially available control serum (Infectrol D, SeraCare Life Sciences, Inc. manufacturer's reference value 108.674 IU/mL) containing 0.05%, 0.10%, 0.5% of several MPC polymers (Biolipidure® from NOF Corporation) as final concentration were prepared with HBs antigen negative serum. The optimal type of MPC polymer and the concentrations added to the sera were determined by comparing the color intensity of their test lines in the evaluation of each test sample with a commercially available test kit (Quick Chaser® from MIZUHO MEDY Co., Ltd.). The color intensities of the test lines were quantified with an immunochromatographic reader.

In addition, the correlation of two different test kits (ESPLINE® from FUJIREBIO and Quick Chaser® from MIZUHO MEDY Co., Ltd.) was evaluated with sera obtained from HBs antigen-positive patients regarding the three types of MPC polymer which were improved the detection sensitivity in the evaluation with the control serum. The measurements were performed in triplicate after 0.5% of MPC polymer as final concentration was added to each diluted serum.

Results: Antigen amount for diluted sera with MPC polymer were determined by CLEIA method, and no difference in the antigen amount was observed regardless of the addition or the types of MPC polymer. In addition, the color intensity in each dilution series was compared to the cutoff value, which is average value of the color intensity of negative control serum without MPC polymer. In the evaluation with control serum, the test line in the case without MPC polymer enabled to detect up to 2-fold diluted control serum while the test line in the case with MPC polymer enabled to detect up to 32-fold diluted control serum. The addition of MPC polymer in the evaluation with sera obtained from HBs antigen-positive patients was also confirmed that the detection sensitivity enhanced up to 8-fold higher in ESPLINE® and up to 4-fold higher in Quick Chaser®.

Conclusion: The color intensity of the test line enhanced by the addition of MPC polymer. In particular, when the highly hydrophilic and anionic MPC polymer was added, the test line was detected narrower and darker, and we believe that the increased color intensity resulted in improved sensitivity. By considering the influence of interfering substances in clinical materials and the physical properties of the MPC polymer (hydrophobicity, molecular size, etc.), it is expected that the sensitivity of the ICA will be increased to be more suitable for clinical materials.

A-365

Carbohydrate-binding module proteins to functionalize paper for lateral flow applications

L. Walker[1], G. Yang[1], O. Drabwell[2], C. Buerki[1], R. Porter[2], R. Greene[1]. *[1]Gemina Laboratories, Burnaby, BC, Canada, [2]RAPIvD, Sharnbrook, United Kingdom*

Background: Lateral flow (LF) tests have employed nitrocellulose membrane since their introduction. Refinement of nitrocellulose and LF production techniques have led to substantial improvements in reproducibility, sensitivity, clarity, and speed of results. The recent surge in demand for LF tests during the COVID-19 pandemic has drawn increased attention to the consequences of using non-biodegradable or non-recyclable materials in test production, requiring disposal via landfill. The nitrocellulose substrate is a critical component for substitution with an equivalent or superior material to address environmental concerns. Additionally, nitrocellulose and the specific molecular recognition reagents, typically antibodies, contribute significantly to the test cost, and whilst LF tests are less expensive than most other formats, challenges remain with respect to providing affordable tests with adequate performance globally in low resource environments. Despite attempts to substitute cellulose for nitrocellulose, principally for reducing cost and more latterly, environmental impact, cellulose is not widely used due to inferior performance, especially in relation to the clarity and quality of the signal line and reagent requirements. Here, we present preliminary data supporting the use of cellulose membrane (paper) as an alternative substrate to nitrocellulose for LF assays. We demonstrate that cellulose-binding module (CBM)-streptavidin fusion proteins for the immobilisation of capture antibodies on cellulose addresses the performance limitations, yielding sensitivity, clarity and speed of result matching current clinical and industry requirements for a qualitative SARS-CoV-2 antigen LF test.

Methods: We expressed and purified CBM-streptavidin fusion proteins from E.coli to a purity of >90%. Binding to biotinylated proteins and cellulose material was confirmed. An inhouse SARS-CoV-2 antigen assay was adapted for use with the CBM-streptavidin fusion protein on cellulose material. Using methods compatible with current LF test production, we conjugated and dried down 40nm gold (BBI) with anti-nucleocapsid antibodies (Hytest). The biotinylated anti-nucleocapsid capture antibody was dried down on a separate pad. CBM-streptavidin fusion protein was plotted on Whatman 43 paper at the test line. We evaluated assay performance using SARS-CoV-2 nucleocapsid protein and UV-inactivated SARS-CoV-2 virus (NIBSC) in artificial mucus. We further evaluated accelerated stability of the CBM-paper system at 45°C.

Results: Plotting of the CBM-fusion protein on the test line (0.3 mg/mL, 1 uL/cm) resulted in a tightly focused line, while the control line antibody, without CBM-fusion protein (1 mg/mL, 1 uL/cm), diffused from its deposition line. Additionally, we were able to substantially reduce the amount of capture antibody needed using the CBM-fusion protein system. The cellulose-based assay exhibited a runtime of 30 minutes and an analytical sensitivity of 10^3 pfu/mL. No significant changes in performance were observed after 9 weeks of incubation at 45°C, representing an approximate accelerated shelf-life of 12 months.

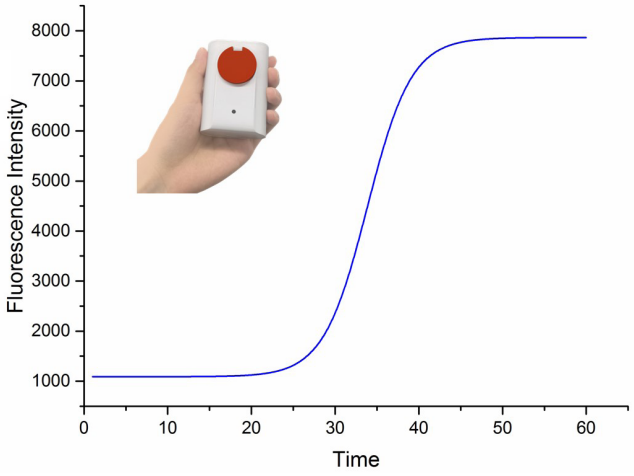
Conclusion: Our results indicate that paper-based tests match sensitivity, clarity, and speed of current qualitative antigen tests on nitrocellulose, with a shelf-life of at least one year. This preliminary investigation demonstrates the viability of using paper as a replacement for nitrocellulose when coupled with CBM-fusion proteins for antibody immobilisation, offering significantly reduced material cost and environmental impact.

A-367

Development of a novel handheld platform for real-time fluorescence-based nucleic acid detection

Y. Hong, Q. Song, Y. Gao, Y. Liu, W. Wen. *The Hong Kong University of Science and Technology, Hong Kong, Hong Kong*

Background: Real-time fluorescent nucleic acid detection systems are usually large and complex to operate. In the past three years, the pandemic outbreak of COVID-19 has sparked a variety of point-of-care testing (POCT) nucleic acid detection platforms. However, most of these platforms need to be paired with specific reagents, and many detection reagents on the market cannot be applied to these POCT platforms. **Methods:** In this research, a novel handheld real-time nucleic acid detection device was invented. The device uses a heating element to adjust the temperature in time with a sensor to ensure accuracy. The optical detection module is highly scalable and can be extended up to 6 fluorescence channels. The FAM and HEX fluorescence channels were utilized in this experiment. This platform is open and can adjust temperature for various types of isothermal nucleic acid detection, such as LAMP, RPA, and RCA. **Results:** We used a commercial COVID-19 reagent kit with our device to get results in just 30 minutes, detecting SARS-CoV-2 virus as little as 500 copies/mL. Our system provides a reliable and efficient tool for COVID-19 testing in a variety of settings.



Conclusion: The handheld device was also used to test for flu A and mycobacterium tuberculosis, with good results. This device can be used not only for pathogen detection of human diseases, but also in pathogen detection for animals and plants, food safety, environmental monitoring, and other fields, thus expanding the application range of nucleic acid detection and making it simpler and more convenient to use.

A-368

Aspergillus Galactomannan Lateral Flow Assay with Digital Reader for the Diagnosis of Invasive Aspergillosis

J. Peng, J. Yan, J. Du, Y. Wang, H. Wang, Y. Su, Z. Zhou. *Tianjin Enterprise Key Laboratory for precision diagnosis technology of invasive fungal diseases, Tianjin, China*

Background: The Novel *Aspergillus* Galactomannan Lateral Flow Assay(Quic GM) from Dynamiker Biotechnology (Tianjin) Co., Ltd is a fluorescent immunochromatographic cassette test using a monoclonal antibody against GM with Digital Reader (Figure 1), which was rapid and easy to operate compared with the traditional GM ELISA assay. Here we conducte a comparative study between the Dynamiker Quic GM and the Platelia GM (Bio-Rad, USA) to validate the performance of the novel assay for the diagnosis of Invasive Aspergillosis (IA).

Methods: A total of 573 serum samples and 97 BALF samples with proven IA (Serum n = 9, BALF n = 2), probable IA (Serum n = 174, BALF n = 25), possible IA (Serum n = 98, BALF n = 16) and patients not fulfilling 2019 EORTC/MSG guideline IA criteria (control, Serum n = 292, BALF n =54) were included. All the samples were tested in parallel using the Quic GM and Platelia GM.

Results: The total coincidence rate of Quic GM compared with Platelia GM was 97.91% (Kappa value was 0.95>0.75), 97.94% (Kappa value was 0.95>0.75) and 97.91% (Kappa value was 0.95>0.75) in serum samples, BALF samples and serum+BALF samples, respectively (Table 1). The concentrations generated by Quic GM and Platelia GM were linearly correlated (Serum: slope=0.97, r^2 = 0.96, p<0.0001; BALF: slope=0.95, r^2 = 0.94, p<0.0001) (Figure 2).

Conclusion: In conclusion, the Dynamiker Quic GM yielded significant consistency with Bio-Rad Platelia GM ELISA, and showed good performances for IA diagnosis, with the benefit of a relatively short handling time (around 20 min).

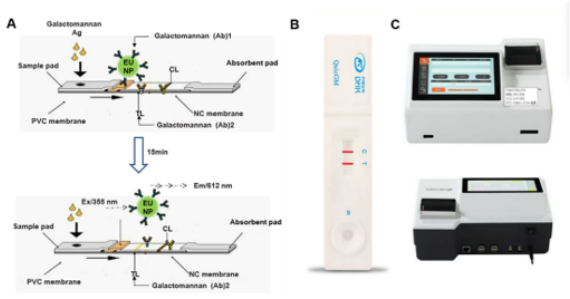


Figure 1 Diagram of the Dynamiker Quic GM (Fig. 1A), Dynamiker Quic GM test (Fig. 1B) and its reader (Fig. 1C).

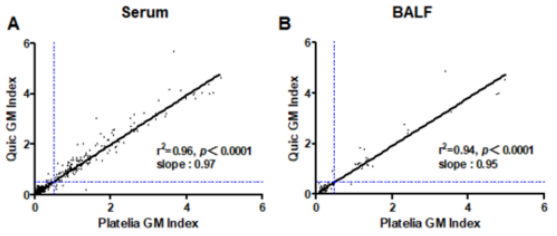


Figure 2 Scatter plot. Data distribution and probability density for all serum samples (Fig. 2A), and all BALF samples (Fig. 2B) individually tested by Quic GM and Platelia GM. The blue dotted lines indicate the manufacturer's cut-off value of each test (I Value=0.5).

Table 1 The coincidence rate of Quic GM compared with Platelia GM

	Positive coincidence rate % (95% CI)	Negative coincidence rate % (95% CI)	Total coincidence rate % (95% CI)	Kappa value (95%
Serum	96.17 (92.32-98.13)	98.72 (97.03-99.45)	97.91 (96.38-98.80)	0.95 (0.91-0.99)
BALF	96.67 (83.33-99.41)	98.51 (92.02-99.74)	97.94 (92.79-99.43)	0.95 (0.85-1.05)
Serum+BALF	96.24 (92.76-98.08)	98.69 (97.17-99.40)	97.91 (96.52-98.75)	0.95 (0.91-0.99)

A-369

Evaluation of a Point-of-Care Whole Blood Creatinine Assay and eGFR Concordance using the CKD-EPI 2021 Equation

Y. Elonda[1], K. Zwaan[1], C. Xu[2]. *[1]Atalmedial, Amsterdam, Netherlands, [2]Werfen, Bedford, MA*

BACKGROUND: Estimated glomerular filtration rate (eGFR) from serum creatinine is considered a better assessment of renal function than serum creatinine alone. Patients with chronic kidney disease have a higher risk of developing contrast-induced nephropathy (CIN). GEM Premier ChemSTAT (Instrumentation Laboratory, Bedford, USA) whole blood (WB) creatinine assay along with eGFR reporting capability per the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2009 eGFR equation is now available and could provide a quick point-of-care (POC) screening test alternative to the lab assay for patient risk management. Currently the CKD-EPI 2009 formula for non-AA population has been used to estimate eGFR in our institution. Recently the CKD-EPI 2021 equation without race adjustment has been recommended by multiple professional societies. The goal of this clinical evaluation was to compare the analytical performance of the ChemSTAT WB creatinine assay and the clinical concordance of eGFR to an established lab method by CKD-EPI 2021 equation.

METHODS: Remnant heparinized WB samples along with age and gender were obtained. The WB samples were analyzed on the GEM Premier ChemSTAT and the plasma assayed on an Architect analyzer (Abbott Laboratories, Lake Forest, USA) as the reference method. The eGFR based on the CKD-EPI 2009 equation were obtained for both WB and plasma creatinine. The eGFR based on CKD-EPI 2021 equation were retrospectively calculated based on WB and plasma creatinine, age and gender. The clinical concordance of categorizing patients with abnormal kidney function (eGFR<60 ml/min/1.73 m²) and overall concordance of patients (eGFR<60 + eGFR≥60) was assessed for WB vs. plasma eGFR. Error grid analysis was performed for eGFR as described by Snaith *et al.* (1) to identify the impact of discordant results between WB and plasma eGFRs.

RESULTS: A total of 60 patient samples were analyzed, median age (SD) = 68.5 (15.2), 53.3% female. The WB ChemSTAT creatinine assay correlated well with the Architect across the tested ranges with regression results of: ChemSTAT_Creatinine (µmol/L) = 1.1099*Architect_Creatinine – 16.0, r = 0.990. A small mean bias (SD) of -3.0 (22.6) µmol/L was observed between ChemSTAT and Architect from the Bland-Altman analysis. When identifying patients with abnormal kidney function (eGFR<60), ChemSTAT WB eGFR showed 84% (21/25) concordance against the plasma eGFR. An overall clinical eGFR concordance of 88% (53/60) was observed for ChemSTAT. The error grid analysis indicated 100% (60/60) of ChemSTAT WB eGFRs (Zones A and B combined) had no implication to clinical management. Mean biases (SD) from reported CKD-EPI eGFR 2021 vs. 2009 equation of 3 (1.6) and 2 (2.6) were observed from Architect plasma creatinine and ChemSTAT WB creatinine, respectively.

CONCLUSIONS: Strong correlations were observed between the ChemSTAT WB POC creatinine assay and eGFR versus the lab method. Based on the high clinical concordance of WB eGFR with CKD-EPI 2021 equation and low mean bias, GEM Premier ChemSTAT demonstrated good performance as POC method for rapid and lab quality renal function assessment.

REFERENCE: (1) Snaith B, *et al.* Clin Chem Lab Med 2018;56:1269–1276.

A-370

High rates of potassium, chloride and hemoglobin discordances in split samples drawn within 1 minute and analyzed on point of care GEM 5000 and central laboratory Roche Cobas 8000 and Sysmex analyzers

G. Cembrowski[1], Q. Xu[2], H. Sadrzadeh[3]. *[1]University of Alberta, Edmonton, AB, Canada, [2]Family Practice, Vancouver, AB, Canada, [3]Alberta Precision Laboratories, Calgary, AB, Canada*

Background: Previously, we used serial intensive care unit patient data as well as split sample glucose comparisons to characterize the analytical performance of the GEM 4000 and its tendency to generate outliers in the early to late evening. In this work, we summarize the discordance rate of 2 years of split specimens (minimally 17,700 patient care samples) that were drawn within 1 minute of each other and analyzed on the GEM 5000, Roche Cobas 8000 and Sysmex analyzers.

Methods: Two years (2020 and 2021) of GEM-central laboratory Cobas 8000 and Sysmex comparisons were obtained for potassium, sodium, chloride, glucose and hemoglobin (Sysmex). We used the GEM's iQM2 control (drift) limits to define discordant differences between the GEM and Roche/Sysmex analyses. We graphed the differences vs time of day and applied Dahlberg's formula to derive the magnitude of the running variation of the differences.

Results: The Table summarizes the drift limits, the number of comparisons performed over the two-year period and the average incidence of discordances over the 24 hour period. What strikes the casual observer is the asymmetric distribution of the discordances. More subtle is the lower frequency of potassium and hemoglobin discordances in the early morning and the higher frequency in the late afternoon and evening. The Dahlberg running variation graphs readily demonstrate this diurnal pattern.

Conclusion: The GEM 5000 overcalls increases in potassium, decreases in chloride and increases in hemoglobin. We recommend that other laboratories verify these findings.

Analyte/Pairs	Potassium: 18,617		Sodium: 19,027		Chloride: 18,632		Hemoglobin: 25,137		Glucose: 17,723	
Outlier Def.	Diff>0.5mmol/L		Diff>4mmol/L		Diff>4mmol/L		Diff>7%		20%(>5mmol/L) or 1mmol/L	
Time Interval	K (low)	K (high)	Na (low)	Na(high)	Cl(low)	Cl(high)	Hgb(low)	Hgb(high)	Gluc(low)	Gluc(high)
12am-4am	1.1%	4.5%	0.2%	2.5%	8.0%	0.8%	0.8%	7.2%	0.2%	0.4%
4am-8am	1.4%	4.1%	1.2%	1.7%	8.8%	0.7%	0.9%	6.8%	0.3%	0.6%
8am-12pm	1.2%	4.7%	1.1%	1.3%	6.8%	0.4%	0.5%	7.0%	0.2%	0.4%
12pm-4pm	1.4%	5.0%	0.8%	1.7%	6.7%	1.0%	0.7%	7.5%	0.2%	0.2%
4pm-8pm	1.4%	5.5%	0.7%	2.6%	5.4%	1.0%	0.7%	8.2%	0.3%	0.3%
8pm-12am	1.5%	5.2%	0.3%	3.1%	6.6%	0.9%	0.6%	7.9%	0.2%	0.3%

A-371

Comparative evaluation of POCT based lysophosphatidylcholine measurement

J. Rim, H. Choi, H. Jang, J. Jang, H. Kang, J. Lim. *Yonsei University College of Medicine, Seoul, Korea, Republic of*

Background: Lysophosphatidylcholine (LPC), also referred to as lysolecithin, is a phospholipid intermediate whose concentrations have been correlated with such diseases as cancer, artherosclerosis and diabetes. Recently, serum LPC concentrations have been reported to be candidate biomarker for bacterial infection and sepsis. In this study, we evaluated the comparative performance of the point-of-care test (POCT) measurement of LPC using CareBuddy Multi Analyzer.

Methods: A total of 120 serum samples were analyzed for LPC by enzymatic spectrophotometry (CareBuddy Multi analyzer, CareBuddy catridge) and mass spectrometry based method (TSQ Altis LC/MS/MS) as the reference method. Additionally, subset of samples with enough remnant volumes were assessed for PCT and CRP using electrochemiluminescence immunoassay (Roche Cobas e801, Roche Elecsys BRAHMS PCT) and immunoturbidimetric assay (Roche Cobas c702, Sekisui Nanopia CRP), respectively. The equivalence and correlation among the methods were evaluated using linear regression analysis.

Results: The LPC measurement by POCT analyzer showed high correlations with the LC-MS based reference method (y=1.02x+1.26). The LPC measurement by POCT showed correlation coefficient for PCT (Pearson's r = -0.316) and CRP (Pearson's r = -0.618). Upon applying clinical cutoffs for qualitative result, LPC POCT measurement showed good agreements to each reference methods.

Conclusion: In summary, our results suggest that the quantitative measurement for LPC via POCT device in human serum samples possess possible clinical feasibility as a novel sepsis biomarker.

A-372

Increased Sensitivity in Detection of SARS-CoV-2 Antigen Using RADI COVID-19 Ag Rapid Test

S. Kee[1], J. Choi[2], J. Jeon[1], M. Heo[3], J. Park[4], H. Choi[1]. *[1]Chonnam National University Hospital, Gwangju, Korea, Republic of, [2]Chonnam National University Research Institute of Medical Science, BMSGP, Chonnam National University Medical School, Hwasun, Korea, Republic of, [3]Seegene Medical Foundation Gwangju & Honam Laboratory Center, Gwangju, Korea, Republic of, [4]Chonnam National University Hwasun Hospital, Hwasun, Korea, Republic of*

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapid point-of-care antigen tests can provide easy and fast results for the diagnosis of coronavirus disease 2019 (COVID-19). However, due to the lower sensitivity of rapid antigen tests relative to reverse transcription polymerase chain reaction (RT-PCR), they might miss many infectious COVID-19 cases. We evaluated and compared a new rapid antigen test with the current approved one. **Methods**: The RADI COVID-19 Ag Rapid Test (KH Medical, Hanam, Korea) was validated with a total of 1,054 preconfirmed residual nasopharyngeal swab samples (258 positives and 796 negatives) by PowerChek 2019-nCoV Real-time PCR (Kogene Biotech, Seoul, Korea). Among them, 258 positives and 546 negatives were used to compare the RADI COVID-19 Ag Rapid Test with the STANDARD Q COVID-19 Ag Test (SD Biosensor, Suwon, Korea). **Results:** Using RT-PCR results as the reference, the overall sensitivity and specificity of RADI COVID-19 Ag Rapid Test were 82.2% (212/258; 95% confidence interval, CI [76.9 - 86.6]) and 99.1% (789/796; 95% CI [98.2 - 99.7]), respectively. The positive predictive value and negative predictive value were 96.8% (212/219; 95% CI [93.5 - 98.5]) and 94.5% (789/835; 95% CI [93.0 - 95.7]), respectively. The accuracy was 95.0% (1001/1054; 95% CI [93.5 - 96.2]) with Cohen's kappa value of 0.86 (95% CI [0.82 - 0.89]). The positive percent agreement and negative percent agreement of the RADI COVID-19 Ag Rapid Test for STANDARD Q COVID-19 Ag Test were 97.0% (159/164; 95% CI [93.0 - 99.0]) and 90.6% (580/640; 95% CI [88.1 - 92.8]), respectively, and those of STANDARD™ Q COVID-19 Ag Test for RADI COVID-19 Ag Rapid Test were 72.6% (159/219; 95% CI [66.2 - 78.4] and 99.2% (580/585; 95% CI [98.0 - 99.7], respectively. The overall percent agreement between the two rapid antigen assays was 91.9% (739/804; 95% CI [89.8 - 93.7]) with Cohen's kappa value of 0.78 (95% CI [0.73 - 0.83]). Among the 65 discrepant samples between the two rapid antigen assays, 56 were positive as per RT-PCR, which showed 53 samples negative for STANDARD Q COVID-19 Ag Test and 3 samples negative for RADI COVID-19 Ag Rapid Test. The median E gene Ct value of the 65 discrepant samples was 28.2 (interquartile range: 26.1 - 32.3). **Conclusion**: RADI COVID-19 Ag Rapid Test provides acceptable sensitivity and specificity according to the validation criteria for KFDA immunological test product approval. Furthermore, RADI COVID-19 Ag Rapid Test shows improved sensitivity compared to the current approved rapid antigen test.

A-373

Comparison of the CoaguChek ® and Coag-Sense ® PT2 Meter Point of Care INR Devices

T. Schumann[1], B. Karon[1], C. O'Connor[2], L. Jennifer[3], J. Blanco[1], L. Hartman[1], A. Kramer[4], J. Uy[3], N. Myhre[1], D. Gayle[1], K. Hartung[1], H. David[5], P. Santrach[1], R. McBane[1]. *[1]Mayo Clinic, Rochester, MN, [2]Mayo Clinic, Eau Claire, WI, [3]Mayo Clinic, Scottsdale, AZ, [4]Mayo Clinic, La Crosse, WI, [5]Mayo Clinic, Jacksonville, FL*

Background: Warfarin monitoring may be done at the point-of-care (POC) at home, in a clinic environment, or be performed using plasma specimens in a central laboratory. With a goal of standardizing International Normalization Ratio (INR) measurements and improving data integrity by enabling electronic transmission of results from any location, we evaluated two POC INR devices against an internal plasma INR reference method.

Methods: We conducted a multicenter study to test the accuracy of two commercially available point of care devices: Coag-Sense PT2 Meter (Coagusense, Inc., Fremont, CA) and CoaguChek XS Pro or XS Plus (Roche Diagnostics, Indianapolis, IN) compared with plasma INR values among warfarin treated patients. Bias and linear regression analysis were assessed for device-plasma INR comparisons. Warfarin dosing decisions were assessed for each device compared to an internal plasma INR reference method (Instrumentation Laboratories ACLTOP 500, 550, 750 analyzers (Werfen Diagnostics, Bedford MA) in one of three CLIA-certified laboratories. The thromboplastin used at our institution for INR measures is HemosIL RecombiPlasTin 2G (R2G) reagent (Werfen Diagnostics, Bedford MA) which has an International Sensitivity Index (ISI) of 1.0.

Results: Two hundred ninety-nine warfarin patients treated across three Mayo Clinic sites agreed to participate in the study. Common anticoagulant indications were atrial fibrillation (63.9%), venous thromboembolism (21.7%) and heart valve prosthesis (15.4%) with a 2.0-3.0 INR target range for 93.6% of patients. For the CoaguChek devices, 81.3% of values fell within 0.2 INR units of plasma INR referent and 95.3% within 0.4 units. The correlation between CoaguChek whole blood and plasma INR was excellent (R^2=0.93). For the Coag-Sense device, 34.1% of values fell within 0.2 INR units and 60.2% within 0.4 INR units of plasma INR values, with a slightly weaker correlation between Coag-Sense whole_blood and plasma INR (R^2=0.83; p<0.0001). Mean (± standard deviation) deviation from plasma INR was 0.04 ± 0.2 for CoaguChek compared to 0.4 ± 0.3 for Coag-Sense (p<0.0001). The difference between POC devices was largely attributed to systematic negative bias between Coag-Sense and ACL TOP plasma INR values. Using the plasma INR as the reference method for dosing decision making, appropriate dosing recommendations would have occurred for 97.7% of CoaguChek and 81.6% of Coag-Sense results.

Conclusion: Compared to our institution's plasma reference method, INR values obtained from the CoaguChek devices (XS Pro and XS Plus) exhibited less systematic bias compared to the Coag-Sense values. This translates to greater percentage of concordant management decisions between POC and laboratory plasma INR methods when the CoaguChek device is used.

A-374

Analytical Performance Assessment and Comparison of Point of Care Testing (POCT) Devices for Blood Lipids Measurements

K. Dahya, F. Pokuah, U. Danilenko, H. W. Vesper. *Centers for Disease Control and Prevention, Atlanta, GA*

Background: Cardiovascular diseases (CVDs) are the leading cause of death in the U.S. and worldwide. According to the World Health Organization (WHO), worldwide, 85% of CVD deaths are due to heart disease and strokes, and over 75% occur in low- and middle-income countries (LMICs). Early detection and treatment are key to preventing the consequences of CVD. Point-of-care testing (POCT) devices offer alternatives to screening large populations for CVD risk. Although POCT are increasingly used for screening, only few information is available about their accuracy and reliability.

Methods: Eight POCT devices for blood lipids that previously successfully participated in the CDC CVD Biomarker Standardization Programs were used in this preliminary study. The analytical performance for all eight devices was assessed using split sample comparisons to the CDC reference measurement procedures for measure-

ments performed in whole blood (WB) and serum. Specifically, the accuracy of total cholesterol (TC) was determined in WB and serum. The accuracy of high-density lipoprotein-cholesterol (HDL-c), and low-density lipoprotein-cholesterol (LDL-c) was determined in serum. The precision of TC, HDL-c, LDL-c, and triglycerides (TG) was determined in WB and serum.

Results: The reference values of the specimens from 20 donors ranged between 154 - 279 mg/dL, 36.9 - 109 mg/dL, and 71.1 - 182 mg/dL for TC, HDL-c, and LDL-c in serum, and between 145 - 210 mg/dL for TC in WB. Preliminary assessments found that the mean bias for each instrument ranged from -7.86% - 13.82% and from -11.90% - 3.24% for TC in WB and serum, respectively. For serum HDL-c samples, the mean bias ranged from -6.81% - 15.43%. The mean bias for serum LDL-c samples ranged from -22.40% to 3.08%. For three devices, approximately 30% of the WB measurements and approximately 50% of the serum measurements were reported as outside of the measurement range. The mean imprecision for each instrument ranged from 2.2% - 13.75% and from 1.88% - 11.76% for TC in WB and serum, respectively. For HDL-c, the mean imprecision ranged from 5.36% - 23.21% and from 2.30% to 26.69% in WB and serum, respectively. The mean imprecision ranged from 4.82% - 20.34% and from 3.06% - 20.54% for LDL-c in WB and serum, respectively. For TG, the mean imprecision ranged from 5.78% - 23.91% and from 1.71% - 11.02% in WB and serum, respectively.

Conclusion: These preliminary findings about the analytical performance of POCT devices indicate notable differences in accuracy and precision for lipids measured in WB and serum, with serum measurements being more likely within performance requirements than WB measurements. Some devices appear to be more likely to report values outside the measurement range even though reference values are within the measurement range of the device. Further studies to verify these preliminary findings with more individual donor samples are underway.

A-375

Evaluation of the CLIA-Waived Afinion HbA1c Point-of-Care Test

E. Villasin, I. Metushi. *Department of Pathology and Laboratory Medicine. University of California Los Angeles, Los Angeles, CA*

Background: Point-of-care (POC) HbA1c tests can be essential in evaluation/monitoring of diabetes provided that the test has acceptable analytical performance. The Abbott Afinion HbA1c is an *in vitro* diagnostic test for quantification of HbA1c in human capillary and venous whole blood. This study aim was to evaluate the performance of Afinion HbA1c in whole blood EDTA patient samples. **Methods:** The Afinion utilizes the boronate affinity principle for measurement of HbA1c. Hemoglobin A1c was tested on the Abbott Afinion™ 2 analyzer. Two levels of quality control (QC) and two levels of whole blood EDTA patient samples (one low and on high) were used to assess imprecision. Within-day precision was determined by assaying the 20 times in one day and between-day precision was determined by assaying the samples in duplicates over a period of 10 days. The analytical measured range (AMR) and method comparison was performed by using whole blood EDTA patient samples. Percent bias plot and Deming regression analysis was performed by EP Evaluator. **Results:** Within-day and between-day precision and accuracy data are summarized in table 1 below. The assay displayed good linearity over the measured AMR. Lastly, the method comparison between Afinion vs. Cobas pro, had a correlation coefficient R^2 of 0.994, slope (m) of 1.14 and % bias was 2.5%. Afinion vs. Trinity Biotech HPLC had R^2 = 0.996, m = 1.04 and % bias was -2.3%. Afinion vs. DCA Vantage had R^2 = 0.991, m = 1.03 and % bias 1.5. **Conclusion:** We conclude that the Abbott Afinion POC device evaluated here is precise and yields results consistent with other Clinical Chemistry analyzers such as Cobas Pro and Trinity Biotech.

Table 1: Within day and Between Day Accuracy/Precision for Afinion HbA1c

	QC Level 1(%)	QC Level 2(%)	Patient Level 1(%)	Patient Level 2(%)
Target Concentration	6.4	8.5	4.8	10.0
Within-Day mean	6.3	8.3	4.6	10.2
Between-Day mean	6.2	8.4	4.8	10.3
Within-Day SD	0.1	0.1	0.1	0.1
Between-Day SD	0.1	0.1	0.1	0.2
Within-Day %CV	1.7	1.1	1.4	1.3
Between-Day %CV	1.5	1.3	2.2	1.7
Within-Day %Bias	-1.3	-1.9	-3.6	1.8
Between-Day %Bias	-2.7	-1.8	0.6	3.2

A-376

Development of a 10-Minute Sample to Answer RT-PCR Respiratory Panel Performed on Point of Care (POC)

R. Peytavi, J. Hambalek, E. Alvarado, Y. Parocua, A. Abeje, F. Foltz, B. Pollack, P. Halle, M. Petrullo, K. Allen, H. Kido, R. Martin. *AMDI, Santa Ana, CA*

Background:

The COVID-19 pandemic has shown the importance of fast and sensitive PCR testing to identify SARS-CoV-2 (SC2) infected patients. Identifying infected individuals with a fast sample to answer test provides the opportunity to limit the spread of the virus and prescribe antimicrobial treatment in the same visit. Additionally, the post pandemic world has seen simultaneous outbreaks of RSV, influenza, and SC2, the so-called tridemic. These higher risk respiratory viruses can appear against a background of lower risk viruses such as rhinovirus, adenovirus, enterovirus, other corona viruses, and bacterial infections that present with similar symptoms and make triage challenging. Fast turnaround molecular testing along with multiplex detection of all key pathogens associated with flu-like symptoms is important for timely selection of treatment, counseling to contain the spread of disease, and provide public health officials information on prevalence of outbreaks. AMDI is developing a cloud-based, ultrafast, fully automated POC molecular technology that enables detection of up to 32 microbial targets in less than 10 min from sample to answer. A 15-second extraction-free hyperbaric heating (HBH) step is performed on the input swab sample to release nucleic acid and neutralize PCR inhibitors in the sample in seconds. The sample is directly transferred without dilution to a chamber where it mixes with dry RT-PCR reagents and then is dispatched to eight thin (<250 µm thick) PCR reaction cuvettes where rapid heat transfer can occur. This is followed by thermocycling on an instrument designed to achieve 10 sec per cycle and detect four fluorescent signals per cuvette. This study first evaluates the HBH method against nucleic acid purification assays using SC2 positive clinical samples. Next, a preliminary LoD on the AMDI system (HBH and fast thermocycling) was performed using inactivated SC2 and RSV whole organism in a nasal swab matrix.

Methods:

To evaluate HBH as a sample prep method, 12 known SC2 positive remnant clinical samples in VTM or saline were diluted 1:1 with AMDI collection buffer, treated by HBH, tested by RT-PCR on QuantStudioTM and compared with the Roche LIAT. A preliminary SC2 and RSV LoD on the full AMDI system was determined by spiking dilutions of inactivated viruses into pooled nasal swab matrix, followed by treatment with HBH, and testing by RT-PCR using the ultra-fast thermocycler.

Results:

In clinical sample testing HBH shows equal or greater sensitivity than LIAT, even for high Ct samples. HBH followed by RT-PCR detected 12/12 (100% sensitivity) samples while Roche LIAT detected 11/12 (92% sensitivity). When HBH is coupled with AMDI ultra-fast PCR thermocycling technology, an SC2 preliminary LoD of 500 copies/mL and an RSV preliminary LoD of 250 copies/mL was obtained in <9 min including a 60 second RT step.

Conclusion:

HBH is an efficient and effective sample prep method and when coupled with ultrafast thermocycling provides sensitive PCR results in 10 minutes sample to answer.

A-377

The Selection and Implementation of POC Illicit Drug Toxicology Testing in Hamad Medical Corporation - Doha, Qatar

E. AL KUWARI[1], D. Al-Wali[1], J. S. Habib[2], Z. Kabir[1], A. Abdelfadel[1], E. Torato[1], A. Dellil[1]. *[1]Hamad Medical corporation, Doha, Qatar, [2]Hamad Medical Corporation, Doha, Qatar*

Background: In 2013, Qatar launched the National Mental Health Strategy which is further supported by National Health Strategy 2018-2022 and Qatar National Vision 2030, where the mental health has been identified as a priority area of development. In doing so, the Ministry of Public Health worked closely with the Ministry of Interior in providing the appropriate treatment rehabilitation. With all considerable advantages of point-of-care testing (POCT) in rapid diagnosis, POC toxicological test has been implemented and used in screening of patients for drugs of abuse. In this study, we highlighted the challenges in selection and implementation process of POC toxicological test in Hamad Medical Corporation (HMC).

Methods: Due to unavailability of all required drug panels, it had to be customized and tested for the verification purpose before starting the evaluation process. Maintaining

HMC procurements regulations and Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologist (CAP) standards for POC toxicology tests, two POC illicit drug toxicology screening tests has been evaluated, comparison study has been performed against the HMC central laboratory. POCT team conducted training session ensuring the appropriate practice in place. The Extendable Laboratory Information System (LIS) functionality has been utilized for seamless data management and reporting for the automated analyzer.

Results: QUIDEL triage meter pro is an automated drug testing analyzer which detects Amphetamines, Barbiturates, Benzodiazepines, Cannabinoids, Methadone Metabolite, Methamphetamine, Opiates, Tricyclic and Cocaine Metabolites, while the customized manual testing kit FASTEP detects Tramadol (TRA), Methylenedioxymeth-amphetamine (MDMA), Phencyclidine (PCP), Buprenorphine (BUP), Oxycodone (OXY), Fentanyl (FYL), Ethyl Glucuronide (EtG), Cotinine (COT), Pregabalin (PGB) and Gamma Hydroxybutyrate (GHB). Both analytical performances were verified. Rapid bedside testing performed for more than 100 patients in last 8 months during admission, observation, and pre- discharge process. There is An Obvious improvement observed in testing turnaround time compared to the laboratory, easy results interpretation and reporting as well as cost-effective.

Conclusion: The implementation of POC illicit drug toxicology testing in HMC aligned with core pillars of the Qatar vision 2030 to deliver the right treatment at the right time and place. Its enhancing patient outcomes and overall Satellite health care sector in Qatar.

A-378

Clinical Evaluation and Implementation of Rapid COVID-19 Antigen Test in Qatar

J. S. Said, F. Afreen, D. Al-Wali, E. A. AL KUWARI, Z. M. Kabir, A. M. Salem. *Hamad Medical Corporation, Doha, Qatar*

Background: In March, the World Health Organization (WHO) declared COVID-19 as a global public health emergency. To curb the spread of virus, timely detection of infected patients and screening of suspected cases in massive population posed a challenge for the healthcare system. Rapid antigen tests (RAT) were high in demand as a fast, accurate and cost-effective screening tool to increasing the testing capacity. In this crucial timing, clinical evaluation and procurement of available COVID-19 RAT following the World Health Organization (WHO) guidelines was a huge challenge for Point of Care testing (POCT) department. The aim of this study is to highlight the challenges overcame in establishing a Point-of-care COVID-19 testing system including personnel training and building a compatible Laboratory Information System (LIS) upholding Hamad Medical Corporation (HMC) cyber security regulation.

Methods: Three regulatory approved RATs were evaluated against real-time polymerase chain reaction (RT-PCR) and analytical performance were verified. Maintaining the Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologist (CAP) standards for COVID-19 waived testing, POCT team initiated to conduct practical and educational training vastly through online platforms and face to face sessions. We utilized the extendable LIS functionality for fast medical decisions in hospitals and enabled "reason of testing" feature in Qatar's COVID-19 tracking application.

Results: Our performance verification showed 98.0% sensitivity and 100% specificity for Panbio™ COVID-19 RAT, 98.0% sensitivity and 100% specificity for Quidel SOFIA2 COVID-19 RAT and Roche COVID-19 RAT yielded a sensitivity of 97.0% and specificity of 100%. Total of 291 training sessions were conducted for 9710 clinical staffs across 17 HMC facilities and 185 private clinics. Using LIS and POC middleware features, obvious increment in RAT of more than 509 tests per day has been reported in a single emergency unit. Overall, 5.5% decrement in incorrect patient identification data using QUIDEL SOFIA2 automated device and LIS functions was recorded in laboratory quality indicator data. COVID-19 tracking application was linked to antigen results for contact tracing, isolating, and breaking the chain of transmission.

Conclusion: The methods established for the COVID-19 implementation showed acceptable performance fulfilling the elements of HMC product procurement specification and CAP Accreditation standards. Synergic actions of HMC-POCT with supportive laboratory management aided prompt decision making to optimize patient management and added efficiency to the public health control measures in Qatar.

A-379

Frequency of reassignment of blood gas sample source at a large tertiary care hospital

R. Kim, M. Altura, K. Gantt, I. A. Hashim. *UT Southwestern Medical Center, Dallas, TX*

Introduction: Arterial blood gases analysis are central to the assessment and management of the critical patient acid-base status and its collection requires expertise and is often collected by trained respiratory therapists, nursing, and medical staff. Venous blood gases, arterialized venous blood gases, and capillary samples are however easier to collect. In this retrospective, study we examined the frequency of reassignment of blood gases results to a different sample type.

Method: Retrospective review of blood gases results requiring sample type reassignment collected during the last three years from January 2020 to December 2022 were obtained. Results were analyzed for concordance with sampling site and workflow analysis.

Results: Over the three years study period (2020 to 2022), a total of 196 samples for blood gases analysis required reassignment to a different collection site representing 0.12 % of all blood gases testing. Among the 123 initial arterial collections, 72 were reassigned as venous, 40 reassigned to mixed venous sample, and one to capillary collection. Whereas, among the initial 24 venous samples collections 16 were reassigned as arterial and 8 to mixed venous collections. Additionally, among the 25 initial mixed venous collections, 16 were reassigned as arterial, 9 reassigned as venous and 3 were reassigned as capillary collections. The frequency of reassignment from arterial to venous was significantly higher compared to venous to arterial ($P<0.05$) or for the reassignment in the mixed venous collections. Although a smaller number of samples were amended to capillary, capillary blood gases reflect arterial samples.There was no significant difference in the frequency of sample type reassignment between the three years of collection with the exception of a slight drop in year 2020 where the majorly of patients were of COVID-19 infection and that collections were likely performed by the same assigned medical personnel.

Conclusion: Common errors in blood gases collection are failure to obtain an arterial sample and accidental collection of venous samples instead often due to close proximity is common. In this retrospective review, the common reassignment is that of arterial to venous followed by arterial to mixed venous and venous to arterial sample types. Artifactual causes that may lead to misclassification of sample type include sample integrity as exposure to room air falsely reduces pCO_2 and elevates pO_2 giving a false impression of an arterial collection, similarly, dilution of arterial blood samples by heparin is common (inadequate blood fill of syringe) falsely lowers pCO_2 both raising doubt as to the sample type.The impact of reassignment of change of sample type on outcomes and on interpretation requires analysis.

A-380

Concordance of Truvian's Point-of-Care Blood Testing Analyzer to Central Laboratory Testing

R. L. Higgins, M. Adams, D. Arndt, P. Desmond, B. Fernandez, G. Grabarek, N. Haase, J. Kim, R. Krupa, G. Mina, R. Morgan, J. Poland, G. Reed, R. Richardson, A. Schroeder, F. Lee, D. Marrinucci. *Truvian, San Diego, CA*

Background: Truvian has developed a fully automated blood testing analyzer (TruAnalyzer) capable of running a panel of clinical chemistry, immunoassay and hematology assays from a low-volume blood sample. Truvian's compact instrument was designed to deliver a convenient and affordable alternative to off-site traditional labs, providing lab-accurate rapid insights from a single, small sample of blood with no upfront pre-processing required to inform health decisions at the point of care. Here, we evaluate the precision and accuracy of a comprehensive wellness panel tested on the TruAnalyzer and compare its performance to FDA cleared devices.

Methods: TruAnalyzer performance was assessed for both precision and accuracy. For the precision studies, two commercially available controls (one for plasma-based assays and one for blood-based assays) were added to the Truvian consumables and loaded into the TruAnalyzer. Six replicates were run on each of the ten instruments (N=60). Coefficient of variance (CV) analysis was performed and compared against pre-established requirements. For method comparison studies, 50 unique donors were consented and enrolled in an IRB approved study and 68 donor samples were collected over a 3-day period. For each donor, three 4-mL tubes of blood were drawn: 1) a Lithium Heparin (LiHep) sample for the TruAnalyzer clinical chemistry and hematology panel, 2) a LiHep gel tube for the Roche Cobas c311 clinical chemistry assays, and 3) an EDTA sample for the Sysmex PocH-100i complete blood count with 3-part

differential. All samples were processed within 1 hour of collection. Using only 300 µL sample volume, the TruAnalyzer reported results for 23 analytes including CMP (no electrolyes), Lipid, HbA1c, and CBC with 3-part differential. Concordance to comparator devices was determined by Bland-Altman analysis.

Results: For the precision study, 13 out of 14 (93%) chemistry analytes had a between-system CV of ≤ 5.8%. For hematology, 5 out of 5 (100%) analytes had a between-system CV of ≤ 6%. For the method comparison study, RBC counts showed 98% of samples tested being within 6% of comparator values. WBC and platelet counts had 97% of samples tested being within 10% and 15% of comparator values respectively. 95% of hematocrit samples were within 6% of comparator values and 98% of hemoglobin samples were within 7% of comparator values. All hematology analytes showed equivalency to Sysmex PocH-100i as determined by Bland-Altman equivalency analysis. For clinical chemistry analytes, 14 out of 15 (93%) analytes showed equivalency to Roche Cobas c311 as determined by Bland-Altman equivalency analysis when CLIA ATE guidelines were applied. 7 out of 15 clinical chemistry analytes had ≥ 90% of results within ATE. 5 out of 15 clinical chemistry analytes had ≥ 80% of results within ATE.

Conclusion: The Truvian blood testing analyzer is the first multi-modality system capable of running hematology, clinical chemistry and immunoassays from a single, low-volume blood sample. Results from this study demonstrate good precision for the majority of panel analytes and excellent concordance to FDA cleared comparators for assays evaluated in this study.

A-381

Laboratory-based Reflexive Algorithm for Identification of Falciparum and Non-Falciparum-Species and its Clinical Utility in the Diagnosis of Malaria in Southern Ghana

K. A. Sarpong[1], L. Bansah[1], E. Armah[1], D. Perkins[2]. *[1]University of Ghana, Legon, Ghana, [2]University of New Mexico, Albuquerque, NM*

Background: Malaria presents a public health menace to millions of people in LMICs, killingmore than 600,000 people with the highest number in children living in sub-Saharan Africa.Laboratory testing provides the basis for >70% of clinical decisions, although their accessibilityand affordability are limited in LMICs and results in syndromic diagnosis and self-medication.The National Malaria Control Program guidelines specify that healthcare facilities perform RapidDiagnostic Testing (RDT) or microscopy for the diagnosis of malaria. This “one size fits all”approach has the unfortunate potential of resulting in a higher number of false positives and falsenegatives. Although *Plasmodium falciparum* is the predominant species in Ghana, emergingevidence suggests that non-*falciparum* species (*P. vivax, P. ovale, P. malariae,* and *P.* knowlesi)contribute to morbidity and mortality, and complicates the diagnosis and treatment for a subset ofpatients. The rationale for this study is the development of a reflexive, stratified diagnosticapproach that combines the use of RDTs and Microscopy/NAAT for the identification of*falciparum* and non-*falciparum* species in malaria endemic areas.

Method: Venous blood was collected from participants and five different RDTs on the Ghanaianmarket were used to screen the blood for the presence of malaria parasites along with microscopyand the LAMP method. The diagnostic performance of the RDTs was evaluated by utilizingmicroscopy as the gold standard. For microscopy, a volume of 6 ��L and 2��L of the blood sampleswere pipetted onto labeled frosted-end slides for the preparation of thick and thin blood films,respectively. Lastly, DNA was extracted from venous blood (QIAamp DNA Mini Kit, Qiagen,Germantown, MD, USA) and analyzed using a modified version of the Nested Malaria PCR assaythat can identify the five species of the Plasmodium parasite including P. knowlesi.

Results: Preliminary data from results from 71 participants showed an overall sensitivity of 90%for the identification of Plasmodium infection among all the RDTs examined in this investigation.The Wondfo® One Step Malaria HRP2/pLDH (P.f/Pan) test was the only RDT that passed theWHO recommended specificity of 90% when compared to microscopy. The WHO recommends aminimum sensitivity of 95%; however, none of the five RDTs utilized in this study met thisrequirement.

Conclusion: Although microscopy remains the gold standard for the identification of *Plasmodiumspp* for malaria diagnosis, it is becoming increasing popular for most healthcare facilities in LMICsto use RDTs for screening of the huge patient numbers that present with febrile illness. From ourpreliminary results, we have shown that positive cases by RDTRs are usually confirmed positiveby microscopy while most RDT negatives are false negatives by microscopy. Our combinatorialapproach with the molecular testing assay using LAMP increases the specificity of the testingprocess that guides treatment for malaria patients.

A-382

Evaluation of the Labcorp TrueSpin™ for Decentralized Blood Sample Centrifugation

G. Iacovetti[1], B. Collier[2], S. Setzer[1], M. Peevler[1], J. Ragar[1], W. Brandon[2], M. Chappell[2], R. P. Grant[2], G. J. Sommer[1], U. Y. Schaff[1]. *[1]Labcorp, Pleasanton, CA, [2]Labcorp, Burlington, NC*

Background

The increased demand for decentralized blood sample collection presents numerous operational challenges for diagnostics providers. Sample degradation including sample hemolysis due to time, temperature, and handling between collection and laboratory analysis leads to limited test menus and unreliable results. Here we evaluate the light-weight, portable Labcorp TrueSpin™ for rapid point-of-care blood separation using commercially available microvolume blood collection tubes. The TrueSpin is a class I FDA registered device designed for layperson use. The centrifuge runs on AA batteries and separates a blood sample in five minutes.

Methods

Here we show results of studies evaluating sample quality and analyte stability in serum samples collected into gel microtubes and processed using the TrueSpin. Hemolysis and residual red blood cell (RBC) concentration are compared between samples separated using the TrueSpin and conventional centrifugation. Additionally, we evaluate serum-based chemistry analyte stability in both separated and unseparated samples stored for three days refrigerated and unrefrigerated.

Results

No significant difference was seen in hemolysis or residual RBC concentration in serum samples prepared by TrueSpin compared to the reference centrifuge method. Furthermore, we show that many common serum-based chemistry analytes have limited (< 1 day) stability if uncentrifuged, but improve to ≥ 3-day stability following TrueSpin separation and refrigerated or room temperature storage (see Table 1).

Conclusions

These findings suggest that the TrueSpin is a simple and effective solution for remote sample separation and may enable broader test menus and increased test result reliability for decentralized sample collection pursuits.

	Stability (days)			
Analyte	Centrifuged 20-25° C	Centrifuged 2-8° C	Uncentrifuged 20-25° C	Uncentrifuged 2-8° C
Albumin	3	3	3	3
ALP	3	3	3	3
ALT	3	3	1 (+)	3
AST	3	3	1 (+)	3
BUN	3	3	0 (+)	1 (+)
Calcium	3	3	3	3
Chloride	3	3	1 (-)	3
Carbon Dioxide	3	3	1 (-)	3
Creatinine	1 (+)	2 (+)	1 (+)	3
Direct Bilirubin	1 (-)	1 (-)	3	3
Glucose	3	3	0 (-)	0 (-)
Phosphate	1 (+)	3	0 (+)	2 (+)
Potassium	3	3	1 (+)	0 (+)
Sodium	3	3	3	0 (-)
Total Bilirubin	3	3	3	3
Total Protein	3	3	3	2 (+)
Uric Acid	3	3	1 (-)	3

Table 1. Days of stability for each analyte in an expanded comprehensive metabolic panel when compared to baseline values using CLIA standards for method bias. (+) indicates a higher than acceptable bias before the three-day incubation was complete and (-) indicates a lower than acceptable bias before the three-day incubation was complete.

A-383

A Novel Lateral Flow Immunoassay for Health Biomarker and Immunosuppressive Drug Monitoring in Fingerstick Blood in a Point-of-Care Setting

T. Nguyen[1], M. Ortega[1], E. Vuu[1], Y. Kulahci[2], F. Zor[2], V. S. Gorantla[2], A. C. Daza Aguilar[3], E. Tantisattamo[3], U. Reddy[3], C. Rhee[3], K. Kalantar-Zadeh[4], J. Gerber[1], R. Ganguli[1]. *[1]Intelligent Optical Systems, Inc., Torrance, CA, [2]Wake Forest for Regenerative Medicine, Winston-Salem, NC, [3]University of California, Irvine, Irvine, CA, [4]Harbor-UCLA Medical Center, Torrance, CA*

Background Rapid, CLIA-waived quantitative testing can help eliminate delays in receiving care, improve telemedicine, enhance the management of chronic diseases and clinical trials, and reduce healthcare costs. Intelligent Optical Systems has developed an innovative Enhanced Lateral Flow Assay (ELFA) platform and partnered with the University of California, Irvine Nephrology Division and Wake Forest Institute for Regenerative Medicine for the clinical validation of the assay. The ELFA platform quantitatively measures the immunosuppressive drug tacrolimus (TAC), and health biomarkers such as proinflammatory cytokines IL6, IL8, matrix metalloproteinase-3 (MMP3), glial fibrillary acidic protein (GFAP, an indicator of traumatic brain injury), and cystatin C (CysC, an indicator of kidney function) from fingerstick blood at the point of care. The testing panel is continuously expanding. **Methods** The ELFA platform is an *in vitro*, quantitative, single plex or multiplexed lateral flow immunoassay that consists of three main components: (i) simple fingerstick whole blood sampling kit; (ii) lateral flow test strip with running buffer and dried fluorescent labeled affinity reagents; and (iii) miniaturized readout device that can be further developed with a controlled user interface, enabling untrained users to easily record results and instantly transmit them to the physician. Depending on the analytes, the ELFA point-of-care platform has undergone bench testing or clinical validation studies, including: (1) Single plex for TAC: Clinical validation using 5 uL of capillary fingerstick whole blood from kidney transplant recipients (KTR, n=50) compared to standard LC-MS/MS laboratory method. (2) Multiplexed ELFA for TAC, IL6, and MMP3: In vitro assessment of limit of detection (LoD), detection range, specificity, and repeatability (n=5) with 10 uL spiked whole blood. (3) Single plex ELFA for GFAP, IL8, and CysC: In vitro determination of the LoD, detection range, CV%, and correlation of spiked and estimated analyte levels with 10 uL of spiked whole blood. For all analytes, a 4-parameter logistic calibration curve with n=3 for each level of analyte was constructed to determine the LoD, detection range, and CV%. Pearson correlation and Bland-Altman analysis were applied to correlate and compare data between spiked and estimated levels, or between ELFA and standard lab test methods. **Results** Study (1) demonstrated excellent correlation between ELFA and LC-MS/MS, with a correlation coefficient r-value of 0.89, and at 95% limit of agreement, a mean difference of 0.7 ng/mL in the clinically compared samples from 50 KTRs. Study (2) showed an LoD and range of detection of 2 ng/mL to 20 ng/mL, 1 ng/mL to 5 ng/mL, and 10 ng/mL to 200 ng/mL for the multiplexed detection of TAC, IL6, and MMP3, respectively. The repeatability assessment showed a <10%CV for all analytes. Study (3) showed an LoD and range of detection of 1 ng/mL to 10 ng/mL, 0.5 ng/mL to 5 ng/mL, and 0 to 5 mg/L for GFAP, IL8, and CysC, respectively. **Conclusion** The ELFA platform delivers minimally invasive, convenient sampling in a POC device using capillary fingerstick blood, and provides accurate, rapid measurements of TAC, which demonstrate good repeatability and strong correlation against LC-MS standard reference measurements.

A-384

Laboratory Evaluation of the SeptiCyte Rapid Host Response Assay In Differentiating Infection-Positive Versus Infection-Negative Systemic Inflammation Patients Suspected of Sepsis

L. Sankari[1], H. Dolin[2], R. Hejal[1], H. Mohammad[2], A. Windau[2], C. Greig[2], D. Savadekar[2], C. Weisenberger[2], K. Remy[1], F. Jacono[1], X. S. Zhang[1]. *[1]University Hospitals Cleveland Medical Center, Case Western Reserve University, Cleveland, OH, [2]University Hospitals Cleveland Medical Center, Cleveland, OH*

Background: Early diagnosis of sepsis is critical for timely treatment but remains challenging. A rapid host response assay, SeptiCyte, was recently approved by the FDA for aiding early differentiation of infection-positive from infection-negative systemic inflammation in patients suspected of sepsis. This test is a fully automated reverse transcription polymerase chain reaction (RT-qPCR) assay that quantifies changes in the expression of two host immune response genes and results are available in about 1 hour. The present study aimed to evaluate the SeptiCyte assay performance in a clinical setting at a large medical center. **Methods:** Eighty-two patients admitted to the University Hospitals Cleveland Medical Center with suspected sepsis from July to September 2022 were included in the study. SeptiCyte samples were obtained at the time of initial evaluation. In addition, healthy donor blood samples and extracted RNA samples were also utilized for assay validation. The SeptiCyte assay was performed according to the manufacturer's instructions using multi-chambered fluidic cartridges and the Idylla Console (Immunexpress, Seattle, WA). The assay result was reported as the SeptiScore (range 0-15), a calculated ratio of the expression of two host immune response genes, and the SeptiCyte band (ranging from 1 to 4, with 1 indicating the lowest risk of sepsis and 4 indicating the highest risk). The assay cutoff values are 5.0, 6.2 and 7.4 respectively for the band 2, 3 and 4. **Results:** Intra- and inter-assay precision was 0.2-6.2% and 0-3.2%, respectively. % Recovery at the cutoff for band 3 (SeptiScore 6.0) was 102.4% with a CV of 2.4%. Method comparison with the Immunexpress lab demonstrated equivalent performance, as evidenced by a slope of 0.99 and an intercept of 0.27 by Deming regression. SeptiScore showed a weak positive correlation with CRP (Correlation Coefficient 0.389, $p<0.01$) but no association with procalcitonin (n=76), lactate (n=78), international normalized ratio (INR), platelet, neutrophil, lymphocyte, or white blood cell (WBC) count (n=82). CRP (n=71) median concentration in patients with band 4 (16.8) was significantly higher than in patients who were band 1 (3.6). The SeptiScore of blood culture positive cases (n=22) was 7.2 ± 2.0 (mean ± SD) and that of pathogen negative non-sepsis (by sepsis retrospective review of event only, n=10) cases was 5.6 ± 1.6 ($p<0.05$). In addition, the median SeptiScore was notably higher in patients with viral infection compared to those without infection (9.2 vs. 6.2, $p<0.05$). Further analysis showed 92% (11/12) of patients with hematologic cancer were categorized as band 4 including one non-sepsis case per the retrospective review. SeptiScore was similar between patients with non-hematologic cancer and those without malignancy ($p>0.05$). **Conclusion:** The SeptiCyte RAPID assay showed good analytical performance. SeptiScore represents an independent factor to quantify host response in patients suspected of sepsis. It may add value in aiding differentiation between infection-positive from infection-negative systemic inflammation with promising results in patients with viral infections or those with positive blood cultures. The negative predictive value of low SeptiScore within band 1 or 2 may need further investigation.

A-385

A Point-of-Care Test to Assess Vaccine Response and Recent Infection by Quantitative Measurement of Neutralizing Antibody, Anti-Nucleocapsid, and Anti-Spike Levels

A. Perebikovsky[1], J. Y. Lim[2], H. Munoz[1], M. C. Caparas[1], A. Fiore[1], A. Srinivasan[1], D. Okrongly[1], Y. Liu[1], H. Janwari[2], G. Murray[2], B. Martinez[1], B. Le[2], K. Rashidi[1], H. Kido[1], A. C. Roberts[1], H. Lu[1], B. Pollack[1], E. Shamloo[1], B. D. Miller[1], D. Wright[2], R. E. Davis[2]. *[1]Autonomous Medical Devices Inc, Santa Ana, CA, [2]Autonomous Medical Devices Inc, Menlo Park, CA*

Background: Following vaccination against SARS-CoV-2 (SC2), the human immune system produces neutralizing antibodies (NAb) that block the virus from entering host cells. These NAb levels are highly predictive of protection against SC2 infection and progression to severe disease [1]. Unfortunately, individual humoral responses vary and NAb titers decay over time, making measurement of NAb important in timing vaccine boosters, especially for at-risk individuals.

Microneutralization assays (MNA) can be used to measure vaccine effectiveness by quantifying virus-specific NAb titers. However, these assays have long turnarounds, require a BSL3 lab, and have high inter-lab variability [2].

The Q-NAb™ test was developed to quantitatively measure NAb levels and correlate with MNA [3]. Q-NAb is available as both an ELISA for central laboratories, and a multiplexed POC centrifugal disc assay that measures NAb, anti-Nucleocapsid protein (NP), and anti-Spike antibodies.

Methods: The heart of the Q-NAb test is a recombinant fusion protein (FP) that blocks anti-RBD non-neutralizing antibodies (NNAb). FP consists of human ACE2 fused to the Wuhan-RBD of the SC2 Spike. Designed to conceal the the NAb binding epitopes of RBD, FP serves as a specific depleting agent for NNAb. The pretreated sample is then incubated with immobilized Wuhan-RBD to obtain a NAb readout.

Both Q-NAb formats were calibrated to WHO international standard 20/136 using a set of 7 calibrators and correlated to MNA results. NAb levels were determined using >200 clinical samples collected 2-12 weeks post monovalent or bivalent vaccine booster and compared across age groups (<60, 60-70, 70-80, 80+ years).

Results: Both Q-NAb Disc and ELISA show correlation to each other (n=167, WLS, $R^2 = 0.85$) and are traceable to international standards. Q-NAb also correlated with MN_{50}: ELISA (n=137, Spearman's $\rho = 0.88$) and Disc (n=208, Spearman's $\rho = 0.91$). After receiving a bivalent booster, no difference in median NAb levels across age groups was observed (Kruskal-Wallis, p=0.3). While 99.4% of samples had a positive

anti-Spike, using a NAb cutoff of 1000 IU/mL derived from the literature [2, 4-5], we found the percentage of individuals below that cutoff increased with age (9.8% for <60 years, 20.4% for 60-70 years, 19.5% for 70-80 years, and 34.1% for 80+ years). In addition, 32% of samples had an anti-NP level indicating recent infection.

Conclusion: The Q-NAb test, available in both central lab and POC formats, measures NAb levels, correlates to MNA, and is traceable to international standards. Q-NAb was used to assess humoral responses following vaccine boosters in a clinical sample set. While virtually all subjects had anti-Spike levels, the percentage of individuals falling below a NAb cutoff of 1000 IU/mL ranged from 9.8% to 34.1%, depending on age. Additionally, about one-third of patients had anti-NP activity, indicating NAb levels resulted from a combination of vaccination and infection. This data shows Q-NAb is an easily accessible and more accurate tool to identify a subset of at-risk patients that anti-Spike antibody tests alone do not discriminate.

A-386

Performance assessment of Point of care testing for blood glucose: a 5 year review of EQA data

M. A. Thomas[1], G. Davies[2], N. Blount[2]. *[1]Cardiff and Vale University Health Board, Cardiff, United Kingdom, [2]Weqas, Cardiff, United Kingdom*

Introduction WEQAS is the largest External Quality Assessment (EQA) provider for the Point of Care Testing (POCT) market within the UK supplying samples and assessment to over 30,000 sites per month with over 20 Programmes across the entire Health care sector. All the Programmes are accredited to ISO 17043.The aim of the programme is to provide support to POCT co-ordinators, to identify non compliant sites and improve the analytical performance of users. Most of the programmes are designed to allow the full assessment of all the devices within the organisation at either monthly, bimonthly or quarterly intervals. A Co-ordinator in each organisation is given a Group Administrator function for the EQA website and maintains the database for its own organisation. The role of performance surveillance is therefore devolved to each individual Co-ordinator at a local level and monitored nationally by the EQA organiser. The powerful database gives POCT Co-ordinators a wealth of information on method and analyser performance both within their own organisation and between organisations. Organisation performance summary reports, distribution letters, non-compliance reports, poor performance reports and cumulative reports are generated from one system. The POCT Users are also provided with a simple traffic light system with clear action limits. For the POCT glucose programme one sample is distributed each month with samples covering a range of 2 to 28 mmol/l annually.

Methods A review of performance was undertaken between January 2018 and January 2023 for the POCT glucose Programmes for a selection of devices. Devices that were no longer available were excluded as were devices with < 50 users in the group. This represented data from 10,360 devices per month in 2018 and 12,277 in 2023. Performance was assessed as the coefficient of variation (CV%) for the device group after outlier exclusion and calculated for a wide range of glucose concentrations.

Results For devices with CV less than 5% in 2018 there was generally no further improvement in performance, however at a glucose of 2 mmol/L an improvement in performance of 0.5to 3.5% was observed for the other devices. In 2018 CVs ranged from 3.7 to 12% and 3.1 to 8.3%% at 2.5 and 26 mmol/L respectively compared with 4 to 10.3% and 3.3 to 7.2% in 2022.

Conclusion Although there were numerous examples where EQA identified poor performance at an individual user level, there was little evidence to suggest that the performance of POCT glucose devices had improved significantly over the last 5 years.

A-387

Evaluation of the analytical and clinical performance of epoc point of care equipment compared to standard blood gas in patients with nephropathy

R. F. Paula[1], P. F. Távora[2], M. M. Gomes[2], J. d. Neto[3], T. C. Leonel[1], L. I. Santos[2], A. D. Midrigal[1]. *[1]Siemens Healthineers, São Paulo, Brazil, [2]i9med laboratory – Diagnostic Medicine, Belo Horizonte, MG, Brazil, [3]Nephrocynics, Belo Horizonte, MG, Brazil*

Backgroud: Chronic kidney disease (CKD) is a public health problem, characterized by progressive loss of function of nephrons with consequent loss of ability to filter blood and maintain homeostasis. Due to the absence of symptoms, CKD usually receives a late diagnosis, which causes the patient to be immediately submitted to the hemodialysis procedure. Therefore, it is important that the patient has a fast and effective diagnosis, however it is necessary that it be done with clinical and analytical safety. **Objective:** This study aimed to evaluate the performance of siemens Healthineers epoc® platform for the quantification of blood gas parameters, glucose, creatinine, ions, hemoglobin, hematocrit comparing this equipment with the reference methodology for blood gases in patients with CKD**. Methods**: Eighty-five blood samples obtained from patients under clinical follow-up with nephrologists were collected and processed at epoc® and compared with the RapidPoint®500e Siemens Healthineers platform. Creatinine was compared with Abbott platform Architect®. Discrepancies related to clinical values and/or analyses were evaluated, considering the reference values determined for each method. For analytical verification, we analyzed the means of the results of each methodology, the percentage differences between the means of the results and Pearson's correlation coefficient. **Results:** For clinical analysis considering the reference values for each method, all analyses were approved. Pearson's correlation coefficient was obtained for pH= 0.97, for pCO2 = 0.953 and pO2 = 0.9947. For glucose, the value of 0.9919 was obtained and for the ions, 0.887 for Na+ (sodium), 0.9727 K+ (potassium) and 0.9406 for Cl- (chloride). For Hb and Hct were 0.825 and 0.8231, respectively. For creatinine it was 0.9339. The analysis showed the differences in mean for Hb = 2.25%, Hct = 2.22%, ph = 0.1505%, pC02 = 10%, p02 = 6.28%, Na+ = 1.84%, Cl- = 3.93%, K+ = 1.77%, while glucose was 2.43% difference and creatinine 1.152%. The range analyzed for Hbt was 5.4 - 20.4, for ph of (7.133 - 7.475), for POC2 (29.8 - 115.8), for pO2 (15.1 - 118.4), for (Na+ 124.0 - 150.0), for (K+ 1.50 - 7.80), for (Cl- 92.0 - 121.0) and for K+ (1.50 - 7.80), for glucose (43.0 - 303.0). For creatinine it was (0.63 - 15.00) and (16.0 - 60.0) for hematocrit. In the accuracy evaluation, was obtained: Na+ (CVg = 2,4/DP = 3,29), K+ (CVg = 17,8/DP = 0,84), Cl-(CVg = 4,5/DP = 4,68), Glucose (CVg = 47,2/DP = 75,83), Creatinine (CVg = 32,6/DP = 3,10), ph (CVg = 0,883/DP = 0,065), pCO2 (CVg = 26,68/DP =12,82), pO2 (CVg = 50,15/DP = 29,42), Hct (CVg = 24,43/DP = 9,0), Hbt (CVg = 24,43/DP = 3,08). Conclusion: The study proved good results for the comparability between the epoc equipment® and the standard methodology. In addition, there was no difference in the clinical interpretation of patient studied, which is essential to ensure the safety in the use of these methodologies in the diagnosis and follow-up of CDK.

A-388

Evaluation of performance of the nBili assay on the RAPIDPoint®500e analyzer compared to the total bilirubin assay for the Dimension EXL200 and Atellica CH analyzers in neonates blood samples

R. F. Paula[1], T. C. Lamo[1], M. S. Lage[2], A. D. Midrigal[1]. *[1]Siemens Healthineers, São Paulo, Brazil, [2]Marcio Cunha Hospital, São Francisco, Ipatinga, MG, Brazil*

Background: Neonatal hyperbilirubinemia is very common, with 60% of term newborns (NB) and 80% of premature neonates developing this condition. If not diagnosed and treated, can lead to severe complications, with bilirubin encephalopathy (BAE) being one of the most feared outcomes. Analysis of whole blood samples measured by the RapidPoint®500 Blood Gas Analyzer (RP500) is a technology option that provides rapid bilirubin results with a small sample volume and can also measure other important analytes. However, it is still unclear whether the blood gas analyzer can be used for biochemical dosage of bilirubin in newborns. **Objective:** This study aimed to evaluate the clinical and analytical reliability of the method for detecting bilirubin in infants in comparison with classic biochemical analyzers such as Atellica® CH 930 and Dimension® EXL200. **Methods:** A comparative clinical study was performed to evaluate the acceptable analytical agreement of total bilirubin results from 41 samples from neonatal patients between Atellica® CH 930, Dimension® EXL200 and RapidPoint®500 analyzers. Data were analyzed using Vericheck® software. **Results:** Forty-one whole blood samples from newborn patients were analyzed for total bilirubin measurement. The range of analyzed results was from 2.7 to 20.8 mg/dL. Were considered for the analysis of clinical data: gestational age, weight, age, visual assessment of the newborn, criteria for hospitalization, whether phototherapy was performed and how long it took. In the regression analysis, it was possible to obtain a Pearson regression coefficient of 0.9806 between the Atellica® CH 930 and RP500 analyzer. The average of the values obtained with the RP500 was 10.8 and for the Atellica® CH930 it was 11.10, obtaining a percentage difference of means of 2.70%. In comparison with the Dimension®EXL200 analyzer, it was possible to obtain a Pearson correlation coefficient of 0.9814. The average of the values obtained with the RP500 was 10.8 and for the Dimension it was 9.32, resulting in a percentage difference of mean of 15.83%. **Conclusion**: The study showed good results for analytical comparability between analyzers. There was no change in the clinical management according to the criteria analyzed in conjunction with the laboratory analyses, making it possible to guarantee the safety of using the RP500 platform in the diagnosis and follow-up of newborns with suspected hyperbilirubinemia.

A-389

Implementation of a solicited laboratory workflow improves quality indicators for point of care blood gas testing by Respiratory Therapists

E. Ballard, H. Stieglitz. *The Ohio State University Wexner Medical Center, Columbus, OH*

Background: Quality indicators (QIs) including turnaround-time (TAT), critical result notification, corrected patient results, and unacceptable specimen are key measures of a laboratory quality management program that interrogates the pre-analytic, analytic, and post-analytic phases of the testing process. It is essential that high quality standards are maintained for critical care testing such as blood gas analysis. Achieving these high standards is difficult enough with specially trained laboratory personnel and can especially challenging with respiratory therapists (RTs) who are tasked with the difficulty of balancing patient testing while providing care. Tracking these QIs can also be cumbersome, especially if using manual methods. This retrospective study evaluates the effect of implementing a solicited laboratory information system (LIS) workflow using EPIC-Beaker on blood gas testing QIs performed at the point-of-care (POC) by RTs.

Methods: Blood gas QIs including TAT, percent of critical results notified, and number of corrected patient reports were compared between two workflows used by RTs. Metrics obtained between 1/1/2021-1/31/22 were from 13 POC blood gas analyzers from manufacturer #1, with manual TAT review of a sampling of specimens (5 days' worth of samples per month) performed in manufacturer #1 middleware; manual review critical result notifications; and manually completed cards to track corrected reports. Metrics obtained between 2/1/22-12/31/22 were from 13 newly implemented POC blood gas analyzers from manufacturer #2, using a solicited EPIC-Beaker workflow with barcode-enabled labels for specimen tracking of collect and received times, automated review of TAT (received to result, collect to result, and collect to receive) with reports, and utilization of the communication log activity to document critical result notifications and unacceptable specimen. LIS reporting tools were used to summarize monthly TAT, critical result notification, unacceptable specimen, and corrected report QIs.

Results: Between the time periods employing the manual vs Beaker workflows, percent of results meeting the TAT were 99.5% (results within 30 minutes of receipt) vs 95.5% (results within 30 minutes of collection); percent of critical results notified was 100% vs 100%; and number of corrected reports was 132 vs 24 (demonstrating an 82% reduction), respectively. Estimated time spent reviewing/compiling QIs by RTs was ~5 hours/week vs ~3 hours/month with the manual vs EPIC-Beaker workflow.

Conclusion: Using an EPIC-Beaker solicited LIS workflow significantly improved the number of corrected patient reports compared to the previous workflow. Of the critical results audited each month, the percent with notification documentation remained the same between the workflows, however, using reporting tools allowed faster auditing of all critical results obtained each month compared to the manual workflow that only audited a sampling of the critical results per month due to the prohibitive time needed for manual review. TATs monitored also did not significantly change, however, the Beaker workflow allowed for simultaneous auditing of multiple measures of TAT, compared to the former workflow that only included electronic documentation of specimen 'receipt' by the RT and not the actual collect time. Employing automated LIS workflows may improve POC blood gas testing QIs while streamlining the process of compiling QIs.

A-390

Implementation of the new GEM Hemochron 100 point-of-care coagulation system for heparin monitoring during cardiopulmonary bypass procedures

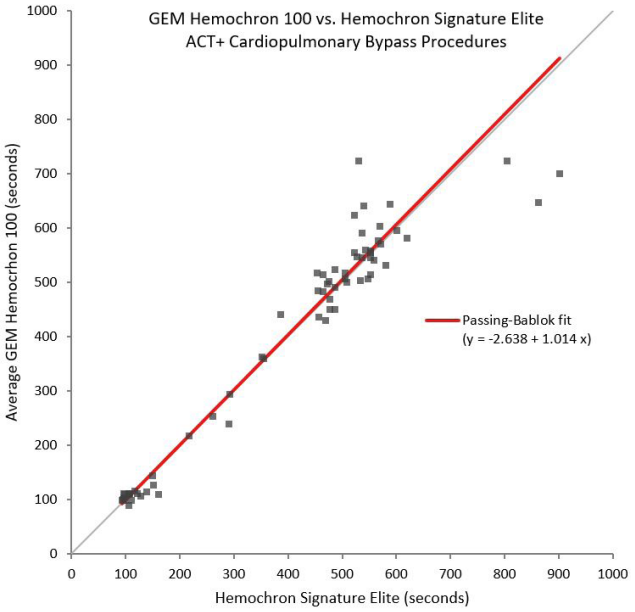
A. Sheffield[1], G. Oldendorp[2], M. Jordan[1], D. Mast[1], M. Losos[1], I. Siddiqui[1], H. Stieglitz[1]. *[1]The Ohio State University Wexner Medical Center, Columbus, OH, [2]The Ohio State University Wexner Medical Center, columbus, OH*

Background: The Ohio State University Wexner Medical Center has monitored the administration of unfractionated heparin (UFH) during cardiopulmonary bypass (CPB) procedures with the activated coagulation time test, using the Hemochron Signature Elite (Elite) instrument (Werfen) with the ACT+ cartridge. An assessment was conducted to evaluate the performance of the newest Hemochron model, the GEM Hemochron 100 (Werfen), to upgrade current instruments.

Methods: Precision and Methods Comparison studies were conducted with nine GEM Hemochron 100 systems. Each GEM Hemochron 100 instrument was analyzed with two levels of directCHECK liquid quality control, five replicates, over four days. The Method Comparison study compared the results from one Elite system's result, against the average result reported from one to three GEM Hemochron 100 systems. This methodology was repeated in multiple groups, using different Elite and GEM Hemochron 100 systems. All tests were performed with the ACT+ assay, which is sensitive to UFH from 1-6 IU/mL.

Results: Precision: All nine GEM Hemochron 100 instruments yielded: Level 1 CV ≤ 9.5%, and Level 2 CV ≤ 2.5%. Method Comparison: Total datasets 66; range 94-902 seconds; estimated bias at the 300, 420, and 480 second ACT+ clinical decision points were 1.6, 3.2, 4.1 seconds respectively; correlation (r) 0.97; slope 1.01; average CV across GEM Hemochron 100 systems 6.0%.

Conclusion: The performance of the new GEM Hemochron 100 system demonstrated acceptable agreement compared to the Elite system during CPB, maintaining existing ACT+ procedural target times. Results of system performance were consistent with the manufacturer's package insert, as well as previously published data. Based on the clinical performance, GEM Hemochron 100 systems have been implemented into our surgical suites.



Wednesday, July 26, 2023

Poster Session: 9:30 AM - 5:00 PM
Animal Clinical Chemistry

B-001

Method Development of C-Reactive Protein in the Serum of Non-Human Primates

A. Kalb. *Charles River Laboratories, Ashland, OH*

Objective: The objective of this study is to validate C-Reactive Protein (CRP) measurement in Non-Human Primate (NHP) serum using the ADVIA 1800 analyzer.

Methods: CRP was validated in NHP serum using the ADVIA 1800 analyzer. Validation testing included intra-assay precision, inter-assay precision, accuracy, linearity of dilution, limit of quantitation (LOQ), limit of detection (LOD), reference interval, carry-over, correlation, and stability. Samples were collected into tubes containing no anti-coagulant. Intra-assay precision was determined by analyzing two biological samples and the two quality control levels of Bio-Rad Lyphochek Immunology Plus Control 10 consecutive times (in duplicate) within a single run. Inter-assay precision was determined by analyzing two biological samples and the two quality control levels of Bio-Rad Lyphochek Immunology Plus Control six times for six days over a 10-day period. Accuracy was calculated using the data obtained from the inter-assay precision testing. For linearity of dilution, calibrator material and diluted specimen samples were analyzed in duplicate. The LOQ was determined by diluting the low-level calibrator material to produce a value at the low end of the reportable range. Diluted material was tested six times within the same run. The LOD was determined by assaying the appropriate blank 10 times within the same run. The reference interval was determined by analyzing 21 serum samples. Correlation between the two ADVIA 1800 analyzers was determined by assaying 10 samples on both analyzers once. The carry-over of the assay was determined by analyzing the high-level quality control material followed by the low-level quality control material. Stability of serum samples was tested at room temperature, refrigerated, and frozen (at -20°C) for various durations of storage.

Results: Results show that measurement of CRP in NHP serum met the acceptance criteria for intra-assay precision, inter-assay precision, accuracy, linearity of dilution, limit of quantitation, carry-over, and stability. For intra-assay precision in specimen and control material, the %CV was within ±20%. The inter-assay precision for specimen and control material %CV was within ±20%. The total error observed (TEobs) was within ±20% for accuracy in control material and the obtained mean was within the acceptable range specified by the manufacturer. For linearity of dilution, the coefficient of determination was ≥ 0.9000 and TEobs for each dilution and calibrator level was lower than 20%. The LOQ was set at the lowest concentration for which the TEobs was within ±20%. The LOD was calculated by adding the mean concentration obtained and three times the standard deviation. The reference interval was set to the 2.5th to 97.5th percentile interval using Excel software. The carry-over was acceptable as it was ≤2%. Correlation between the two ADVIA 1800 analyzers was determined to describe any bias in result interpretation. Stability at all storage conditions was found to be acceptable with a mean %difference within ±20%.

Conclusion: CRP in NHP serum met the acceptance criteria for all required validation parameters. The ADVIA 1800 analyzer can be used for preclinical studies to test CRP in NHP serum.

B-002

Method Validation of Cystatin-C in the Serum of Dogs

A. Sringeri. *Charles River Laboratories, Ashland, OH*

Objective The objective of this study is to validate the new Siemens Cystatin-C reagent for analysis of dog serum on the ADVIA 1800 analyzers. **Methodology** The ADVIA 1800 analyzer was used in the validation of Cystatin-C in Dog serum. Whole blood samples were collected in tubes free of anti-coagulant and centrifuged for 10 minutes at 2400 rpm. Serum was separated after centrifugation using appropriate separation techniques. Testing disciplines for the validation included intra-assay precision, inter-assay precision, accuracy, linearity, limit of quantitation (LOQ), limit of detection (LOD), reference interval, carryover, correlation, and stability. Intra-assay was determined by analyzing two biological samples and the three quality control levels of BioRad Liquichek Immunology Control 10 consecutive times (in duplicate) within a single run. Analysis of two biological samples and three levels of BioRad Liquichek Immunology Control six times for six days over a 10-day period was conducted to determine inter-assay precision. Accuracy was calculated using the data from the inter-assay precision testing. Specimen samples and Siemens Cystatin C-2 Calibrator were diluted and analyzed in duplicate for linearity. To determine LOQ calibrator was diluted to produce a value at the low end of the reportable range and tested six times within the same run. LOD was determined by assaying the appropriate blank 10 times within the same run. The reference interval was determined by analyzing 21 serum samples. Through analyzing 10 serum samples on both ADVIA 1800 analyzers correlation between analyzers could be determined. The carry-over of the assay was determined by analyzing the high-level quality control material followed by the low-level quality control material three consecutive times. Stability of serum samples was tested at room temperature, refrigerated, and frozen (at -20°C) for various durations of storage. **Results** The measurement of Cystatin-C in Dog serum met the acceptance criteria for intra-assay precision, inter-assay precision, accuracy, linearity of dilution, LOD, LOQ, carry-over, and stability as articulated in the results. The intra-assay precision and inter-assay precision in specimen and control material %CV was within ±20%. For accuracy the total error observed (TEobs) was within ±20% and the mean was within the acceptable range specified by the manufacturer. For linearity of dilution, the coefficient of determination was ≥ 0.9000 and TEobs for each dilution was lower than ±20%. The LOD was calculated by adding the mean concentration and 3 times the standard deviation. LOQ was set at the lowest concentration measured at which the %CV, %bias, and TEobs was within ±20%. The reference interval was set to the 2.5th to 97.5th percentile interval. The carry-over was accepted as it was ≤2%. Correlation between the two ADVIA 1800 analyzers was determined to describe any bias in result interpretation. Stability was found to be acceptable at all storage conditions with a mean difference within ±20%, except freeze/thaw #2. **Conclusion** Cystatin-C in Dog serum met the acceptance criteria for all required validation parameters. The ADVIA 1800 analyzer can be used for preclinical studies to test Cystatin-C in dog serum.

B-003

Decrease in serum apoE-rich HDL-cholesterol may be associated with cholesterol accumulation in the liver of rats fed a high-cholesterol diet

R. Shinohata[1], R. Takeuchi[2], S. Usui[2]. *[1]Okayama University, Okayama, Japan, [2]Tottori University, Yonago, Japan*

Background: Free cholesterol accumulation in the liver has been suggested to be one of risk factors for the progression of non-alcoholic fatty liver to non-alcoholic steatohepatitis. Therefore, a non-invasive approach to assess hepatic cholesterol accumulation in the early stage without elevation of liver enzymes (AST and ALT) may be useful in clinical setting. In this study, we show that serum apoE-rich HDL-cholesterol level is a possible indicator for predicting hepatic cholesterol accumulation in rats with diet-induced simple liver steatosis. **Methods:** Male Sprague-Dawley rats (8 weeks) were purchased from Charles River Laboratories. Four different diets were obtained from Oriental Yeast; control diet (MF), high-fat diet (HF; 68% MF, 30% palm oil, and 2% cholic acid), high-cholesterol diet (HC; 95.5% MF, 2.5% cholesterol, and 2% cholic acid), and high-fat/high-cholesterol diet (HFC; 81.75% MF, 15% palm oil, 1.25% cholesterol, and 2% cholic acid). The rats were randomly divided into 4 groups and fed as follows. The control and HFC groups received the MF and HFC diets for 8 weeks, respectively. The HF/HC group received the HF diet for the first 4 weeks and the HC diet for the next 4 weeks. The HC/HF group received the HC diet first, then the HF diet. Blood and liver tissue specimens were collected under anesthesia. Total HDL fractions were isolated from whole sera by the polyethylene glycol precipitation method, and applied into a cation-exchange column (HiTrap SPHP, GE healthcare) for determining apoE-rich HDL-cholesterol levels. Correlations between variables were assessed with Spearman's correlation coefficient (r_s). **Results:** Total cholesterol content of the liver was significantly higher in the HFC (11.1-fold, P<0.001), HF/HC (9.1-fold, P<0.001), and HC/HF (5.8-fold, P<0.001) groups than the control group. Hepatic free cholesterol was also higher in the HFC (5.5-fold, P<0.001), HF/HC (3.9-fold, P<0.001), and HC/HF (3.3-fold, P<0.001) groups. Hepatic triglycerides were slightly increased in the HC/HF (1.6-fold, P<0.01) and HFC (1.5-fold, P<0.05) groups. Serum apoE-rich HDL-cholesterol was significantly decreased in the HF/HC (0.21-fold, P<0.001), HC/HF (0.25-fold, P<0.001) and HFC (0.42-fold, P<0.01) groups. The HFC group had significantly higher serum ALT (3.0-fold, P<0.05) and AST (2.3-fold, P<0.01) levels, compared with the control groups. There was a significant increase in hepatic mRNA expression of TNF-α (24.8-fold, P<0.01) and TGF-β (3.8-fold, P<0.001) only in the HFC group, indicating liver inflammation and stellate cell activation. To exclude the effects of hepatocyte injury on apoE-rich HDL metabolism, rats with ALT less than 100 U/mL (n=16) were included in the following analysis. ApoE-rich HDL-cholesterol levels were significantly and inversely correlated with hepatic total cholesterol (r_s=−0.535, P<0.05) and free cholesterol (r_s=−0.532,

P<0.05) levels but not with hepatic triglycerides. Furthermore, the ratio of ALT to apoE-rich HDL-cholesterol was more highly correlated with hepatic total cholesterol (r_s=0.626, P<0.01) and free cholesterol (r_s=0.632, P<0.01). There was no such correlation for apoE-poor HDL-cholesterol. **Conclusion:** The present observations suggest that serum apoE-rich HDL-cholesterol levels may be a useful indicator for cholesterol accumulation in the liver of cholesterol-fed rats.

B-005

Blood pH and phosphate tend to follow an inversely correlated homeostatic status

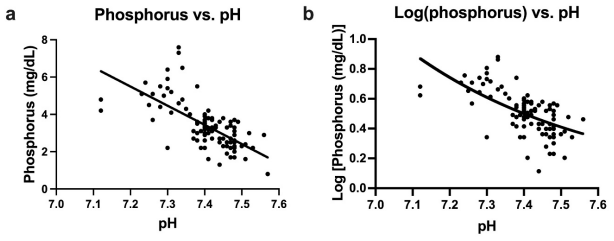
S. Islam, B. Osa-Andrew, J. Cao, A. R. Muthukumar, P. M. Jones, I. Hashim. *UT Southwestern Medical Center, Dallas, TX*

Background: Phosphorus containing biomolecules perform an essential biochemical function in maintaining extra and intracellular acid-base status. Maintaining normal concentration (2.5-4.5 mg/dl) of phosphorus is indispensable for optimal cellular function and homeostasis. Serum concentration of phosphorus (mainly inorganic orthophosphates) is an indicator of $H_2PO_4^-/HPO_4^{2-}$ acid-base buffer condition. Disturbance of pH could be an immediate impact of many respiratory and metabolic morbidities. Extra/intracellular redistribution of phosphates in response to an abnormal pH is an established homeostatic phenomenon. Failure to maintain an appropriate phosphate level to support the extra/intracellular redistribution may result in a pH shock. Therefore, it is important to have a clear understanding on how blood phosphate and pH levels are interconnected which could assist in guiding therapeutic interventions to fix phosphate abnormalities and acid-base disturbances. This study examined the homeostatic correlation between blood pH and phosphate level among hospitalized and ambulatory patients.

Methods: Serum phosphorus concentration and pH were retrieved from the biochemical profiles and arterial blood gas data of hospitalized and ambulatory patients who presented or admitted our tertiary care level university hospital during the first week of July, 2021. Logarithmic (base ten) values of phosphorus concentration were determined to normalize the distribution of the phosphorus data more uniform. Correlation analyses between phosphorus/log[phosphorus] and pH were performed by GraphPad PRISM version 8.

Results: A total of 101 corresponding blood gases and phosphate levels were obtained. Both phosphorus and log[phosphorus] values of the patients displayed an inverse correlation with arterial pH (figure 1 a,b). Pearson r values demonstrated a negative correlation between phosphorus/log[phosphorus] and pH (-0.6737 and -0.6450, respectively).

Conclusion: Monitoring of phosphate levels provide an early indication of acid base status. Phosphorus level maintains an inverse correlation with pH profile. Therefore, a therapeutic manipulation of phosphorus or pH may have a mutual effect requiring further investigation.



B-007

Measurement of cytokines in bronchoalveolar lavage (BAL) fluid and CSF supports model development for discovery programs.

T. Lambert, K. Lynch, R. Cortina, M. Schultz, T. Lewandowski, T. Sellers. *GSK, Collegeville, PA*

In our laboratory, increased demand for cytokine measurements to support both safety and efficacy endpoints across discovery programs has also resulted in cytokine analyses in biological fluids other than routine serum or plasma samples. Recently we have evaluated cytokines in bronchoalveolar lavage (BAL) fluid and CSF to support a mouse respiratory infection and a rat neuroinflammatory model, respectively. Fit-for-purpose validation of these sample types is necessary to confirm cytokine methods are suitable for use, including selection of optimal sample dilutions to reduce or eliminate any matrix effects or interferences. Meso Scale Discovery® (MSD Gaithersburg, MD) methodology was used for measuring cytokines and validation parameters included precision and accuracy of calibration curves (CV% and %Relative Error <20%) to confirm LLOQ and ULOQ for each cytokine method, precision of results for quality control and study samples (CV≤ 20%) and linearity of response in samples. In development of the mouse respiratory infection model, cytokines IL-1β, IL-6, KC/GRO and MCP-1 were increased in BAL fluid (up to 2892-fold) 20 hours post-infection compared to sham and vehicle control groups. Once the model was established, treatment of mice with Compound A demonstrated statistical decreases in these cytokines in BAL fluid compared to an infected control group. Dilutional linearity demonstrated that a minimal required dilution of 1:2 was required for mouse BAL fluids. In support of development of the rat neuroinflammatory model, cytokines IL-1β, IL-6, KC/GRO, MCP-1 and TNF-α were increased (325X, 13X, 28X, 23X and 7.7X, respectively) in CSF compared to plasma at 24 hours post treatment. Dilutional linearity evaluations demonstrated that CSF samples can be run neat but a 1:4 dilution was required for plasma samples. In conclusion, fit-for-purpose validation confirmed MSD methods were acceptable for use in supporting these drug discovery models.

Automation and Analytical Techniques

B-009

Accuracy validation of Elecsys HBsAg II Quant assay to resolve equivocal qualitative HBsAg results

D. Kim, J. Lee, J. Park, N. Kim, S. Lee, Y. Cho. *Jeonbuk National University Medical School and Hospital, Jeonju, Korea, Republic of*

Background: There are reports of false qualitative HBsAg results because of several causes, such as samples with low HBsAg concentrations that may produce false positives. The main aims of this study are to validate the analytical accuracy and operational cutoff value of the Elecsys HBsAg II Quant assay. The secondary goal is to assess the role of the Elecsys assay in resolving equivocal quali-tative HBsAg results compared to those of the qualitative HBsAg assay in the medical laboratory.

Methods: The limit of blank (LoB), limit of detection (LOD), limit of quantification (LoQ), and linearity were first estimated to validate the analytical accuracy of the Elecsys HBsAg II Quant assay. To validate the linearity of the assay according to CLSI EP06-A, five expected diluted as-sayed samples, including 0.01 (dilution ratio of minimum to maximum value = 4:0), 11.90 (3:1), 23.70 (2:2), 35.50 (1:3), and 47.30 IU/mL (0:4), were prepared within the concentration of the WHO 3rd IS (47.3 IU/mL) and measured for five replicates at each level. A total of 449 serum samples showing initial equivocal results (1-50 index) were evaluated by El-ecsys HBsAg II Quant and ADVIA Centaur HBsAg II assays.

Results: The LoQ of the assay was determined to be 0.050 IU/mL, as provided by the manufacturer. The Kappa agreement between the two assays was almost perfect at 0.9669 despite the seven dis-cordant results. The ROC curve analysis of this assay showed an AUC of 0.965 (P < 0.001), with a specificity of 100% and sensitivity of 24.15% at the new COI value of 5.42. Those HBsAg samples with index values < 5.42 need to be confirmed with an approved method or via additional HBV marker assays without further duplicate tests. The Kappa agreement between the two assays was almost perfect at 0.9669 despite the seven discordant results (three by ADVIA Centaur HBsAg II Negative and Elecsys HBsAg II Quant Positive; four by ADVIA Centaur HBsAg II Positive and Elecsys HBsAg II Quant Negative). The concordance rate and accuracy of the ADVIA Centaur HBsAg II as-say relative to the Elecsys HBsAg II Quant assay from the confirmed results were 98.44% and 98.55%, respectively, when the HBsAg seroprevalence of 5.3% from the Western Pa-cific region, including South Korea, was applied.

Conclusion: We confirm that the Elecsys HBsAg II Quant assay is a very accurate and sensitive for HBV infec-tion and recommend it as an alternative confirmatory HBsAg assay for resolving equivocal qualitative HBsAg results.

B-010

Comparison of Manual Urine Dipstick and Microscopy With Automated UC-3500 and UF-4000 Sysmex UN Series Urinalysis; A Comparative Experimental Research Approach at an Urban Referral Hospital in Ghana.

E. Toboh[1], R. K. Ephraim[2], K. Osei Darkwah[3], N. Afriyie Gyamfi[4], L. Duah Agyemang[5], E. Kumah Forkuo[5], P. Antwi[6], K. Appiagyei[7]. *[1]Ghana Health Service, Dansoman Polyclinic, Accra, Ghana, [2]University of Cape Coast, Cape Coast, Ghana, [3]Jeonbuk National University (JBNU) College of Natural Sciences Institute of Molecular Biology and Genetics, Jeonbuk, Korea, Republic of, [4]St. Elizabeth Hospital, Hwidiem, Ghana, [5]Komfo Anokye Teaching Hospital, Kumasi, Ghana, [6]Teeside University, Middlesborough, United Kingdom, [7]37 Military Hospital, Accra, Ghana*

Background: Urine analysis is one of the most common tests for assessing urinary-tract and kidney diseases. In recent years several automated instruments examining urine have been introduced. Though the introduction of automated urine analyzers is expected to reduce the labour involved, turnaround time and potential assay variations, manual urine analysis remains the gold standard or reference method for the analysis of urine. The new Sysmex UN-Series is believed to offer superior performance than other automated systems and manual methods. **Methodology:** A total of 67 routine freshly voided midstream urine samples were analyzed within 1 hour in our study. The results of the automated system were compared with the results of the manual processing which consisted of physical, dipstick chemistry and sediment analysis. Correlation and concordance/agreement between the automated and the manual system were assessed by Bland-Altman plot and Cohen's Kappa analysis respectively. **Results:** There was a significant fair agreement between the manual analysis and Sysmex UF-3500, in the determination of urine physicochemical parameters (k=0.193, p=0.004). Both showed a significant moderate correlation (r=0.593, p <0.001) and a significant fair agreement (κ=0.109, p <0.001) for specific gravity and pH determination respectively. The best concordance between the two methods was in the nitrates (κ=1.000, p <0.001). Both systems recorded strong positive correlation for RBC (r = 0.951 and R^2 = 0.904) and WBC (r = 0.91 and R^2 = 0.822, p <0.001) counts. Together, they had a significant good agreement [(65/67(97.0%), κ=0.734, p <0.001)] and a significant fair agreement [(48/67 (71.6%) κ=0.065, p=0.491)] for cast and bacteria counts correspondingly. **Conclusion:** We conclude that the new automated Sysmex-UN series urine analyzer can be safely used in our laboratory since it demonstrated good performance and compatibility with the manual method. We recommend that microscopy results of the automated platform should be confirmed by manual microscopy, particularly in pathological samples.

B-011

Analytical Validation of a New Vasoactive Intestinal Peptide Radioimmunoassay

S. Ashrafzadeh-Kian, J. Bornhorst, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

Background: Vasoactive intestinal peptide (VIP), a potent vasodilator, is produced by neuronal cells located in the central nervous system, the digestive, respiratory, and urogenital tracts, and the exocrine, thyroid, and adrenal glands. Increased circulating VIP is clinically useful to detect VIP-producing tumors in patients with chronic diarrheal diseases. **Objective:** Develop a sensitive replacement radioimmunoassay (RIA) for VIP quantitation in human plasma due to the diminished supply of the antibody used in the previous in-house method. **Method:** Patient VIP in EDTA plasma competes with labeled (^{125}I) VIP for a limited number of primary rabbit polyclonal antibody (BMA Biomedicals, Augst, Switzerland) binding sites. Antibody-bound VIP is then complexed with a goat anti-rabbit secondary antibody and precipitated out of the solution with the use of polyethylene glycol and centrifugation. After the supernatant is discarded, the ^{125}I signal from the pellet is counted on the Wizard2 gamma counter (Perkin Elmer, Waltham, MA). The measured signal is inversely proportional to the amount of VIP present in the patient's sample. Method validation of the new assay included determination of imprecision (pooled human EDTA plasma), limits of detection and quantification, analytical measurement range (AMR), accuracy by spike recovery, interferences, cross-reactivity, stability, reference interval determination, and agreement with the current Mayo laboratory-developed assay. **Results:** Stability studies on freshly collected EDTA plasma showed VIP is stable for 3 days ambient, 7 days refrigerated, and 14 days frozen. Intra-assay imprecision studies showed 8.3, 2.3, and 1.3%CV for mean VIP concentrations of 41, 144, and 304 pg/mL. Inter-assay imprecision was 12.9, 7.6, 5.2, and 7.5%CV for mean VIP concentrations of 30, 81, 180, and 308 pg/mL. The assay limit of detection was 29 pg/mL. The limit of quantitation was 30 pg/mL (%CV=13%) and was established via precision profile. The AMR was 30-500 pg/mL (slope of 0.98, intercept of 3.0 pg/mL, and R^2 of 0.97). The mean % spike recovery using a different preparation lot of calibrator material was 89% (range 83-93%). The assay was not affected by up to 1000 mg/dL hemoglobin, 1000 mg/dL triglycerides, or 10 mg/dL bilirubin. Peptide histidine methionine 27, neurotensin, gastric inhibitory peptide, motilin, secretin, and glucagon do not cross-react in the assay. The 97.5th reference limit was <86 pg/mL (95% CI 81-90, n=127). Positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) with the current Mayo VIP assay were 97, 82, and 90% respectively. **Conclusion:** We have developed a replacement VIP RIA. This assay has a lower limit of quantitation and a broader AMR than the current assay. Concordance with the current assay was acceptable.

B-012

Validation of a urine ammonium assay on Roche Cobas c502 chemistry analyzer

L. Song[1], k. H. Tong[2], M. I. Fournier[2]. *[1]David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, [2]Ronald Reagan Hospital, UCLA Healthsystem, Los Angeles, CA*

Background: Urine ammonium (uNH4+) excretion is critical for the maintenance of acid-base balance and reduced $uNH4^+$ excretion can cause retention of hydrogen ions resulting in metabolic acidosis in patients with chronic kidney disease (CKD). In addition, study found that declined urine ammonium excretion is an independent factor for predicting the worsening of kidney function. However, urine ammonium assay is not readily available on automated chemistry analyzers. We developed a protocol for the determination of urine ammonium levels using an ammonia assay for plasma samples on an automated chemistry analyzer. In this report, we present the urine ammonium protocol used in our laboratory and the validation of the urine ammonium assay. **Methods** All urine samples were diluted 100 times with deionized water and ammonium in the diluted samples was measured using the NH2L2 ammonia II assay on Roche cobas c502 chemistry analyzer. When a urine sample containing ammonium level less than 1.06 mmol/L, a 50-fold dilution was performed. Recovery was determined in six urine samples when 9 parts of the urine were mixed with one part of a standard 200 mmol/L ammonium solution (made with Ammonium Chloride from Thermo Scientific). Linearity was determined by serial dilutions of a sample containing 187 mmol/L ammonium. Limit of quantitation was determined using a 50-fold dilution protocol. The reference interval of 3-65 mmol/L was verified using 64 urine samples from volunteer donors and patient without CKD and metabolic acidosis. The stability was determined by testing two samples kept at room temperature for 0, 8, and 24 hour, respectively. The within-run precisions were determined by testing three urine samples 20 times in a run, and the between-run precisions were determined by testing three samples 2 times a day for 10 days, respectively. **Results**: Recovery was 95.8% to 102.2% in six urine samples contained varying amount of ammonium ranged between 6.0 mmol/L to 22.2 mmol/L. Linearity was 0.5-99 mmol/L. Limit of quantification was 0.5 mmol/L. The reference interval of 3-65 mmol/L was verified. Urine ammonium was stable up to 24 hours at room temperature. For within-run precision, the coefficient of variation (CV) was 1.51%, 0.49%, and 0.54% for samples containing 6.0 mmol/L, 27.7 mmol/L, and 75.7 mmol/L ammonium, respectively. For between-run precision, the CV was 2.54%, 5.98%, and 6.13% for samples containing 4.4 mmol/L, 27.9 mmol/L, and 55.1 mmol/L ammonium, respectively. **Conclusion**: Urine ammonium levels can be determined using the NH2L2 ammonia II assay on Roche cobas c502 chemistry analyzer after manual dilution of urine samples 100 times with deionized water. The limit of quantitation is extended to 0.5 mmol/L when urine samples are diluted 50 times with deionized water.

B-013

Association between laboratory parameters analysis and clinical severity in patients with coronavirus disease (Covid 19) at Hospital Universitario Fundación Santa Fe.

O. Martinez Nieto[1], H. A. Gonzalez[2], C. E. Saavedra Andrade[2], D. M. Bertel Rodriguez[2], P. A. Rodriguez Urrego[2], J. P. Vargas Gallo[2]. *[1]Fundacion Santa Fe de Bogotá, Bogota, Colombia, [2]Fundacion Santa Fe de Bogota, Bogota, Colombia*

Background:

Clinical management of COVID-19 disease was challenging. Laboratory parameters could be useful to classify patients in risk scores and probability to progress to a severe stage at the emergency room. This study aims to determine the association

between laboratory parameters of COVID-19 patients and clinical disease severity to provide applicable information to improved treatment strategies. Thus, we described the laboratory findings that can work as a predictive marker of severity, in a cohort of patients form our institution from Colombia diagnosticated with COVID-19 during a period of one year.

Methods:

This retrospective cohort descriptive study was conducted at one quaternary hospital at Bogota Colombia, where laboratory parameters on peripheric blood samples in 298participants with COVID-19 diagnosis were evaluated. For comparison between two groups' data, the chi-square and Mann-Whitney U test were used. For correlation between variable a Spearman and a Kruskal Wallis test were performed as data didn't normal distribution. A p-value lower than 0.05 was considered statistically significant in all analyses.

Results:

Primary outcomes shown significant correlation between more severe disease and interleukin IL-6 elevation, by the Kruskal Wallis test. Lower counts of lymphocytes percent, ferritin, fibrinogen, neutrophils, basophils and coagulation factors were found to be associated with severe disease (p<0,0001). Creatinine, D dimer, LDH, WBC count, creatinine and erythrosedimentation were significantly higher and were correlated with more severe disease on scores (p<0,001). Higher levels on PCR parameters were associated with severe disease (p<0,001).

Conclusion:

Laboratory parameters such as IL-6 can be used as a severity marker in order to help physicians find both adequate treatment and hospitalization location of COVID-19 patients. This information can be used to manage future pandemics, and to evaluate complementary strategies that should be efficient on epidemic management and patient care having an impact on public health, mortality rates and hospitality capacity

Table: Multinomial logistic regression analysis of laboratory parameters for clinical severity

Variable	Mild vs. Moderate		Mild vs. Severe	
	OR (95% IC)	p-Value	OR (95%) IC	p-Value
LDH	4,167 (3,873-4,561)	**<0,001**	1,774 (1,242-2,082)	**<0,001**
D-Dimer	2,754 (1,987-3,123)	**<0,001**	2,684 (2,042-2,934)	**<0,001**
Ferritin	0,918 (0,671-1,213)	**<0,001**	2,606 (2,340-2,890)	**<0,001**
IL-6	1,27 (1,032-1,987)	**<0,001**	2,934 (2,605-3,109)	**<0,001**
Leukocyte	1,756 (1,032-2,032)	**<0,001**	1,614 (1,023-1,903)	**<0,0001**
Lymphocytes	2,025 (1,972-2,344)	**<0,001**	5,404 (5,132-5,603)	**<0,001**
Neutrophiles	2,952 (2,400-3,230)	**<0,001**	3,115 (2,987-3,320)	**<0,001**
Plaquettes	2,346 (1,965-2,873)	**<0,001**	1,787 (1,501-1,903)	**<0,007**
PCR	2,670 (2,098-3,121)	**<0,002**	1,483 (1,322-1,543)	**<0,002**
PTT	1,833 (1,023-1,987)	**<0,001**	2,945 (2,608-3,032)	**<0,001**
TGP, ALT	0,752 (0,234-1,321)	**<0,002**	1,047 (0,932-1,192)	**<0,0002**
Troponin I	1,446 (1,021-1,986)	**<0,001**	1,724 (1,593-1,965)	**<0,002**

B-014

Impact of centrifugation time reduction in Laboratory Automation System

P. Choosongsang. *PSU, Hat Yai, Thailand*

Background: The centrifugation of specimens is an important preanalytical process which will be impact the most on turnaround time of the laboratory results. To verify the automation systems, we tested different combinations of tube and centrifugation settings to determine the most efficient for routine chemistry procedure. The outcomes of each combination were then compared to the benchmark to find out the best setting to reduce turnaround time for laboratory results.

Methods: In this study, we evaluated a total of 40 leftover blood samples that were collected on 4 mL plastic Vacutainer® (Becton Dickinson) lithium heparin tubes and 2 mL plastic Vacuette® (Greiner bio-one) lithium heparin tubes. All tubes were centrifuged on Abbott Automation GLP systems. The first set of samples was centrifuged according to the manufacturer's guideline (10 min at 1300 × g, room temperature for Vacutainer® tubes and 10 min at 2200 × g, room temperature for Vacuette® tubes). The second set was centrifuged 7 min at 2700 × g, room temperature. And the third set was centrifuged 5 min at 2700 × g, room temperature. Then, analyzed all samples with 22 routine chemistry parameters on Alinity ci analyzers. The allowable total error and correlation coefficient (R) were used to determine the significance of the differences between 3 protocols of the centrifugation time.

Results: The study showed that the centrifugation times of 7 min and 5 min were not significantly different from the manufacturer's protocol (10 min) after testing with 22 routine chemistry parameters (albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, direct bilirubin, total bilirubin, calcium, chloride, cholesterol, creatinine, low-density lipoprotein-cholesterol, iron, potassium, lactate dehydrogenase, sodium, phosphorus, total protein, triglycerides, unsaturated iron-binding capacity, high-density lipoprotein-cholesterol, urea nitrogen and uric acid) in both 4 mL plastic Vacutainer® lithium heparin tubes and 2 mL plastic Vacuette® lithium heparin tubes. Overall, the results of the study indicate that shorter centrifugation times of 7 min and 5 min at 2700 x g able to use for routine chemistry.

Conclusion: The study concluded that the centrifugation time did not affect the analytical results of 22 routine chemistry parameters. This suggests that shorter centrifugation times able to use with no impact on accuracy or reliability. The results of the study will support laboratory to have efficiency improvements by shorten the turnaround time on the results release.

B-015

Magnesium Fractionation using Ultrafiltration System

H. Badr[1], O. Anthony O[2]. *[1]UTMB, Galveston, TX, [2]Department of Pathology , University of Texas Medical Branch, Galveston, TX*

Background: Total magnesium (tMg) comprises 3 fractions: ionized (iMg), protein-bound (pbMg), and complexed magnesium (cMg). Serum iMg can be measured directly using ion-selective electrode method, but determination of pbMg and cMg has not been addressed. The aim of this study is to examine the efficiency of a newly introduced ultrafiltration system to fractionate tMg and validate proposed its reference intervals for Mg fractions. **Methods:** Leftover whole blood samples were collected from 40 subjects. iMg and tMG were measured in whole blood using Stat Profile Prime® ES, and ultrafiltrate using VITROS 5600 Analyzer, respectively. Serum fractionation was done by ultrafiltration system (Centrifree®, Sigma-Aldrich, MA, USA). pbMg and cMG were calculated by subtracting tMg ultrafiltrate from tMg in whole blood, and subtracting iMg from tMg concentrations in the ultrafiltrate, respectively. **Results:** Reference intervals of iMg, pbMg, and cMg were proposed as mean± 2 SD of 0.93-1.61 mg/dL, 0-0.85 mg/dL, and 0.07-0.57 mg/dl, respectively. iMg, pbMg, and cMg represented 65%, 20%, and 16% of the tMg concentration in normo-magnesemic subjects with slight variation in hypo- and hyper-magnesemic subjects. iMg showed weak positive correlation with tMg, (r=0.5, 0.03, 0.005), in normo-magnesemic, hypo- and hyper-magnesemic subjects, respectively. iMg showed weak positive correlation with cMg (r=0.11) and strongly with pbMg (r=0.41) in hypermagnesemic subjects. iMg was not dependent on protein concentration or pH, however it correlated positively with blood sugar (r=0.5 and 0.2) and serum creatinine levels (r=0.3 and 0.5) in hypo- & hyper-magnesemic subjects, respectively. **Conclusions:** The ultrafiltration system was a promising means to assess cMg and pbMg in critical care patients. iMg measurement provides better discrimination in ill patients and was associated with lifestyle factors including alcohol consumption, hypertension and diabetes. Stat Profile Prime® ES point-of-care analyzer provides rapid and precise direct measurement comparable to current tMg standard method. Due to its rapid turnaround time, it can help to efficiently manage workload of high complexity laboratories, and alleviate the burden on the critical care team.

B-016

Automated Next Generation Sequencing Fluidic-Based Device For Sample to Library Preparation: A Flexible Solution For Rapid Decentralized Sequencing for Infectious Disease Diagnostics

S. Chan, J. Tan, S. Tee, J. Park, Y. Ng, G. Michel. *Helaxy Pte. Ltd., Singapore, Singapore*

Background:

In addition to the established use of Next Generation Sequencing (NGS), such as drug resistance monitoring in HIV, the recent COVID19 pandemic has shown the crucial need of NGS technologies as tools for identification of clinically relevant variants of the SARS-CoV-2 virus.

Typically, in these settings, to be cost-effective most NGS analyses were conducted using larger batch sizes often resulting in long turn-around times of 2-3 weeks the need of trained personnel, and centralized testing facilities.

Methods:

We have developed an easy to use automated fluidic-based benchtop device to bridge a perceived gap in NGS library preparation and provide a solution for decentralized testing that does not require specially trained personnel.

With a batch size of 6, smaller hospitals and laboratories can perform NGS library preparation on fresh or archived samples with a fast turn-around-time of two days, facilitating earlier reporting to speed up diagnosis and treatment.

The Helaxy NGS fluidic device performs sample nucleic acid extraction and a 2-step PCR in a plastic fluidic card, which is the sole consumable required for the extraction and library prep process.The sample is applied to the fluidic card by manual pipetting followed by loading of the card into the device.

Nucleic acids are extracted with a combination of magnetic bead pull-down and centrifugal force in a purification chamber to give an eluate that is spun to a separate PCR chamber that uses the concept of thermal induction and cycling. Barcoding for library preparation follows within the same PCR chamber and the resulting product is transferred into another fluidic card for size selection purification, normalization and pooling to generate a library for input into compatible NGS sequencers, e.g., but not confined to, Illumina instruments.

Results:

In order to demonstrate clinical utility we have shown that the Helaxy NGS fluidic device can successfully extract nucleic acid from at least three sample types: SARS-CoV-2 RNA from nasopharyngeal swabs, HIV RNA from plasma and 16S RNA from stools.

Using in-house and commercial assays, we have generated libraries of target amplicons using the fluidic device workflow followed by sequencing with Illumina sequencers iSeq and MiSeq. Sequencing results analysed and reported by the Helaxy NGS Bioinformatics Reporting Pipeline revealed expected variants and mutations in the samples.

We correctly identified all the unique mutations such as L211del and G446S in commercially available inactivated Omicron BA1 variant of the SARS-CoV-2 virus as well as characterised drug-resistant mutations such as L90M in the Protease gene and D67N and Y181C in the Reverse Transcriptase gene in commercially available inactivated HIV-1 virus.

Conclusion:

The Helaxy NGS fluidic device can thus effectively provide an easy-to-use automated solution for sample library preparation in decentralized facilities to reduce the turn-around-time from sample collection to result reporting for clinical diagnosis and treatment.

B-017

The effect of temperature on the performance of streptavidin-coated paramagnetic particles in immunoassay applications

B. Wu[1], L. Li[1], D. Wang[1], Y. Xiong[2], W. Lu[2]. *[1]Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China, [2]Mindray IVD Innovation Center Minnesota, Oakdale, MN*

Background: Streptavidin-coated paramagnetic particles (streptavidin particles) are widely used in clinical immunoassays. Streptavidin has the highest affinity with biotin among all known non-covalent interaction we know today. With biotinylated molecules, be they small or large, strong binding of such molecules can be brought to streptavidin in a reliable and robust manner. In addition, streptavidin itself is a stable molecule, more resistant to heat, proteolysis, and chemical denaturation than a typical protein [1]. Many publications illustrate the binding sites, binding forces, and configurations of streptavidin with or without biotin [2][3]. From our experience working with streptavidin particles in immunoassays, we know that the challenges to their applications are mainly with baseline signal, biotin interference, and thermal stability. This abstract describes our findings on the thermal stability of assay reagents made from streptavidin particles. **Methods:** Intrigued by a recent publication that reports the effect of temperature on the affinity and stoichiometry of streptavidin with biotin [1], suggesting streptavidin's binding capacity as a function of temperature in the range of 4ºC to 40ºC, we coupled streptavidin particles (Dynabeads T1 type) with biotinylated thyroxine hormone (T4), estradiol (E2), folate binding protein (FBP), and anti NT-proBNP antibody at varied temperatures between 4ºC and 40ºC, and then evaluated their coating capacity and thermal stability at 30ºC or 37ºC. The detection was alkaline phosphatase conjugates with anti T4 antibody, folate analogue, anti E2 antibody, and anti NT-proBNP antibody, respectively. The evaluation used Mindray's automated chemiluminescent analyzer, CL6000i. **Results:** Our data indicate that temperature plays a critical role in enhancing the performances of streptavidin particles in these immunoassays. From pre-coupling treatment of the particle to coupling with biotinylated ligands and subsequently post-coupling blocking and aging, temperature shows great impacts on the stability and tolerance to heat of the coupled particles at elevated temperatures. In contrast to the effectiveness of pre-coupling washing at 50ºC or 55ºC, coupling of streptavidin particles with biotinylated ligands favors a lower temperature, for example, ambient temperature and 4ºC. Coupling at 37ºC to 40ºC results in slightly lower RLU signals in all four assays. Coupling at lower temperatures improves signal level and, more importantly, enhances thermal resistance of coupled particles. Post-coupling aging and optimization of diluent compositions can additionally improve accelerated stability data obtained at 37ºC. **Conclusion:** There are two mechanisms that negatively impact the stability performance of streptavidin particles coupled with ligands: One is gradual loss of loosely bound streptavidin molecules and the other streptavidin's configurational and structural changes triggered by temperature changes. Both mechanisms can be countered by proper control of temperature in the multiple steps of making the solid phase reagents. Our findings can help immunoassay developers to design more robust assay reagents involving streptavidin particles. **References:** [1] K.L. Mpye; S. Gildenhuys, and S. Mosebi. *AIMS Biophysics, vol. 7(4), pp 236-247, 2020*. [2] P.C. Weber; D.H. Ohlendorf; J.J. Wendoloski and F.R. Salemme. *Science, vol. 243, pp 85-88, 1989.* [3] W.A. Hendrickson; A. Pähler; J.L. Smith; Y. Satow; E.A. Merritt; R.P. Phizackerley. *Proc. Natl. Acad. Sci. USA. Vol. 86, pp 2190-2194, 1989*.

B-018

Compact all in one, Fully Automated Molecular Diagnostic System "ExiStation™ FA 96" in 100 minutes

K. Lee[1], D. Im[2], H. Chae[2], Y. Cho[2], J. Kim[2], E. Kwon[2], H. Kim[2], D. Jang[2], S. Park[2], S. Koo[2], S. Kim[2], S. Eom[2], H. Lee[2], S. Park[2], H. Park[2], H. Park[2]. *[1]Bioneer corp., Daejeon, Korea, Republic of, [2]Bioneer corp, Daejeon, Korea, Republic of*

Background:

Traditional PCR method's instruments are discrete the processes of sample collection, nucleic acid(NA) extraction, and NA amplification. As human labor is required between the instruments, there is a risk of human error, infection and contamination. Biosafety facilities such as biosafety cabinet, level D protective clothing, and negative pressure facilities are required for ensuring safe testing. To improve the testing environment, the molecular diagnostic market has developed and started using automated instruments, though, which take up larger space and take longer time to test. As a result, it is necessary to develop instrument that takes up less space and reduces testing time to a minimum, so, space and time can be saved.

Methods:

In this study, we developed an automated systematic genetic diagnostic instrument. In order to carry out the entire process within one integrated instrument, automatic mechanism of de-capping and re-capping of sample tube, sample aspiration, and sample transfer were developed. The system also includes integrated key steps of NA extraction and amplification(qPCR). We developed a primer/probe design technology that can accurately detect viruses with high accuracy in performing PCR tests using extracted genes, as well as precision temperature control algorithm and optimized real-time nucleic acid amplification using reverse transcription enzymes and gene amplification enzymes. In order to carry out all these functions within one device, we maximized user convenience by developing software for instrument operation, result analysis, and a Graphic User Interface for automation.

Results:

ExiStation™ FA 96 is largely composed of sample processing, gene extraction, and gene amplification (qPCR). *(Photo to be in poster)* Sample Processing automatically opens a cap of sample tube, then transfer a required quantity of sample to cartridge. While opening the cap, the barcode attached to sample tube is automatically identified and the information required for a test is entered. After sampling, the cap is automatically re-capped and placed back in the sample tube rack. When collecting samples, it measures the sample level in the tube and indirectly check the viscosity by detecting the pressure inside the tip, minimizing aspiration errors. The Extraction step performs the function of extracting genes. It consists of three modules of 32 wells, and the number of samples can even be adjusted in eight units, so the instrument can be operated flexibly upon the number of samples requested. Then, the qPCR step follows. The *ExiStation*™ FA 96 setup allows simultaneous testing of up to 20 targets for 94 samples in approximately 100 minutes. The instrument is equipped with a HEPA filter and UV lamp to maintain clean, and since the sample tubes are inserted directly on the instrument, it is no of necessity or save the costs such as of negative pressure rooms, biosafety cabinet, and level D protective clothing.

Conclusion:

ExiStation™ FA 96 is a fully automated molecular diagnostic test instrument that can quickly and accurately process large amounts of samples, while greatly reducing user risks and shortening molecular diagnostic test time.

B-020

Fully Automated and Scalable cfDNA Extraction Solution from up to 10 mL Samples

J. Lysen, J. Baggs, T. Butts, K. Durvasula. *Omega Bio-tek, Norcross, GA*

Background: The use of circulating, cell-free DNA (cfDNA) is rapidly evolving in the clinical landscape, becoming one of the fastest growing areas in recent years. It offers tremendous clinical potential as a screening method for tumor, cancer, disease progression, treatment response, and more. Circulating DNA is found in low quantities and often requires large sample input volumes to isolate the less abundant cfDNA to sufficient amounts needed for downstream analysis. The existing methods for extraction are often silica spin column-based, which involve an extensive amount of hands-on work, and they require large volumes of binding buffers, making it unsuitable for extractions involving sample volumes as large as 10 mL. These existing workflows impose certain limitations in terms of the sample volume and number of samples that they can process.

Methods: To tackle this necessity, Omega Bio-tek has developed a fully automated solution using Mag-Bind® cfDNA Kit (M3298) to extract cfDNA from up to 10 mL volumes from 48 samples in 2.75 hours when integrated on Hamilton's Microlab® STAR™ platform.

Results: The system is scalable and can extract from sample volumes ranging from 1-10 mL without any hardware modification or additional, expensive accessories on the Hamilton workstation. Here, we elucidate the workings of this automated protocol using Omega Bio-tek's Mag-Bind® cfDNA Kit and compare its performance to a manual extraction protocol from 4 mL of plasma processed using the same kit. We also present additional solutions using the Mag-Bind® cfDNA kit to showcase extraction of fragments as small as 50 bp with minimal genomic DNA contamination.

Conclusion: Omega Bio-tek's custom workflows provides the customers with different protocols to fit their different application needs. Thus, this kit offers researchers and scientists the flexibility to use the same kit for not only large volume sample processing but also for recovering short cfDNA fragments.

B-021

Analytical performance of thyroid function test on DxI800 system

J. Jang, H. Kang, H. Jang, J. Rim, J. Lim. *Yonsei University College of Medicine, Seoul, Korea, Republic of*

Background Thyroid function tests (TFT) which usually include thyroid stimulating hormone (TSH), total triiodothyronine (TT3), and free thyroxine (FT4) are widely applied in clinical practice for screening, diagnosis and monitoring of thyroid disease. We evaluated analytical performance of TSH, TT3, and FT4 using DxI800 (Beckman Coulter, USA) including comparison to Alinity I (Abbott, USA). **Methods** Precision was determined by percent coefficient of variation (%CV) measuring low, medium, and high levels of quality control material for 5 days with one run per day, 5 replicates per run. Linearity was evaluated using 5 levels of patient serum samples and concentrations were determined by the mean value of two replicates of each level. Method comparison was performed by Passing-Bablok regression using 40 patient serum samples. **Results** The within-run imprecision at low, medium and high level were 1.50%, 1.77%, and 2.59% for TSH; 4.10%, 2.45%, and 2.84% for TT3; and 3.23%, 2.76%, and 2.19% for FT4. The within-laboratory imprecision at low, medium, and high level were 1.61%, 1.91%, and 2.87% for TSH; 4.33%, 3.73%, and 3.70% for TT3; and 3.36%, 2.76%, and 2.20% for FT4. Linearity of TFT on DxI800 was demonstrated in the range of 0.01 - 44.68 µIU/mL, 0.09 - 7.40 ng/mL, and 0.18 - 5.52 ng/dL for TSH, TT3, and FT4, respectively. Method comparison between DxI800 and Alinity I showed a slope of 1.21 and intercept of 0.05 (correlation coefficient R=0.994); a slope of 0.95 and intercept of 0.13 (correlation coefficient R=0.971); and a slope of 1.81 and intercept of -0.88 (correlation coefficient R=0.969) for TSH, TT3, and FT4, respectively. **Conclusion** DxI800 showed acceptable performance in terms of precision and linearity for TFT assay including TSH, TT3, and FT4. This study demonstrated that TFT on DxI800 are suitable in clinical laboratory.

B-022

Technical evaluation of urine chemistry testing on the Beckman DxC700AU Chemistry Analyzer

C. Gea, F. Wong, M. Lim, B. Quek, J. Wong, C. Yeo. *Department of Clinical Pathology, Singapore General Hospital, Singapore, Singapore*

Background:

The Singapore General Hospitals' clinical biochemistry laboratory constantly reviews and optimizes its automated chemistry testing through workflow enhancements and advancement of technology. The laboratory had evaluated 11 urine chemistry analytes on a standalone Beckman Coulter DxC700AU in view of upgrading its existing Beckman Coulter AU680.

Methods:

The laboratory evaluated the following urine chemistry analytes: urea, sodium, potassium, chloride, creatinine, total protein, calcium, inorganic phosphate, magnesium, amylase and uric acid. The parameters evaluated were: linearity, limit of detection (LOD), carryover, imprecision and patient comparison against the AU680. Linearity was verified by analyzing Validate-It linearity materials in triplicate, and assessed by generating linearity statistics using Analyze-It v2.03. 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 verified by sequentially analyzing a low concentration sample in triplicates followed by a high concentration sample in duplicates and lastly the same low concentration sample in duplicates. Imprecision was performed in triplicates across 5 days using quality control material from Bio-Rad, and assessed against the vendor's imprecision claims. Patient comparison was performed using 60 to 80 samples for each analyte between the DxC700AU and the AU680. Passing Bablok Regression, Spearman Correlation and Altman-Bland Bias Plots was generated using the Analyze-It program.

Results:

All analytes were verified to be linear within their respective AMRs; all linearity material recovered within ±10% of its expected target value. LOD was assessed to be satisfactory and below the lower limit of measurement. No significant carryover was observed for any analyte. Total imprecision for all the analytes were generally <4.8%. Passing Bablok Regressions, where X is the AU680 and Y is the DxC700AU, generated slopes ranging between 0.98X and 1.04X with constants ranging between -1.13 and 31.18. The large constants were partly due to analytes with large AMR (e.g. creatinine and uric acid urine). Altman-Bland Bias Plots presented bias ranging between -3.6% to 1.7% for different analytes. All analytes had excellent correlation between the 2 analyzers with R values of 1.0.

Conclusion:

The performance characteristics of the DxC700AU in analyzing urine chemistries was found to be acceptable and highly comparable to the AU680. The upgrade of the AU680 to DxC700AU was seamless in view of excellent comparability in assay performances. Although the DxC700AU was expected to be the technological successor to the AU680, the quality-of-life improvements of the analyzer were the highlights of the DxC700AU. Improvements included a large clear LED indicator that highlights the status of the instrument and on-the-fly loading of reagent kits. Future improvements that the laboratory is looking forward to is the removal of the need to manually load specimens onto the instrument by connecting the standalone equipment onto its total laboratory automation track.

B-023

Optimizing measurement of homocysteine in an emergency care pathway

G. GRZYCH, D. MARCHAND, D. Clarence, G. Emeline, R. Schult, E. VAN NIEUWENHUYSE, F. Zerimech. *CHU Lille, Lille, France*

Background/Context: New indications for homocysteine measurement have emerged in recent years, and homocysteine is now being used as a marker of nutritional deficiency as well as a marker of nitrous oxide intoxication and there is a need to return quickly results to adapt the treatment and orientate diagnosis. Current rapid methods are not adapted to extremely high values encountered which are frequently greater than 50 µmol/L that need use of mass spectrometry that is unfortunately not adapt to emergency. Hence, we evaluated here a new immunological method that allows for the reliable and rapid rendering of homocysteine results in order to integrate it into our care pathways.

Method: We prospectively measured plasma homocysteine using both liquid chromatography-mass spectrometry (UPCL - Sciex AP 3200) and the Chemiluminescence

Immunoassay (CLIA) method (Maglumi-Snibe). To limit the bias for each assay, the analyses were performed on the same EDTA plasma sample with the same pre-analytical treatment. Bland-Altman plots and Deming regression were used to assess agreement between the homocysteine LC-MS/MS method and homocysteine CLIA method.

Results: Our study included 169 paired samples. The intra- and inter-assay imprecision was comprised between 0.73-2.21% and between 2.75 - 3.73%, respectively. Linearity, without sample dilution, was acceptable in the range of 5 - 70 mol/L. This CLIA assay correlated with the HCY LC-MS/MS assay with a slope of 0.9334, intercept of 1.750, R2 = 0.9637 and average bias of -0.6867 µmol/L.

Discussion: The CLIA method produces results comparable to mass spectrometry while significantly reducing analysis time and result delay. This method is thus more appropriate when homocysteine is used not only for emergency diagnosis but also for nutritional monitoring of the patient. This method also allows for the measurement of extremely high concentrations, which are becoming more common in laboratories.

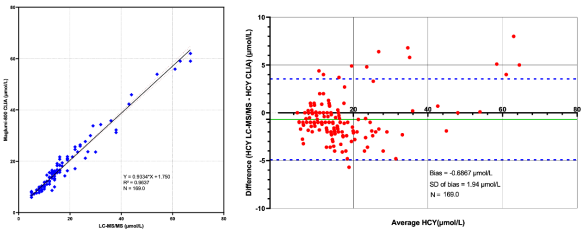


Figure: Comparison of homocysteine by LC-MS/MS and homocysteine by CLIA in plasma samples by Deming regression. Bland–Altman plot showing the differences between homocysteine by LC-MS/MS and homocysteine by CLIA. Hcy concentrations are expressed in µmol/L

B-024

An Evaluation of the Analytical Performance of the New Beckman Coulter DxC 500 AU Clinical Chemistry System

D. O'Meara[1], S. O'Donnell[2], K. Lynch[2], F. Laura[2], M. O. Meade[2]. *[1]Beckman Coulter Ireland, O'Callaghans Mills, Ireland, [2]Beckman Coulter Ireland, O'Callaghan's Mills, Ireland*

Background: The Beckman Coulter DxC 500 AU clinical chemistry analyzer* is the latest system from Beckman Coulter. It is a fully automated, random-access analyzer, designed for low to medium throughput laboratories, with a throughput of 800 tests/hour including ion selective electrodes. The purpose of this study was to evaluate the analytical performance of the new DxC 500 AU and to compare the performance against the current AU480 and DxC 700 AU analyzers.

Methods: To assess the performance of the DxC 500 AU, Beckman Coulter assays were selected for evaluation that covered a range of sample types and assay methodologies. Precision was assessed using pooled human samples over 20 days following CLSI guideline EP05-A3. The linear range was assessed following CLSI EP06-A. The DxC 500 AU was compared to the AU480 and DxC 700 AU analyzers using a minimum of 100 samples spanning the dynamic range based on CLSI guideline EP09c-ED3.

Results:**IgG Serum: OSR6x172** Estimates of repeatability and within laboratory precision were assessed following EP05-A3 at multiple critical analyte concentrations. The IgG serum assay exhibits total imprecision of < 6 % with a within run imprecision of ≤ 3.5 %. The IgG serum assay demonstrated acceptable linearity throughout the analytical measuring range of 75 to 3000 mg/dL following CLSI EP06-A. A method comparison study following CLSI EP09c-ED3 compared the IgG serum assay on the DxC 500 AU against the AU480 and DxC 700 AU. Serum patient samples (n>100) measured across the analytical range were analyzed using Weighted Deming regression and yielded a slope of 1.046, an intercept of -20.2884 mg/dL and correlation coefficient R=0.9979 against the DxC 700 AU and yielded a slope of 1.005, an intercept of -3.6578 mg/dL and correlation coefficient R=0.9973 against the AU480.**CRP Serum (Highly Sensitive): OSR6x99** Estimates of repeatability and within laboratory precision were assessed following EP05-A3 at multiple critical analyte concentrations. The CRP serum assay exhibits total imprecision of ≤ 10 %CV or ≤0.02 SD with a within run imprecision of ≤ 5 %CV or ≤0.02 SD. The CRP serum assay demonstrated acceptable linearity throughout the analytical measuring range of 0.2 to 80 mg/L following CLSI EP06-A. A method comparison study following CLSI EP09c-ED3 compared the CRP serum assay on the DxC 500 AU against the AU480 and DxC 700 AU. Serum patient samples (n>100) measured across the analytical range were analyzed using Weighted Deming regression and yielded a slope of 0.990, an intercept of 0.0421 mg/dL and correlation coefficient R=0.9997 against the DxC 700 AU and yielded a slope of 0.995, an intercept of -0.0008 mg/dL and correlation coefficient R=0.9998 against the AU480.

Conclusion: The results of the study demonstrated excellent analytical performance of the new Beckman Coulter DxC 500 AU analyzer and confirms comparable performance to the AU480 and DxC 700 AU analyzers. *Product In development. Pending clearance by the United States Food and Drug Administration and achievement of CE compliance. Not currently available for *in vitro* diagnostic use.

B-025

Evaluation of Bias Between Alzheimer's Disease Blood-Based Biomarkers Assays and Their Concordance With Amyloid-PET on the Fujirebio Lumipulse and Quanterix Simoa Platforms

D. Figdore, J. Bornhorst, C. Jack, R. Petersen, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

Background: Blood-based biomarkers (BBBs) are a growing area of study for improving Alzheimer's disease (AD) clinical evaluation. BBBs have been demonstrated to correlate with AD CSF biomarkers as well as neuroimaging results for assessment of amyloid pathology. Currently BBBs may be used as a screening tool for patient selection in clinical trials, and alongside more well characterized biomarkers like amyloid-PET and CSF markers for evaluation of individuals presenting with cognitive impairment. A comparative study of several AD BBBs assays was performed to assess bias between two platforms, as well as correlation to markers of amyloid pathology.

Methods: The study included 146 plasma samples from 112 cognitively normal (CN), and 34 mild cognitive impairment (MCI) or AD dementia individuals. Plasma Aβ42, Aβ40, and pTau181 concentrations were obtained using the Fujirebio Lumipulse and Quanterix Simoa immunoassays. Assay comparison between Fujirebio and Quanterix were performed for Aβ42, Aβ40, Aβ42/Aβ40 ratio, pTau181, and pTau181/Aβ42 ratio using Passing-Bablok regression and regression slopes were obtained. Amyloid-PET was performed using Pittsburg Compound B, and a standard uptake value ratio (SUVR) of 1.48 was used to classify amyloid status. In the CN group, 69 were amyloid negative, and 43 were amyloid positive. In the MCI/AD dementia group, 8 were amyloid negative and 26 were amyloid positive. Spearman's rank correlation coefficient (rho) was used to determine correlation between assays and correlation of each with Amyloid-PET SUVRs.

Results: Between Fujirebio (y) and Quanterix (x) assays, measured Aβ42 and Aβ40 concentrations showed a large bias with y = 5.23x - 14.25, and y = 4.42x - 264.87, respectively. A moderate to strong correlation was observed between assays for Aβ42 and Aβ40 with rho values of 0.641 (p <0.0001), and 0.498 (p <0.0001), respectively. The ratio of Aβ42/Aβ40 corrected this bias with y = 0.96x + 0.03 and rho = 0.509 (p <0.0001). pTau181 showed little bias between methods with y = 0.92x + 0.48, and a rho = 0.592 (p <0.0001). However, the pTau181/Aβ42 ratio showed a large bias driven mostly by differences in the Aβ42 concentrations with y = 0.27x + 0.04 and rho = 0.574 (p <0.0001). Quanterix assay results correlated with amyloid-PET SUVR yielded rho coefficients of AB42/40 ratio = -0.219 (p = 0.079), pTau181 = 0.380 (p <0.0001), and pTau181/Aβ42 ratio = 0.466 (p <0.0001). Fujirebio assay results correlated with amyloid-PET SUVR showed AB42/40 ratio rho = -0.551 (p <0.0001), pTau181 rho = 0.370 (p <0.0001), and pTau181/Aβ42 ratio rho = 0.391 (p <0.0001).

Conclusion: This study demonstrates that some BBBs methods differ significantly between manufacturers. Little bias was observed between the Aβ42/Aβ40 and pTau181 determinations for the evaluated platforms; however, significant bias was observed in Aβ42, Aβ40, and the pTau181/Aβ42 ratio. The Fujirebio platform demonstrated stronger correlation of the Aβ42/Aβ40 ratio with amyloid-PET SUVR, while Quanterix showed better correlation of the pTau181/Aβ42 ratio. The pTau181 assays showed similar correlation to amyloid-PET SUVR. Bias between assays as well as clinical correlation differences may preclude the generalization of clinical performance of AD BBBs assays between platforms.

B-026

Evaluation of Colorimetric and Electrolyte Test Assays on the Fuji Dri-Chem NX700I Clinical Chemistry Analyzer

P. Theologou[1], R. Wichert[1], F. Zacchini[1], R. Moennikes[1], H. Wahl[2]. *[1]Medizinisches Labor Wahl, Luedenscheid, Germany, [2]Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics Philipps University, Marburg, Germany*

Background The FUJI DRI-CHEM NX700i holds 28 colorimetric and 3 electrolyte test assays and analyzes up to 190 tests/hour (colorimetry + electrolytes). A STAT

testing position is available. The colorimetric method slide is a multilayered slide composed of dry chemical ingredients needed for the reaction quantifying enzymes and substrates by colorimetric methods. The potentiometric method slide contains ion selective film electrodes. Calibration is done with QC cards except for CRP working with a calibrator. Each test needs 10µL of sample except for CRP (5µL) and ISE (50µL for all 3 tests). **Methods** In this study the Fuji Dri-Chem NX700i was evaluated using serum samples. The parameters investigated were albumin (ALB), blood urea nitrogen (BUN), creatinine (CRE), total bilirubin (TBIL), aspartate aminotransferase (AST/GOT), Alanine Aminotransferase (ALT/GPT), γ-glutamyltransferase (GGT), C-reactive protein (CRP), lipase (LIP), triglycerides (TG) and ammonia (NH3). Interferences from hemolytic, lipemic, icteric or highly elevated total protein samples were excluded according to the manufacturers method specific declarations and studied separately. Method comparison was done by measuring 50 samples for each analyte at the same time on the NX700i and the Siemens Atellica CH analyzer. For each parameter inter- (controls, n=10) and intra-assay (patient samples, n=10) coefficients of variation (CV) were calculated for low, medium and high concentrations. **Results** Inter-assay CVs (n=10) were 1.1 to 7.7% (Low), 0.7 to 3.9% (Medium) and 0.8 to 5.5% (High). Intra-assay CVs (n=10) were <0.01 to 9.5% (Low), <0.01 to 3.7% (Medium) and 0.7 to 3.3% (High). Method comparison (Passing-Bablok Regression) of routine samples from hospital patients revealed good agreement of the two methods with correlation coefficients of r = 0.98 and higher except for LIP (0.83), NH3 (0.87) and Alb (0.93). **Conclusion** The FUJI DRI-CHEM NX700i shows good intra- and inter-assay precision with low CVs and excellent correlation with the Siemens Atellica CH analyzer.

B-027

Adaptation of Neonatal Dried Blood Spot Assays for Use on Automated Microplate Instruments

G. Van Slooten. *Awareness Technology, Palm City, FL*

Background: Newborn screening (NBS) is routinely conducted to identify various inherited metabolic disorders which can affect a child's long-term health or survival. The assay methods used include enzyme-linked immunosorbent assays (ELISA) as well as enzymatic biochemical assays. Dried blood spots (DBS) prepared by applying whole blood to filter paper cards are typically used as the sample for these screening assays. In this study we have adapted an openly programmable automated ELISA processing instrument to perform these types of assays with a high level of accuracy and precision.

Method: The Awareness Technology (Palm City, FL) ChemWell-2 instrument was chosen for this study. This instrument is a two plate ELISA processing system which has the capability of performing the DBS extraction in an uncoated round bottom 96-well microplate and transferring a small volume of extracted sample to be assayed to a second flat bottom 96-well microplate. The following enzymatic assay kits manufactured by ZenTech (Liège, Belgium) were used in this study: total galactose, phenylalanine, glucose-6-phosphate dehydrogenase (G-6-PD) and biotinidase.

Results: In order to evaluate the assay performance controls and additional standard DBS were run as samples. Three to four different sample concentration levels were run four to five times in each assay. The runs were repeated 4 times each to determine inter-assay (between run) precision. The results are summarized in the table below:

	Results of 4 Assay Runs with 4 Sample Replicates	
Assay	**Inter-assay Precision Range (% C.V.)**	**Sample Recovery Range (%)**
Total Galactose	5.6 – 8.9	92.2 – 94.8
Phenylalanine	4.5 – 10.3	100.5 – 118.2
G-6-PD	3.0 – 3.1	99.9 – 107.5
Biotinidase	5.7 – 9.8	88.2 – 95.4

Conclusion: Enzymatic NBS methods for total galactose, phenylalanine, G-6-PD and biotinidase including the DBS sample extraction step can be processed with clinically useful accuracy and precision using the Awareness Technology ChemWell-2 automated analyzer.

B-028

Clinical performance of a fully-automated Fecal Calprotectin Chemiluminescence Immunoassay (CLIA)

J. A. Petzke, B. McDermott, C. Thienes, K. Veenstra, C. Wisherd. *ALPCO, Salem, NH*

Background: Fecal calprotectin is a noninvasive marker for inflammatory bowel disease (IBD). Traditionally, calprotectin is measured by the well-established enzyme linked immunosorbent assay (ELISA) method. More recently, advances in fully automated platforms allow for comparable performance and diagnostic accuracy whilst improving laboratory efficiency. In this study, we assessed the diagnostic performance of the fully automated ALPCO calprotectin chemiluminescence immunoassay (CLIA), and compared it to the predicate, ALPCO's 510(k) cleared manual calprotectin chemiluminescence ELISA.

Methods: Fecal calprotectin levels were measured with the ALPCO Calprotectin Chemiluminescence ELISA and the recently developed ALPCO Calprotectin CLIA, utilizing glow and flash chemiluminescence, respectively. An IRB approved study included over 400 individuals with clinical suspicion of IBD. The estimates of clinical sensitivity, and clinical specificity of the ALPCO Calprotectin Chemiluminescence ELISA and the ALPCO Calprotectin CLIA were determined by comparing analytical test results of the prospectively collected stool specimens against the clinical diagnosis made by the clinical investigator/gastroenterologist with ileocolonoscopic and histopathological sampling (reference standard).

Results: When distinguishing IBD from irritable bowel syndrome (IBS) and healthy persons, the sensitivity (70/76) and specificity (322/348), at a fecal calprotectin cut-off level of 50 µg/g, were 92.1% and 92.5%, respectively for the ALPCO Calprotectin Chemiluminescence ELISA. Sensitivity (73/78) and specificity (319/342), at a fecal calprotectin cut-off level of 50 µg/g, were 93.6% and 93.3%, respectively for the ALPCO Calprotectin CLIA.

Conclusion: The ALPCO Calprotectin CLIA showed comparable clinical performance for detection of IBD compared to the predicate 510(k) cleared ALPCO Calprotectin Chemiluminescence ELISA. The automation of the ALPCO calprotectin immunoassay allows for increased laboratory efficiency without sacrificing diagnostic accuracy.

B-029

Validation of an Automated Analyzer for Urine Microscopic Examination

L. Majoy[1], H. Riedy[1], M. Chappel[1], M. O. Nakashima[2]. *[1]Cleveland Clinic, Cleveland, OH, [2]Cleveland Clinic, Cleveland Heights, OH*

Background:

Microscopic examination of urine sediment can be time-consuming and labor intensive. Automated analyzers can help to standardize resulting and reduce manual work. Urinalysis has traditionally utilized semi-quantitative grading scales, eg. "0-3/high power field". The Sysmex UF 5000 (Symex, Kobe, JP) uses flow cytometry to enumerate microscopic particles including white and red blood cells (WBC, RBC), bacteria, casts, and epithelial cells (EC). We sought to validate the counting of these microscopic particles against our reference manual method, and also determine if the research parameters from the analyzer could be used to accurately measure other particles in urine (eg. crystals).

Methods:

Residual urine samples were analyzed for microscopic particles both by the UF 5000 and manually using KOVA tubes (KOVA International, Garden Grove, CA). Particles were enumerated using the laboratory's current semi-quantitative grading system. Exact agreement and agreement +/- one level were calculated.

Results:

Microscopic Results		
Particle	Exact agreement	+/-one grade
RBC	76.6%	90.1%
WBC	63.1%	85.6%
Bacteria	71.2%	90.1%
Casts	61.3%	88.3%

Conclusion:

The analyzer results were comparable to manual resulting; in discrepant cases, the UF result was typically higher than manual, which may reflect the increased sensitivity of this method. Additional studies are ongoing to determine if the UF 5000 research parameters are accurate for semi-quantitative reporting of pathologic casts, crystals, and yeast.

B-031

Digital elisatotal 25hydroxyvitamin D

S. CHAE[1], j. CHUNG[1], H. Huh[1], S. Moon[1], Y. Seo[2]. *[1]Dongguk University Ilsan Hospital, Goyang, Korea, Republic of, [2]Small Machines Co.,Ltd, Seoul, Korea, Republic of*

Evaluation of digital ELISA using a high-resolution computational optical system for measurement of total 25hydroxyvitamin D

BACKGROUND: Biomarkers such as vitamin D occur at very low concentrations in blood, and sensitive detection methods are required in order to measure them. It is difficult to accurately measure low-concentration biomarkers using conventional enzyme-linked immunosorbent assays (ELISA) with fluorescence staining. By developing a digital ELISA (DELISA, Small Machines, Seoul, Korea) platform, a low concentration substance was realized by analyzing the digitalized signal through a high-resolution computational optical system in a microfluidic chip. We evaluated DELISA for measurement of total 25hydroxyvitamin D (25OHD) in a fluidic chip

METHODS The sample with monoclonal antibody bead conjugates mixture was incubated for 20 min at 37°C, then 30uL sample mixture was applied to inlet of the chip. After additional incubation for 2 min at room temperature and 2 min after sample application, 120 uL of washing buffer was applied onto the washing hole and the chip was incubated for 10 min. 25OHD_molecules in the sample bond to magnetic beads coupled with anti-25OHD at the end of the membrane. Chips were inserted into DELISA, and 25OHD results were derived by analysis of the computational method of microscopic imaging, proportional to the concentration of 25OHD._Assay performance characteristics of the limit of detection (LoD), precision and analytical

measurement interval (AMI) using Vitamin D Depleted, Low and High Serum (Molecular Depot, San Diego, CA) were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. 87 Samples for method comparison studies were obtained from routine clinical samples submitted for 2 OHD determination or from apparently healthy normal volunteers. The study was approved by the Institutional Review Board of Dongguk University Ilsan Hospital (202207024).

RESULTS LoD was 10.3 ng/mL and within-laboratory precision was 11, 12, and 10% coefficient of variation at 14.1, 56.6, and 113.2 ng/mL, respectively. The best fit polynomial was a cubic equation from 14.1 to 113.2 ng/mL and the difference between the third-order equation and the first-order equation at Level 3 and 4 concentrations was outside the non-linearity limit. Comparison with chemiluminescent assay (Elecsys Vitamin D total II, Roche Diagnostics, Mannheim, Germany) yielded a 0.904 of correlation coefficient and 0.921 slope in the range from 7.0 to 57.9 ng/mL.

CONCLUSIONS: The DELISA 25OHD assay has a suitable LoD and precision, however, it is necessary to improve the AMI at high concentration values and correlation with the automated analyzer.

B-032

Total lab Automation Validation/Verification Protocol: First Step of Process Excellence Journey in Smart Core Lab in Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute, Mumbai, India

B. Das, L. Bhat, R. Patil, V. Tawde, S. Kamath, P. Pal. *Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute, Mumbai, India*

Background: The total laboratory automation (TLA) involves the integration, automation, consolidation and digitalization of pre-examination, examination, and post-examination processes. It is far simpler to introduce TLA in a lean, highly standardized smart core lab setting than it is to replicate complex procedures based on manual workflows, therefore harmonization/standardization is a requirement. In this era of modern clinical laboratory, a very important step with respect to the quality of the entire process is method validation/ verification. Hence, it is fundamental to verify/ validate TLA process flow, new techniques, new methods and new test parameters. During laboratory accreditation process, the most frequently cited deficiency involves inappropriate PQ (Performance Qualification) and verification protocol. The main principles to the PQ laboratory test validation or verification protocol are Accuracy, Precision, Reference Interval, Detection Limit (LOD), Analytical Measurement Range (AMR) and Clinically Reportable Range (CRR), etc. Hence a clinically appropriate turnaround time is guaranteed via validation, which also provides accurate and precise readings.

Methods: Around 250 parameters, of biochemical and immunoassay for samples like serum/plasma/cerebrospinal fluid/urine have been validated/verified on this automated system. Clinical Laboratory Standards Institute (CLSI) and best practise checklist as per international and national accreditation bodies served as the foundation for verification. Following are the steps for verification: **Analytical Accuracy:** Run samples by both methods to evaluate bias. **Verify Precision:** Run 5 replicates per run for 5 days, at all levels of control sera. **Check Reference Intervals:** Representative 20 healthy individuals are selected. **Verify LOB and LOD Values:** Run 20 blank (zero calibrator) or low patient samples. **Pneumatic System Verification Protocol:** 20 samples carried manually by health care assistants and 20 samples transported via the pneumatic system. We have done sigma metrics of 250 parameters in the lab.

Results: With particular reference to Thyroid Stimulating Hormone (TSH), we have obtained the following results: For the Reference interval study, out of 353 volunteers recruited, 8 volunteer data were excluded (7 outliers, 1 abnormal TPO values). We obtained the reference interval of 0.53 - 4.489 µIU/mL, which is within the range specified by the manufacturer's reference range. A good level of analytical accuracy is demonstrated between the two instruments with $r^2 = 0.9938$ having a coefficient of variation of 4.32%. The coefficient of variation values of precision for inter laboratory method comparison for lower and high-level values is 1.39% and 1.76% respectively, which is comparable with the manufacturers claims values. The coefficient of repeatability is 1.3442 is higher than 0.7 and hence considered as the two instruments having a good retest reliability. We observed more than six sigma performance of TSH. Based on Operational Specification (OPSpecs) Chart and Method Decision Chart sigma values of all the parameters are evaluated and quality control strategies have been defined.

Conclusion: Thus, validation of all biochemistry & immunoassay parameters in TLA set up of the smart core laboratory promotes an environment, emphasizing patient safety and quality patient care.

B-033

Impact of red blood cell sediment on HbA1c measurement

T. Nanthakhan, M. Jaru-arayanun. *King Chulalongkorn Memorial hospital, Bangkok, Thailand*

Background:

HbA1c testing using EDTA whole blood as specimens is essential for monitoring glycemic control in diabetic patients. In the laboratory that using total laboratory automation system, the transportation process might be impact the quality of HbA1c result according to red blood cell sedimentation. This study, we aimed to evaluate the impact of HbA1c result from red blood cell sediment which happen in transportation process of total laboratory automation system.

Methods:

HbA1c samples (n = 50) were mixed once and HbA1c levels were determined by using an Alinity c analyzer at an initial time point (T0 = 0 min.), followed by three additional time points (T1 = 7 min., T2 = 15 min., and T3 = 30 min.). The HbA1c levels and samples analyzed at each time point were then used to calculate correlation coefficient, slope, intercept values. The percent difference in these parameter values from all time points was calculated using the analytical criteria of 5% bias according to the National Glycohemoglobin Standardization Program (NGSP) criteria.

Results:

We discovered that the correlation coefficient, slope, and intercept values obtained from the samples at T1 are 0.999, 0.994, 0.008, respectively, T2 are 0.999, 0.982, 0.042, respectively and T3 are 0.999, 0.979, 0.028, respectively. The percent difference of all criteria was 0.00% which were not significantly different from those at the initial time point (T0).

Conclusion:

This study shows that, the time that using in transportation process in total laboratory automation system at 7, 15 and 30 minutes will not impact quality of HbA1c result even though red blood cell might be sediment before aspirate in the analyzer.

B-034

Evaluation of the analytical performance of Sebia CAPI 3 HbA1c assay

E. T. Laryea, M. Bates, S. Fan. *Vanderbilt University Medical Center, Nashville, TN*

Background: HbA1c has been the most important marker for glycemic control and for the diagnosis of diabetes mellitus. The NGSP has invested significant efforts in the standardization of the HbA1c assays, and several analytical methods have been developed. We evaluated the analytical performance of the Sebia CAPI 3 HbA1c assay, which is determined by capillary electrophoresis. Additionally, samples with and without hemoglobin variants are analyzed, and results are compared to two HPLC-based methods.

Methods: Control materials are used in the precision study, which are included in the Sebia CAPI 3 HbA1c kit (Lisses, France). Linearity study is assessed with the linearity materials from LGC Maine Standards (Cumberland Foreside, ME). Carryover is determined by the EP Evaluator (Colchester, VT) with total 21 aliquots of a high and a low HbA1c sample assayed in a specific order. Accuracy study is determined by whole blood patient samples without hemoglobin variants (n=40) and with hemoglobin variants (n=8, mostly with Hb S of 30-40%). Results from Sebia CAPI 3 are compared to the current laboratory method (BioRad Variant II Turbo) (Hercules, CA) and the TOSOH G8 (Tokyo, Japan). Both are HPLC-based methods.

Results: The within-run precision at a mean of 5.5% HbA1c has a CV at 1% (n=24), and the between-run precision at the level of 5.5% and 8.7% HbA1c has a CV at 1% (n=20) and 2% (n=20), respectively. Linearity study demonstrates the analytical measuring range is between 3.1% and 20.8%, although each laboratory might establish its own clinical reportable range. Carryover is passed based on the automatically calculated error limit in EP Evaluator (3 times the SD of the Low-Low results). The accuracy study shows the Sebia results correlate well with percent bias at 2% for BioRad Variant II Turbo and 3% for TOSOH G8 in samples with (n=8) and without (n=40) hemoglobin variants.

Conclusion: The Sebia CAPI 3 HbA1c assay is based on capillary electrophoresis principle, which is a recognized method by NGSP. The assay has demonstrated good precision, a wide analytical measuring range and minimum carryover. The results are comparable to two HPLC-based methods and are well within the total allowable error of 6% by NGSP in samples with and without hemoglobin variant.

B-035

Maintaining the analytical performance of calcium and phosphorus in 24-hour urine without addition of 6n hcl in the pre-analytical process

D. G. Canali[1], G. Zampieri[2], C. M. Motta[2], D. C. Oliveira[2], C. Rosin[2], S. V. Argolo[3], W. Satsuma[2]. *[1]Diagnóstico da America, São Jose, Brazil, [2]Diagnóstico da America, São Paulo, Brazil, [3]Diagnóstico da America, Rio de Janeiro, Brazil*

Background: The measurements of Calcium (Ca) and Phosphorus (P) in 24-hour urine samples are associated with the evaluation of hyperparathyroidism, renal tubular acidosis, and monitoring of Falconi Syndrome and Nephrolithiasis. The aim is to demonstrate the maintenance of analytical performance without the addition of 6N hydrochloric acid (HCl) in the pre-analytical process. **Methods:** The study was divided into stages and carried out for both analytes. In the first stage, correlation tests were performed on 40 samples with and without HCl within the range of linearity. Imprecision was processed at 3 clinically relevant concentrations for 5 days, repeated 5 times. In the second stage, stability testing of Ca and P was performed on 7 samples over 5 days, without HCl (pH 5-6.7), with HCl (pH <3), alkalized (pH>8), and neutral (pH 7-7.5). In the last stage, interferent testing was performed on 286 samples, where pH was measured after collection and after acidification with 200uL of HCl. A pH meter and pH strips were used to measure pH, and a Siemens® - Advia 2400 equipment was used for analytical measurement of Ca and P. **Results:** In the correlation results, the variance test for both analytes resulted in F-test: 1.01 <1.69, the difference analysis was less than 2.02, correlation coefficient and Bias% of 1.00 and 0.55 for Ca and 0.99 and 0.27 for P, respectively. Total error (TE) obtained for Ca was 9.69 and 9.41, lower than the allowed TE for both analytes. The repeatability and intralaboratory imprecision of the three levels of each analyte were less than 13.8% for Ca and 9.0% for P. There was 100% correlation of the results when the analyses were performed with samples at pH up to 7; at pH values > 7, the concentrations decreased by 60%. All 268 samples with different pH values, subjected to 200uL of HCl, were suitable for Ca and P measurement with pH below 5.7. **Conclusions:** Samples with a pH greater >or= to 7 necessarily need to be acidified with HCl 6N. There is no analytical alteration if all samples in the routine of urinary Ca and P are submitted to 200uL of HCl 6N, even the most alkaline samples. The non-provision of preservative containers to patients reduces the risk of accidents, costs, and the need for legal licenses for handling in healthcare facilities.

B-036

Multi-biomarker approach to latent tuberculosis diagnosis using microring resonator sensors

K. Meserve[1], C. Chapman[1], P. Escalante[2], R. C. Bailey[1]. *[1]University of Michigan, Ann Arbor, MI, [2]Mayo Clinic, Rochester, MN*

Background: Tuberculosis (TB) is an infectious disease that induces a complex response from the host immune system. Most carriers of the bacteria experience the asymptomatic, non-infectious phase, latent tuberculosis infection (LTBI), but have the potential to reactivate to active, contagious TB infection. Current diagnostics for TB are not sufficient for LTBI and are focused on using one biomarker, interferon-gamma (IFN-g). To build better diagnostics for the complex disease, multi-biomarker approaches to profile the immune system, coupled with machine learning algorithms, are necessary to diagnose LTBI and determine patients' risks of reactivation to active TB. We aim to use a multiplexed silicon photonic microring resonator sensing platform to profile thirteen biomarkers in patients with LTBI infection and use machine learning data analysis to identify biomarkers relevant for diagnosing LTBI and predicting reactivation risk.

Methods: We employed microring resonators coupled with a sandwich-style detection format to simultaneously detect thirteen cytokines and chemokines in plasma in under 40 minutes. Chip-integrated silicon photonic microrings are small in size and easily multiplexed with up to sixteen different capture agents. Biomolecular binding events are measured by monitoring the resonant wavelength within the sensing cavity, which shifts as binding events occur. Quantitation of each biomarker is possible with calibration curves that correlate standard protein concentrations to wavelength shifts. The multiplexed assay has been optimized for each individual biomarker, tested for cross-reactivity, and calibrated in the plasma matrix of interest. The LODs vary, depending on biomarker and matrix dilution, but range from 4.9 pg/mL to 691 pg/mL. The patient plasma samples are left over from the TB screening IFN-g release assay, QuantiFERON-TB Gold Plus (QFT), putting this method within the current clinical workflow. We use precision normalization approaches from the four QFT stimulations to account for immunologic differences among the subjects. The biomarker concentrations are evaluated using a random forest machine learning model to identify which biomarkers are important for identifying patients with LTBI and predicting their reactivation risk.

Results: We used this sensor method to profile 42 patients, 24 LTBI negative controls and 18 LTBI positive controls, with 13 being considered at high risk of reactivation. Using a precision normalization approach, a combination of nine normalized conditions using five of the thirteen biomarkers (CCL4, CCL8, IP-10, IL-2, IL-17) discriminated between LTBI positive and negative subjects with a ROC AUC of 0.90. Additionally, eight normalized conditions using four of the same biomarkers discriminated between high and low-risk subjects with an AUC of 0.83. Currently, we have expanded the sample cohort to include an additional 72 subjects, 25 LTBI positive and 47 LTBI negative. Preliminary analysis shows CCL8, IL-2, and IP-10 alone are able to distinguish between LTBI status (p values < 0.01) using raw target concentrations prior to precision normalization.

Conclusion: Overall, we show the importance of multi-biomarker approaches to develop diagnostic and prognostic tools for a complex disease, such as TB. Further work to use the initial machine learning algorithm with the expanded cohort to validate biomarker importance is underway.

B-037

Comparison of Performance between Siemens Clinitek + Status Analyzer to ARKRAY AUTION MAX AX-4030 for Suspected Urinary Tract Infections

M. Ali[1], L. Yang[2], K. Forbush[3], D. Rohr[3]. *[1]The MetroHealth System/ Case Western Reserve University School of Medicine, Cleveland, OH, [2]The Metrohealth System/ Case Western Reserve University School of Medicine, Cleveland, OH, [3]The MetroHealth System, Cleveland, OH*

Background: The purpose of the study is to evaluate the performance and agreement of the rapid point-of-care (POCT) urine dipstick testing in the emergency department (ED) to a laboratory-automated platform. Methods: A total of 30 urine specimens were collected from the emergency department at our hospital and were simultane-

ously tested for urinalysis (UA) on the POCT Siemens Clinitek Status+ analyzer using the Multistix 10SG strip. In the laboratory, we performed the tests on the automated ARKRAY Aution MAX™ instrument, using the Aution Sticks 9EB strips. Samples with more than a trace of leukocyte esterase and/or nitrates are considered positive for a urinalysis and further, the samples were reflexed to urine culture. The performance of both platforms was analyzed using EP Evaluator (Data Innovations LLC.). Results: A total of 30 urine specimens were analyzed for UA using both the POCT and automated laboratory analyzers. The agreement (95% CI) between leukocyte esterase, nitrites, and blood are as follows: 53.3% (36.1 to 69.8%); 96.7% (83.3 to 99.4%), and 70.0% (52.1 to 83.3%) (see Table 1.). Furthermore, out of the 30 specimens that were tested on both platforms, there was prominent discordance between the POCT and lab UA test. The 7 urine samples that were negative on POCT were positive on the laboratory UA platform. All 7 were sent for urine culture, out of 7 only 1 sample was positive for culture which was abnormal: >100,000 CFU/ml *Escherichia coli.* Out of 30 urine samples specimens that were tested on both platforms, 23 urine samples were in concordance with both platforms out of which 8 urine samples tested positive for culture (5 were *E. coli*, 1 was *E. coli* combined with *Klebsiella pneumoniae*, 1 was solely *Klebsiella pneumoniae*, 1 was *Staphylococcus saprophyticus*, and 1 was *Streptococcus agalactiae).* The remaining 15 urine samples resulted in no significant growth of bacteria or suggesting contamination. The sensitivity of POCT UA and laboratory-performed UA was 89% and 100% respectively. Furthermore, the specificity of POCT UA and laboratory-performed UA was 36% and 7% respectively. Conclusion: Our data shows although POCT UA is rapid, a prominent disparity is observed between the POCT UA and automated lab testing. The lab-performed UA has greater sensitivity and specificity compared to the POCT UA. Clinicians should interpret the urine dipstick test results in a clinical context that includes the patient's history and symptoms, especially when using POCT in the ED.

B-038

Applying Robotic Process Automation technology to automate the release of pathology results and reduce costs, time and labor in a clinical laboratory

J. R. Oliveira, I. B. Escalante, I. R. Fernandes, V. S. Milani, P. G. Costa, R. H. Jacomo, L. F. Abdalla. *Sabin Medicina Diagnostica, Brasilia, Brazil*

Background: The need for pathological anatomy tests has increased considerably in recent years, especially in one of our regional branches, where the total operational time to release the report to the patient is crucial to obtain competitive advantages in the market. As a result, the branch´s technical team observed the high lead time of the process, the inefficiency of the use of internal resources and the impact of the various fully manual activities in order to deliver the service offered to the customer and, therefore, sought out the Production Planning and Control (PPC) team with the objective of implementing improvements, in order to optimize the total operation time and automate the activities involved, adding more value to the patient and highlighting the laboratory's brand against competitors.

Methods: In order to implement continuous improvements in the operation of anatomical pathology samples, the involved teams held several brainstorming sessions and mapped out the supply chain for these types of tests. Firstly, the samples were received and outsourced to a partner laboratory and as the results were ready, the reports were made available in their Laboratory Information System (LIS). These reports were then retrieved manually by a capacitated employee whose only job was to download the reports from the partner´s website, revise it and upload it to our LIS so that our patients had access to the result report. Through the application of the principles of the Value Stream Map (VSM) tool, it was possible to determine the time needed for each stage of the process and identify the use of resource in exclusive functions that negatively impact productivity such as the manual activity of retrieving and uploading files. Considering the analysis carried out, it was observed that the best way to automate the process and reduce lead time would be the development of an RPA (Robotic Process Automation) that would login in the partner laboratory´s LIS, search for the patients´ reports, download it, and upload it into our LIS. Once the report was available in our system, the analyst had only to revised it briefly for matching information and release the results.

Results: With the creation of RPA, the process was redesigned along with the teams involved, manual activities were automated and the need for resource exclusivity was mitigated. Thus, it was possible to obtain a 50% optimization of the designated employee's time, a 75% reduction in the total lead time of the internal operation of the laboratory team and a gain of approximately 38% in the productivity of the technical area, making the process faster and more efficient.

Conclusion: With the implementation and validation of RPA in the external unit, the manual process of using the systems was eliminated and the new automated process obtained excellent results when compared to the previous scenario, while now we have greater agility and speed in activities and a continuous flow of information that includes the efficient release of pathological anatomy exam results, strengthening the laboratory's competitiveness in the market and ensuring customer loyalty.

B-039

Method Performance Evaluation of the Siemens Atellica Cystatin C Assay on the CH930 Instrument

R. Maynard, N. Korpi-Steiner, S. Cotten. *UNC, Chapel Hill, NC*

Background: The National Kidney Foundation and American Society of Nephrology Task Force on Reassessing the Inclusion of Race in Diagnosing Kidney Disease published recommendations on the implementation of the CKD-EPI 2021 equation to calculate $eGFR_{cr}$ without a race coefficient. Clinical practice recommendations also suggest increasing availability of Cystatin C testing which can be a more reliable marker of renal function than creatinine for select adults with $eGFR_{cr}$ near medical decision points and in situations where non-GFR factors may have a large effect on serum creatinine. This study evaluated the method performance characteristics of the Cystatin C assay on the Siemens Atellica CH930 analyzer. The assay measures agglutination of latex particles coated with anti-cystatin C antibody at 571/805 nm and is approved for determination of Cystatin C in serum and plasma (lithium heparin, and EDTA).

Methods: Cystatin C reagent lot 2114231 and calibrator lot 560956 were used in the study. Intra-run precision was evaluated using BioRad IntelliQ Immunology Control (Level 1 lot 68991, Level 2 lot 68993). Inter-run precision was evaluated over 20 consecutive days. Mean, SD, and CV were calculated and compared to manufacturer precision claims in the IFU and BioRad UNITY™ Interlab Reports. Linearity was evaluated using AUDIT MicroControls Cystatin C Linearity LQ (Lot 06901). Accuracy was evaluated by measuring 40 serum samples on the Atellica CH930 and Roche Diagnostics Tina-Quant Cystatin C Gen 2 assay. Acceptable criteria were defined as a calculated slope between 0.9-1.1 and r greater than 0.995. Appropriateness of the adult reference interval was evaluated with 20 female and 20 male presumably healthy donors. Acceptability was defined as less than 10% of results outside the proposed range.

Results: Intra-run CV ranged from 2.3-9.2% (Level 1 QC) and 1.2-2.6% (Level 2 QC) across four different CH930 instruments. Level 1 precision did not verify the manufacturer claim of ≤ 3.6% CV on 3 out of 4 instruments. Level 2 precision did not verify the manufacturer claim of ≤ 1.7% CV for any instrument tested. Using BioRad UNITY™ peer performance as targets, 3 of 4 instruments had CV ≤6.7% for Level 1 and all instruments had CV ≤2.8% for Level 2. Inter-run precision for Level 1 QC was ≤5.94% CV which was less than the BioRad Peer Group CV. Linearity material spanned 0.490-5.893 mg/L and results were within manufacturer's expected ranges. Method comparison with the Roche Tina-Quant Gen 2 assay had a calculated slope of 0.954, intercept of 0.094, and r value equal to 0.9993. Thirty-nine of 40 results were within 10% of the reference method (range 0.74-5.45 mg/L). Verification of the reference interval using presumably healthy donors resulted in 5.3% (2/38) of results outside the proposed range.

Conclusion: With the exception of precision, all other manufacturer-claimed performance specifications for the Siemens Atellica Cystatin C assay were verified using four CH930 instruments at our institution.

B-040

Performance Evaluation of Five Reproductive Endocrinology Assays on the Atellica CI 1900 Analyzer

H. Zhang, J. Frenna, L. Halik, N. Ozgen, M. Quintanilla, J. Rhea-McManus, H. Leipold. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput, integrated chemistry and immunoassay analyzer utilizing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica IM Luteinizing Hormone (LH), Progesterone (PRGE), Follicle Stimulating Hormone (FSH), Prolactin (PRL), and Total hCG (ThCG) assays* on the Atellica CI 1900 Analyzer.

Methods: The Atellica CI 1900 LH, PRGE, FSH, PRL, and ThCG assays use the same reagents and calibrators as the comparable Atellica IM assays. Precision and method comparison (MC) studies were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 using quality control material and native and contrived human serum samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days with one reagent lot on two systems. MC studies were

performed according to CLSI EP09-A3. Individual native human serum samples were analyzed using the Atellica IM assays on both the Atellica IM and Atellica CI 1900 Analyzers.

Results: Representative precision and MC results are listed for each assay in the table. Over the five assays tested, repeatability and within-lab %CVs were <7% and <13.5%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1.

Conclusion: Evaluation of the Atellica IM LH, PRGE, FSH, PRL, and ThCG assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on the Atellica IM Analyzer.

		Precision			Method Comparison	
Analyte (Assay)	Unit	Sample Range	Repeatability %CV range	Within Laboratory %CV range	Sample Range	Regression Equation for Atellica IM Comparative Assay
Luteinizing Hormone (LH)	mIU/mL	1.35–166.54	1.3–2.4	2.9–5.9	0.57–173.16	y = 1.04x – 0.14 mIU/mL (IU/L)
Progesterone (PRGE)	ng/mL	0.55–45.16	1.8–6.7	4.6–12.2	0.34–52.38	y = 1.00x + 0.02 ng/mL
Follicle Stimulating Hormone (FSH)	mIU/mL	1.61–179.82	1.3–5.5	2.6–13.2	1.20–182.10	y = 0.97x + 0.15 mIU/mL
Prolactin (PRL)	ng/mL	2.56–158.89	1.1–5.8	2.4–8.8	1.98–154.51	y = 1.07x – 0.05 ng/mL
Human Chorionic Gonadotropin (ThCG)	mIU/mL	7.4–739.8	1.2–3.7	5.1–8.1	7.0–930.3	y = 1.02 x – 0.7 mIU/mL

*The products/features mentioned here are not commercially available in all countries. Their future availability cannot be guaranteed.

B-041

Evaluation of a Nuclear Magnetic Resonance Spectroscopy-Based Method for Estimating Glomerular Filtration Rate Using Creatinine, Cystatin C, Myo-Inositol, and Valine

L. Branson, D. Southard, B. Hadder, W. Katrangi, R. Alturkmani. *Labtech Diagnostics, San Antonio, TX*

Background: Timely and accurate monitoring of renal dysfunction is critical for patients with chronic kidney disease (CKD). Most equations currently used for estimating glomerular filtration rate (GFR) incorporate serum levels of creatinine and/or cystatin C. Here, we evaluate a recently introduced method for estimating GFR that uses nuclear magnetic resonance spectroscopy (NMR) to measure creatinine, myo-inositol, and valine in combination with cystatin C measured by immunoassay, and patient's age and sex ($eGFR_{NMR}$).

Methods: Serum creatinine, valine, and myo-inositol measurements were performed simultaneously by an NMR assay on a Bruker Avance III HD 600 MHz NMR instrument (Bruker Corporation, MA). Cystatin C testing was performed using an immunoturbidimetric assay (Diazyme Laboratories, CA) on a cobas 8000 analyzer (Roche Diagnostics, IN). Within-run and between-day precision studies were performed across 5 days. Method comparison was carried out by testing 86 split serum samples from adult individuals against the numares AG GFR (NMR) assay. Of the 86 samples, 20 were spiked with various concentrations of creatinine, myo-inositol, and valine to reach a wide range of GFR results. An additional method comparison study was performed against Mayo Clinic Laboratories (Rochester, MN) on 59 serum samples from adult patients that had already been tested using the same assay. Measured GFR (mGFR) was also performed on these patients by Mayo Clinic Laboratories using urinary iothalamate clearance.

Results: Within-run and between-day coefficients of variation (%CV) for $eGFR_{NMR}$ did not exceed 6%. The $eGFR_{NMR}$ measurement range was confirmed at 10 to 157 mL/min/1.73m^2 based on verified analytical measurement ranges of creatinine (0.28-9.8 mg/dL), myo-inositol (39-430 µmol/L), and valine (30-1,250 µmol/L). Method comparison studies against both numares AG and Mayo Clinic Laboratories demonstrated excellent concordance (r^2=0.95 and r^2=0.97, respectively) and minimal bias (4.1% and -0.3%, respectively) with results ranging from 21 to 157 mL/min/1.73m^2. When the 59 patients who had their GFR measured were staged by their mGFR results according to the CKD classification ranges of <15, 15-29, 30-44, 45-59, 60-89, and ≥ 90 mL/min/1.73m^2, $eGFR_{NMR}$ showed higher classification concordance with mGFR (69%) than the CKD-EPI 2021 creatinine-cystatin C equation (63%).

Conclusion: Our evaluation confirms the excellent analytical and clinical performance of the recently introduced $eGFR_{NMR}$ assay, supporting its integration into clinical practice for improved estimation of GFR.

B-042

Evaluation of the Analytical Performance of Three Prostate-specific Antigen Immunoassays on the Atellica CI 1900 Analyzer

M. Quintanilla, K. Thakur, E. Clark, M. Smith, G. Arrode-Bruses, J. Rhea-McManus, H. Leipold. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput, integrated chemistry and immunoassay analyzer employing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica IM Prostate-Specific Antigen (PSA)*, Complexed Prostate-Specific Antigen (cPSA)*, and Free Prostate-Specific Antigen (fPSA)* assays on the Atellica CI 1900 Analyzer. **Methods:** The Atellica CI 1900 PSA, cPSA, and fPSA assays use the same reagents and calibrators as the comparable Atellica IM assays. Limit of quantitation (LoQ), precision, and method comparison (MC) were used as performance indicators for the Atellica CI 1900 Analyzer. LoQ studies were performed in accordance with CLSI EP17-A2 using native and contrived human serum samples. Precision studies were performed according to CLSI EP05-A3 using contrived human serum samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. MC studies were performed according to CLSI EP09-A3. Individual native human serum samples were analyzed using the Atellica IM assays on both the Atellica IM and Atellica CI 1900 Analyzers. **Results:** LoQ is defined as the lowest amount of analyte in a sample at which the within-laboratory %CV is ≤20%. Representative precision and MC results for each assay across indicated sample ranges are listed in the table. Over the three assays tested, repeatability and within-lab %CVs were ≤8.0% and ≤15.5%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1. **Conclusion:** Evaluation of the Atellica IM PSA, cPSA, and fPSA assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on the Atellica IM Analyzer.

Detection Capability	**PSA Assay**	**cPSA Assay**	**fPSA Assay**
LoQ, ng/mL (µg/L)	0.04	0.03	0.01
Precision	**PSA Assay**	**cPSA Assay**	**fPSA Assay**
Sample range, ng/mL (µg/L)	0.05–85.18	1.72–84.80	0.05–20.13
Repeatability, %CV range	1.3–8.0	1.0–1.6	1.2–7.0
Within laboratory, %CV range	2.1–15.2	1.7–4.3	2.2–9.1
Method Comparison	**PSA Assay**	**cPSA Assay**	**fPSA Assay**
Sample range, ng/mL (µg/L)	0.18–92.85	0.18–94.08	0.03–23.67
Regression equation for Atellica IM comparative assay	y = 1.03x – 0.00 ng/mL (µg/L)	y = 1.03x + 0.01 ng/mL (µg/L)	y = 1.00x + 0.00 ng/mL (µg/L)

*The products/features mentioned here are not commercially available in all countries. Their future availability cannot be guaranteed. fPSA assay is not available for sale in US

B-043

Thermal evaluation and field study of Labcorp TrueTherm™packaging system for protecting sub-milliliter blood samples

U. Schaff[1], G. Iacovetti[1], B. Collier[2], S. Setzer[1], M. Peevler[1], J. Ragar[1], W. Brandon[2], M. Chappell[2], R. P. Grant[2], G. J. Sommer[1]. [1]*Labcorp, Pleasanton, CA,* [2]*Labcorp, Burlington, NC*

Background:

Many diagnostic assays require temperature-controlled sample storage and shipment to ensure accuracy. Here we present, the Labcorp TrueTherm, designed for thermal-controlled shipment of small volume blood samples. The reusable TrueTherm combines a stainless steel vacuum bottle with a custom internal cold pack and insulating stopper. Users refrigerate the cold pack to protect against warm or cold temperature excursions. The whole system is relatively compact, measuring about 10in (25cm) in length, 3 inches (8cm) in diameter, and 2.5 pounds (1kg) in weight.

Methods:

In order to evaluate thermal performance, the TrueTherm system was exposed to both ISTA-7D summer and winter conditions and internal temperature was recorded for 96hrs. Subsequently, venous blood samples were collected from n=40 subjects, and immediately aliquoted into BD SST (Serum separator) Microtainers®. For each subject, sub-samples were subjected to centrifugal separation (provided by the Labcorp TrueSpin™ battery-powered centrifuge), serum removal, and 24hr insulated shipping

on ice packs compared to a variety of 72hr cross-country shipping conditions with and without centrifugal separation and with and without insulated packaging (including TrueTherm) during August 2022.

Results:

TrueTherm maintained temperature between 2 and 20C for 72hrs under simulated summer and winter conditions. Under actual cross-country summer commercial shipping conditions, both centrifugal separation and thermal control via TrueTherm expanded the number of valid analytes compared to unprotected whole blood (see Table 1).

Conclusion:

The results suggest that TrueTherm is an effective system for protecting small blood samples from thermal extremes during extended shipping. Portable thermal moderation was complimentary with portable centrifugal separation to maximize stability of common analytes.

Analyte		Centrifuged Unprotected	Un-Centrifuged Unprotected	Centrifuged Conv Insulation	Centrifuged TrueTherm	Un-Centrifuged TrueTherm
Electrolyte	CA	+	-	+	+	+
	CL	+	-	+	+	-
	CO2	-	-	+/-	+/-	-
	K	-	-	-	-	-
	NA	+	+	+/-	+	+
	PHOS	-	-	-	-	-
Metabolite	D-BILI	+	-	+	+	+/-
	T-BILI	-	+	+/-	+/-	+
	BUN	+	-	+	+	+/-
	CREJ	-	-	-	-	-
	GLUC	+/-	-	-	-	-
	UA	+	-	+	+	+
Protein	ALB	+	+/-	+	+	+
	T-PROT	+	+	+	+	+
	ALP	+	+	+	+	+
	ALT	+	-	+	+	+/-
	AST	+/-	-	+/-	+/-	-
Lipid	CHOL	+	+/-	+	+	+
	HDL	+	+	+	+	+
	TRIG	+	+	+	+	+
Cardiac	CRPhs	+	+	+	+	+
	ProBNP	+	+/-	+	+	+
Hormone	B-HCG	-	+/-	+/-	+/-	+/-
	LH	+	+	+	+	+
	PRL	+	+	+	+	+
	PROG	-	-	-	-	-
	SHBG	+	+	+	+	+
	TESTO	+/-	+	+/-	+	+

Table 1. Analyte stability at day 3 following cross-country shipping. Bias in n=40 aggregated results compared to baseline values was evaluated against CLIA limits. (+) indicates an acceptable bias, (-) indicates a non-acceptable bias, and (+/-) indicates a borderline acceptable bias.

B-044

Analytical validation of an serum glycerol measured by nuclear magnetic resonance spectroscopy NMR

Q. Wang, R. Scott, J. Meeusen, L. Donato. *Mayo Clinic, Rochester, MN*

Background

Glycerol kinase deficiency (GKD) is a rare X-linked recessive disorder biochemically characterized by hyperglycerolaemia. Adults with GKD are usually asymptomatic. However, they often present with pseudohypertriglyceridemia because most clinical triglyceride methods measure enzymatically generated glycerol from triglycerides. Currently, assays for measuring plasma glycerol are lacking. We describe a preliminary validation of glycerol measured by nuclear magnetic resonance spectroscopy (NMR).

Methods

Serum glycerol was measured by NMR (Numares AG, Regensberg, Germany) using a Ascend 600 NMR (Bruker, Billerica, MA). Stability and matrix effects were determined by repeat measurement of 10 freshly collected samples following various storage conditions. Normal donors were recruited for reference interval (n=143). Analytical specificity was assessed by spiking red cell lysate, bilirubin and intralipid. Precision was assessed by measuring three pools five times for five days. Accuracy was performed by spiking known concentrations of glycerol. Accuracy was assessed using an enzymatic method (Randox Laboratories, Ltd. County Antrim, UK) on a Roche Cobas c501 (Roche Diagnostics, Indianapolis, IN) as reference.

Results

Measured glycerol increased over time in both serum and plasma, however, prepared samples were stable on the instrument for up to 24 hours. Measured glycerol was not impacted by hemoglobin (1000 mg/dL) or bilirubin (625 mg/dL), however, spiking Intralipid® prevented any measurement. NMR and enzymatic assays strongly correlated, with a constant bias (Table). Repeatability and reproducibility were <10% CV and % recovery from glycerol standard were >80% for all concentrations.

Conclusion

Pre-analytical stability, imprecision and accuracy were acceptable for clinical measurement of glycerol using NMR. While there is a significant systematic bias with enzymatic methods, further studies in normal and disease populations will be necessary to determine clinical impact.

Table 1. Analytical validation of serum glycerol by NMR.

Parameter	Acceptance Criteria	Study Results		
Imprecision	Within Run: CV ≤ 20% Between Run: CV ≤ 20%	Mean	Reproducibility %CV (SD)	Repeatability %CV (SD)
		272	4.5% (12)	7.0% (19)
		672	4.7% (32)	9.7% (65)
		833	3.0% (25)	7.7% (64)
Accuracy	Slope 0.9 – 1.1, R2 >0.85	Method comparison NMR vs. Enzymatic N = 26, range = 78-259 mcmol/L (by NMR) Regression: $Glycerol_{NMR}$ = 1.04 x $Glycerol_{ENZ}$ + 141; R^2 =0.898		
	Recovery >90%	Measured (mcmol/L)	Expected (mcmol/L)	Recovery
		265	271	98%
		478	543	88%
		>993	1086	92%*
Reference Interval	Upper 95%-tile	N =143 (51 males and 92 females, ages: 18-85y, range: <73 – 324 mcmol/L) Upper 95% reference limit (95% confidence interval) - ≤ 226 mcmol/L (200, 263 mcmol/L)		
On-Board Stability	Minimum 24 h	12 prepared sera measured after sitting in NMR Time 24h 48h 72h Average % Change 7.1% 1.8% 19.5%		
Analytical Specificity	Hemoglobin 1000 mg/dL Bilirubin 20 mg/dL	Concentration Hemoglobin 1000 mg/dL Bilirubin 625 mg/dL		Max Difference % 9.2% -4.9%
Specimen Type	Acceptable: Mean % <10%, slope 0.9 – 1.1, R2 >0.85	Paired serum (SST) and Na Heparin plasma (N =11; range: 85 - 149 mcmol/L Mean % difference (min, max): 7% (-32% to 40%) Regression: y = 0.85x + 25		

*Recovery calculated using 994 mcmol/L

B-045

A descriptive study of the clinical impacts on COVID-19 survivors using telemonitoring (The TeleCOVID Study)

J. S. Chow. *South Western Sydney Local Health District, Liverpool, Australia*

Background There is increasing evidence that COVID-19 survivors are at increased risk of experiencing a wide range of cardiovascular complications post infection; however, there are no validated models or clear guidelines for remotely monitoring the cardiac health of COVID-19 survivors. This study aims to test a virtual, in-home healthcare monitoring model of care for detection of clinical symptoms and impacts on COVID-19 survivors. It also aims to demonstrate system usability and feasibility. **Methods** This open label, prospective, descriptive study was conducted in South Western Sydney. Included in the study were patients admitted to the hospital with the diagnosis of COVID-19 between June 2021 and November 2021. Eligible participants after consent were provided with a pulse oximeter to measure oxygen saturation and a S-Patch EX to monitor their electrocardiogram (ECG) for a duration of 3 months. Data was transmitted in real-time to a mobile phone via Bluetooth technology and results were sent to the study team via a cloud-based platform. All the data was reviewed in a timely manner by the investigator team, for post COVID-19 related symptoms, such as reduction in oxygen saturation and arrhythmia. This study was designed for feasibility in real clinical setting implementation, enabling the study team to develop and utilise a virtual, in-home healthcare monitoring model of care to detect post COVID-19 clinical symptoms and impacts on COVID-19 survivors. **Results** During the study period, 23 patients provided consent for participation. Out of which 19 patients commenced monitoring. Sixteen patients with 81 (73.6%) valid tests were included in the analysis and amongst them seven patients were detected by artificial intelligence to have cardiac arrhythmias but not clinically symptomatic. The patients with arrhythmias had a higher occurrence of supraventricular ectopy, and most of them took at least 2 tests before detection. Notably, patients with arrhythmia had significantly more tests than those without (t-test, t (13)=2.29, p<0.05). **Conclusions** Preliminary observations have identified cardiac arrhythmias on prolonged cardiac monitoring in 7 out of the first 16 participants who completed their 3 months follow-up. This has allowed early escalation to their treating doctors for further investigations and early interventions.

B-046

Curious Thing, an artificial intelligence (AI) based conversational agents in COVID-19 patient management

J. S. Chow. *South Western Sydney Local Health District, Liverpool, Australia*

Background There are no clear guidelines or validated models for artificial intelligence (AI) based approaches in monitoring of COVID-19 patients who were isolated in the community, in order to identify early deterioration of their health symptoms. **Methods** Developed in partnership with Curious Thing (CT), Sydney-based AI conversational technology, a new care robot technology was introduced in South Western Sydney (SWS) in September 2021 to manage the large numbers of low to medium risk patients with a COVID-19 diagnosis and isolated at home. The CT interface made contact with patients via their mobile phone, following a locally produced script to obtain information recording physical condition, wellness and support. **Results** Between 2nd September to 14th December 2021, there were 32,001 conversations (every phone call that resulted in a conversation with a patient) conducted with 6,323 unique patients (patients included in call lists identified by phone number) engaged. Over half of the completed calls (n=13,143, 53.7%) identified that individuals needed no further support (freeing up valuable clinician time). 4.24% (n=1,077) identified as needing urgent support (getting to them faster than a standard clinician dialling pattern). The AI assisted phone calls effectively identified the patients requiring further support, saved clinician time by monitoring less ailing patients remotely and enabled them to spend more time on critically ill patients, thus ensured that service and supply resources could be directed to those at greatest need. **Conclusion** Based on the severity of the COVID-19, different approaches have been adopted by the healthcare professionals for handling the patients in the most effective manner. This paper describes the AI assisted conversational agent which can act as an alternate approach to identify the deteriorating patients requiring further support while they are isolated at home. The findings are intended to inform policy makers and health professionals about the implications of the use of these AI-based technologies for the management of current or any future pandemic.

B-047

Performance Evaluation of Enzymatic HbA1c Assay on Newly Launched Mindray BS-600M Chemistry Analyzer

M. Li[1], W. Xie[1], Y. Zeng[1], A. Xu[1], L. Ji[1], J. Dai[2]. *[1]Peking University Shenzhen Hospital, Shenzhen, China, [2]Mindray IVD Innovation Center Minnesota, Oakdale, MN*

Background: HbA1c has important reference value in risk stratification, diagnosis, therapeutic monitoring and drug selection for diabetic patients. Therefore, the ability to detect HbA1c has attracted much attention. This study was designed to evaluate the performance of the enzymatic HbA1c assay on a newly launched Mindray BS-600M chemistry analyzer, which can be used to measure whole blood samples directly after mixing without centrifugation. **Methods:** Total 309 clinical samples (EDTA anticoagulation) were analyzed using Mindray BS-600M (enzymatic method), Tosoh G8 (HPLC method) and Roche c501 (immunoassay method) analyzers for the performance comparison. And also, the analytical performance of BS-600M, such as precision, accuracy, linearity, interference of conventional interferents, hemoglobin variants, hemoglobin derivatives and common drugs, were evaluated using other fresh whole blood samples, eight frozen calibrators (IFCC) and forty frozen venous whole blood samples (NGSP). **Results:** The within-run and between-run precision of the enzymatic method for HbA1c determination were less than 1% and linearity of HbA1c (%, NGSP unit) was excellent from 2.96% to 20.23%. The deviations between the results of 48 accuracy samples (IFCC and NGSP) and target values were within ±5%, and the sigma metrics of HbA1c at the high and low concentration levels were between 9.9~19.0, which belongs to the "world-class" level. The results were excellently correlated with those obtained by Tosoh HLC -723 G8 and Roche Cobas c501 (r=0.997, 0.992). The regression equations of HbA1c was y=0.979x+0.005 (n=198) between BS-600M and G8 and y=0.934x+0.317 (n=112) between BS-600M and c501. The deviation of interference from conventional interferents (bilirubin, Intralipid, vitamin C), hemoglobin variants (HbS, HbC, HbD, HbE), hemoglobin derivatives (LAlc, CarHb, AcetylatedHb), and commonly used antipyretic analgesics and hypoglycemic drugs were all within ±6%, except for the high level HbF (>10%); and also, BS-600M cannot be applied to severe anemia patients (HGB<45 g/L), which may lead to falsely high HbA1c results. **Conclusion**: The Mindray BS-600M proved to be a robust and reliable method for HbA1c measurement, and was suitable for routine clinical chemistry or hematology laboratory use. In addition, attention or comment need to be added for samples having high HbF concentrations and severe anemia patients in reporting HbA1c% result.

Clinical and Diagnostic Immunology

B-048

Performance Evaluation of the New Anti-Streptolysin-O Assay on Mindray BS-2800M System

W. Chen[1], S. Liu[1], M. Jiang[1], J. Wang[1], J. Dai[2]. *[1]Shenzhen Mindray Bio-Medical Electronics Co.,Ltd, Shenzhen, China, [2]Mindray IVD Innovation Center Minnesota, Oakdale, MN*

Background: Elevated serum concentrations of Anti- Streptolysin-O(ASO) can be used to provide serologic evidence of past or present infection by β-Hemolytic Streptococci. Increasing serum concentrations of ASO antibodies are produced in response to ASO exotoxins secreted by the bacteria. Measurement of ASO antibody levels in serum can be used as an aid in the diagnosis of diseases such as glomerulonephritis, rheumatic fever, bacterial endocarditis, tonsillitis, and scarlet fever, etc. The New ASO assay has been evaluated on Mindray BS-2800M that is an automated, high throughput, and low cross-contamination system. The evaluation of this assay included precision, linearity, dilution accuracy, interference, high dose hook, and method comparison studies. **Methods:** The new ASO reagent contains R1 buffer and R2 with latex particles conjugated with in-house made ASO antigen. The R1 is incubated with human serum sample containing ASO antibodies for 5 min at 37°C and then R2 is added. After another 5 min of incubation, immune-aggregation complex is formed. The increased turbidity is measured at 570 nm. By constructing a six-level calibration curve (water is used as reagent blank) from the absorbances of calibrators, the ASO antibody concentration of the sample is determined. **Results:** The precision study was run according to EP05A3 with two commercial controls (approximately 80 and 300 IU/mL) and two serum sample pools (approximately 150 and 400 IU/mL) on the Mindray BS-2800M system. The repeatability and within-lab CVs ranged from 0.8% to 2.0% and 2.0% to 6.0%, respectively. The analytical linearity of the assay was determined as from 20 IU/mL to 1000 IU/mL with the limit of blank of 2 IU/mL and limit of detection of 10 IU/mL. The dilution accuracy was evaluated by diluting high ASO level samples at upper region of assay range using saline with 5-fold and run neat and diluted samples. The deviation in recoveries between diluted sample after calculation and the neat sample was found to be less than ±10%. The new ASO assay showed <±10% interference with bilirubin (conjugated or free) up to 40 mg/dL,hemolysate hemoglobin up to 1000 mg/dL,Intralipid up to 1000 mg/dL,and Triglycerides up to1000 mg/dL. The assay on the Mindray BS-2800M system(y) correlated well with the ASO assay(x) on the Beckman AU5800 system:y =1.01 x+1.55(r =0.9514,n = 95,range:50 - 971 IU/mL). No prozone was observed with the assay on the Mindray BS-2800M system up to the highest ASO antibody concentration of 5000 IU/mL. **Conclusion:** We conclude that the New ASO assay with in-house developed ASO antigen raw material, when used on the Mindray BS-2800M system, can measure serum ASO antibody concentrations precisely and accurately over a broad range. It is suitable for use in routine clinical laboratories.

B-050

A Versatile Immunoassay Platform for Fast Multiplex Detection of Protein Biomarkers Based on A Novel Magnetic Particle System

Y. Cho[1], D. Moon[1], K. Han[1], H. Choi[1], J. Kim[1], Y. Ro[2], B. Oh[3], S. Song[4], Y. Jung[1]. *[1]R&D Center, EzDiaTech Inc., Anyang-si, Gyeonggi-do, Korea, Republic of, [2]Department of Emergency Medicine, Seoul National University Hospital, Seoul, Korea, Republic of, [3]Department of Rehabilitation Medicine, Seoul National University Hospital, Seoul, Korea, Republic of, [4]Department of Laboratory Medicine, Seoul National University Hospital, Seoul, Korea, Republic of*

Background: A major challenge of clinical diagnostic applications in the field of emergency department is the not only fast but also accurate measurement of multiple protein biomarkers with broad dynamic range spectrum. Here, we describe the development of a versatile immunoassay platform, the VEUS (Versatile, Easy, and User-friendly System), with compact size which occupies minimal space.

Methods: The VEUS is designed to enable fast multiplex detection (i.e., 15~60 minutes) of target proteins at a concentration with a low limit of detection (i.e., < 1 pg/mL) by automation of overall immunoassay processes.The system possesses four core

technologies enabling entirely automated rapid diagnostics: 1) novel magnetic particles for enabling multiplexed assay using various length of rod-shaped magnetic particles (RSMPs) with conjugated individual antibodies, 2) RSMP movement controlling (up & down/well to well) for increasing antibody-antigen reaction and washing efficiency, 3) a unique optical module for highly sensitive imaging under large field of view for a whole imaging well, and 4) an artificial intelligence (A.I.) based intrinsic program for fast and accurate fluorescent signal analysis which covers detection and classification of RSMP based on their length, and then measurement of fluorescent signal and conversion to biomarker concentration.We evaluated clinical performance of the VEUS platform to diagnose traumatic brain injury (TBI), an emergency disease, as a proof of concept of the versatile immunoassay platform. Among TBI, mild TBI (mTBI) is prevalent in younger population with high level of physical activities that have been linked to increased rate of incidences. Although mTBI is rarely resulted in death, unidentified by the Computed Tomography (CT) and prolonged mTBI as a stealth pathology, may cause life-long disability.

Results: We determined the assay cut-off value from critical concentration of GFAP (Glial fibrillary acidic protein) and UCH-L1 (Ubiquitin carboxy-terminal hydrolase L1) as of 24 pg/mL and 138 pg/mL, respectively. The VEUS performance for a TBI clinical test showed 94.38 % sensitivity and 98.99 % specificity with the clinical serum samples of 89 TBI patients and 97 healthy controls within 50 minutes from the multiplexed assay of GFAP and UCH-L1.

Conclusions: As a versatile immunoassay platform, the VEUS system showed high specificity and sensitivity from the clinical test for the TBI diagnosis. Thus, the VEUS system can contribute to proper treatment for the TBI patients with rapid and accurate clinical results and be also applied for an independent and a precise diagnosis of multiple emergency diseases, such as sepsis, myocardial infraction, heart failure, and stroke which are common in the field of emergency department.

B-052

Establishment of new antibodies and ELISA system to detect the potato alkaloids α-solanine and α-chaconine

K. Okada[1], K. Matsuo[2]. *[1]Kyoto Tachibana University, Kyoto, Japan, [2]Kyoto Tachibana University Graduate School of Health Sciences, Kyoto, Japan*

Background: Food poisoning by potato alkaloids, α-solanine (SO) and α-chaconine (CHA), currently occurs worldwide, however laboratory methods have not yet been established to detect these toxins in biological samples. In this study, we established new antibodies to detect SO and CHA and evaluated the practicality of enzyme-linked immunosorbent assays (ELISAs) using these antibodies in laboratory medicine.

Methods: Solanidine, a chemical compound found in both SO and CHA, was conjugated with bovine albumin, and this complex was repeatedly injected into two rabbits for two months. Two types of polyclonal solanidine antibodies were then purified from rabbit serum using affinity column chromatography. Using these antibodies, two forms of direct ELISAs (Sold ELISA-1, 2) were constructed. The basic detection performance of the ELISAs was confirmed by measuring the levels of SO and CHA diluted in phosphate-buffered saline (PBS). To simulate biological samples from patients with potato food poisoning, SO and CHA powders were mixed with commercially available human serum and urine, and SO and CHA levels were then measured using ELISA. Lastly, SO and CHA levels were detected in extracts from rotten potato (*Irish Cobbler*) sprouts, peels, and tubers.

Results: In samples of SO diluted in PBS, the limit of quantification (LoQ) was determined as 2.56 ng/mL and 9.55 ng/mL for Sold ELISA-1 and -2, respectively. In CHA diluted in PBS, the LoQs of Sold ELISA-1 and -2 were 1.36 ng/mL and 4.8 ng/mL, respectively. The LoQs of SO in the serum were calculated as 14.25 ng/mL and 19.41 ng/mL using Sold ELISA-1 and -2, respectively. In contrast, the LoQs of CHA in the serum were 12.48 ng/mL and 16.92 ng/mL using Sold ELISA-1 and -2, respectively. The LoQs of SO in the urine were determined as 30.28 ng/mL and 45.16 ng/mL using Sold ELISA-1 and -2, respectively. Conversely, the LoQs of CHA in the urine were 22.92 ng/mL and 38.15 ng/mL using Sold ELISA-1 and -2, respectively. Using Sold ELISA-1, both SO and CHA in the extracts from rotten potato tubers and peels were detected as approximately 1.7- and 2.3-fold higher, respectively, in each part than those in the extracts from fresh potatoes. When both SO and CHA were measured in the same samples using Sold ELISA-2, the levels were approximately 1.6- and 2.4-fold higher, respectively, in the tubers and peels than those in fresh potato samples. Furthermore, the potato sprout extract contained approximately 80-fold more SO and CHA than the tuber and 8-fold more than the peel.

Discussion and Conclusion: Based on our results, Sold ELISA-1 presented greater performance in detecting SO and CHA than Sold ELISA-2. The non-specific reactions in the serum samples and influence of urea in the urine samples may underlie the reduced detection performance of SO and CHA in ELISA, respectively. Although the sensitivity depends on the sample type, these ELISAs may be effective as future clinical and food testing methods.

B-054

Potential of Autotaxin in the Early Diagnosis of Sinusoidal Obstruction Syndrome after Allogeneic Hematopoietic Cell Transplantation

K. Takemura[1], M. Nakamae[2], H. Okamura[2], K. Sakatoku[2], K. Ido[2], Y. Makuuchi[2], M. Kuno[2], T. Takakuwa[2], A. Hirose[2], M. Nishimoto[2], Y. Nakashima[2], H. Koh[2], K. Igarashi[3], H. Kubota[1], M. Hino[2], H. Nakamae[2]. *[1]Osaka Metropolitan University Hospital, Osaka, Japan, [2]Osaka Metropolitan University Graduate School of Medicine, Osaka, Japan, [3]TOSOH Corporation, Kanagawa, Japan*

Background: Sinusoidal obstruction syndrome (SOS) is a life-threatening complication after allogeneic hematopoietic cell transplantation (allo-HCT). Early intervention leads to a good prognosis, but it is often difficult to differentiate SOS from other hepatobiliary complications. Liver biopsy is the gold standard for diagnosing SOS, but it is difficult to perform invasive testing early in the transplant process, and no specific biomarker for SOS has been established. Autotaxin (ATX) is useful as a liver fibrosis marker, the serum levels of which are elevated by metabolic inhibition of liver sinusoidal endothelial cells. Considering the pathophysiology of SOS, which begins with injury of liver sinusoidal endothelial cells, serum ATX levels may be elevated early in the onset of SOS.

Methods: A retrospective study was conducted of 247 patients who had received allo-HCT, including 11 patients who developed SOS and met the EBMT criteria (male, 7; female, 4). Of 11 patients with SOS, 9 had pathologically proven SOS. The median time from allo-HCT to SOS diagnosis was 31 days (range, 8-141 days [male], 16-31 days [female]). This cohort included 39 patients with hepatobiliary complications without SOS (total bilirubin > 2 mg/dL; male, 23; female, 16). Serum ATX levels were measured in stored samples before conditioning and at days 1, 3, 5, 7, 14, 28, 60, 90, 180, and 365 after allo-HCT by a two-site enzyme immunoassay with an AIA-2000 analyzer. The area under the receiver operating characteristic (AUROC) of serum ATX levels for the diagnosis of SOS was analyzed.

Results: We first investigated the variability of serum ATX levels during the allo-HCT period. Serum ATX levels fluctuated as follows: decreased on day 1, increased and peaked on days 7-14, and then decreased again on days 28-60. A variety of factors associated with allo-HCT such as the conditioning regimen and administration of steroids affected serum ATX levels. Serum ATX levels before and after allo-HCT tended to be higher in patients with SOS. In male patients, the AUROCs of serum ATX levels on day 3 after allo-HCT were 0.75 (in all patients; 95% confidence interval [95%CI], 0.49-1.00) and 0.75 (in patients with hepatobiliary complications; 95%CI, 0.48-1.00). In female patients, the AUROCs of serum ATX levels on day 14 after allo-HCT were 0.88 (in all patients; 95%CI, 0.77-0.98) and 0.94 (in patients with hepatobiliary complications; 95%CI, 0.82-1.00). These findings indicated that serum ATX levels before the diagnosis of SOS had a diagnostic capacity. In addition, focusing on severe SOS cases, three of six patients had serum ATX levels above the upper reference limit before conditioning (a total of 18 patients exceeded the upper reference limit in this cohort). Of the remaining three patients, two had elevated post-transplant ATX levels near or above the upper reference limit prior to diagnosis of SOS.

Conclusion: Although validation by further studies with larger numbers of SOS cases is needed, the serum ATX level may be a useful biomarker for early diagnosis of SOS after allo-HCT.

B-055

Comparison of Sensitivity Between Tissue Anti-Transglutaminase and Anti-Endomysium IgA and IgG in Serological Screening for Celiac Disease in a Large Clinical Laboratory in Brazil

J. F. de Souza[1], D. do Sacramento Santos[1], F. A. Pinto[1], A. F. Justino Feo[1], V. Vidotto Frade de Oliveira[1], G. Zampieri[1], M. H. Costa Gurgel Castelo[2], A. Correa Wengerkievicz Lopes[3]. *[1]DASA, Sao Paulo, Brazil, [2]DASA, Fortaleza, Brazil, [3]DASA, Florianopolis, Brazil*

Background: Celiac Disease (CD) is a genetic and autoimmune disorder characterized by permanent intolerance to gluten found in foods, resulting in small intestine injury in children and adults. Anti-tissue transglutaminase (anti-tTG) and anti-endo-

mysium (anti-EM) antibodies are important serological markers of this disease. The aim was to analyze the analytical sensitivity of these markers in a large-scale Clinical Laboratory routine.

Methods: We employed a pure sample and a series of dilutions in the analysis of the functional sensitivity of each marker (anti-tTG IgA/IgG and anti-EM IgA/IgG) and a database survey of a large Clinical Laboratory in Sao Paulo/Brazil, from January to December 2021, analyzing 60,000 samples with concomitant results of anti-tTG IGA and anti-EM IgA and 7,075 samples with results of anti-tTG IgG and anti-EM IgG, from patients with no defined clinic condition. The anti-tTG tests were performed by fluorescence enzyme immunoassay (FEIA) and the anti-EM tests by indirect immunofluorescence (IFI), with an initial dilution of 1:10, following the manufacturer's recommendations.

Results: The determination of functional sensitivity by checking the maximum dilution of antibody detection showed higher sensitivity of the anti-tTG IgA and IgG test (dilution factor 0.3 for both) compared to the anti-EM IgA and IgG (dilution factor 0.7 for both). Of the 60,000 samples analyzed for IgA markers, 59,083 (98.5%) were negative for both tests. Of the remaining 917 samples (1.5%), 609 (66.4%) were positive for both anti-tTG IgA and anti-EM IgA, 308 (33.6%) were positive only for anti-tTG IgA, and we did not observe any sample anti-tTG IgA negative with anti-EM IgA positive. Of the 7,075 samples analyzed for IgG markers, 7,032 (99.4%) were negative for both tests. Of the remaining 43 samples (0.6%), 18 (41.9%) were positive for anti-tTG IgG and anti-EM IgG, 14 (32.6%) were positive only for anti-tTG IgG, and 1 (2.3%) were positive only for anti-EM IgG.

Conclusion: The study showed greater sensitivity of anti-tTG compared to anti-EM for both IgA and IgG, in agreement with the literature data. The anti-tTG IgA test, in individuals without immunoglobulin A (IgA) deficiency, is recommended as the initial test in suspicion of celiac disease by current scientific guidelines on celiac disease. Based on our findings and literature data, in individuals with IgA deficiency, we suggest a screening using two automated IgG markers, tTG IgG and deamidated gliadin IgG (DG IgG). In addition to the lower sensitivity of anti-EM, indirect immunofluorescence (IFI), compared to the fully automated FEIA method, is a more subjective, observer-dependent, and labor-intensive methodology to be used as screening in large-scale Clinical Laboratory routines.

B-056

Performance characteristics of a new, fully automated planar microarray immunoassay for the detection of IgG auto-antibodies against Centromere Protein B (CENP-B)

C. Daubrosse[1], P. Ghillani-Dalbin[1], V. Mercier[1], R. Pasion Galvan[2], D. Bijlsma[2], E. Lukasik[2], V. Botti[2], G. Gomez[2], E. Moreau[2], M. Hausmann[2], C. C. Ginocchio[2], M. Miyara[1]. *[1]Sorbonne Université, Inserm, Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Pitié-Salpêtrière, Paris, France, [2]Quotient Suisse SA, Eysins, Switzerland*

Background: Detection of auto-antibodies against Extractable Nuclear Antigens (ENA), including CENP-B, is key in identification of autoimmune (AI) diseases. Many current test methods are manual, slow, labor intensive or do not facilitate the simultaneous detection of several auto-antibodies, that could reduce time to provide comprehensive results to clinicians. We report the performance characteristics of MosaiQ™ CENP-B (Quotient Suisse, Eysins, CH), a new planar microarray immunoassay designed for use with the fully automated, continuous random access, high throughput MosaiQ system for qualitative serological detection of CENP-B IgG auto-antibodies. CENP-B assay is one parameter of a multiplexed MosaiQ connective tissue disease panel in development. **Methods**: MosaiQ CENP-B microarrays were prepared by printing CENP-B antigens onto epoxy-silane glass chips and assembled into magazines for processing on the MosaiQ 125 instrument (Fig.1). Primary patient serum tubes and reagents are loaded on the instrument and automatically processed to generate immunoassay spot signals, interpreted by the instrument using a proprietary image analysis algorithm. Anonymized human serum samples (Hôpital Pitié Salpetrière, Paris, France), characterized as CENP-B auto-antibodies non-reactive or reactive with ≥1 CE-marked devices, were included in the study. **Results**: After discordant analysis, MosaiQ system correctly identified 96/99 samples characterized as reactive by comparators and all 199 non-reactive samples: positive percent agreement 97.0% (95% CI, 91.4%, 99.4%), negative percent agreement 100% (95% CI, 98.2%, 100%), overall percent agreement 99% (95% CI, 97.1%, 99.8%). A reproducibility and repeatability evaluation showed, respectively, overall agreement of 99.7% and 100%. **Conclusion**: MosaiQ CENP-B microarray provides a high level of clinical concordance with other CE-marked assays for detecting CENP-B auto-antibodies. The MosaiQ System has the potential to advance AI disease testing by increasing laboratory efficiency and productivity, with the ability to multiplex the detection of various auto-antibodies on a single microarray, and a capacity to automatically process thousands of samples per day.

Figure 1

B-058

Development and Evaluation of the Assay for Detection of Human Specific IgE Against Animal Dander Allergens on a Fully Automated Chemiluminescence Immunoassay Platform - Autolumo® A2000 Plus

S. Rezaei[1], X. Yang[1], I. Xi[1], D. Zhao[2], J. Han[2], K. Zhu[2], Z. Li[2], C. Lee[1]. *[1]AdvanBio, Irvine, CA, [2]Autobio Diagnostics Co, Zhengzhou, China*

Background:

The detection of specific IgE is a key method for diagnosing allergic diseases. An accurate, user friendly and cost-effective way to clinically diagnosing allergies is beneficial for the patients. Our detection kit together with the Autolumo A2000 Plus chemiluminescence fully automated platform provide a new and more effective (40 minutes) way for detection of specific IgE with simple operation, high sensitivity and specificity.

Methods:

The Animal Dander Allergen Mix Assay kit detects specific IgE against cat dander (E1), horse dander (E3), cow dander (E4) and dog dander (E5). Performance evaluation of the animal dander mix, E1, E3, E4 and E5 assay kits for the detection of specific IgE in serum was performed on our automated chemiluminescence platform (Autolumo A2000 Plus) and compared to a well-characterized platform (ImmunoCAP, Phadia). During detection, magnetic particle suspension, antigen solution, patient sample, and enzyme conjugate were added sequentially. A streptavidin-biotinylated antigen-sIgE antibody-anti-IgE-HRP conjugate complex is formed, wherein the amount of the anti-IgE-HRP conjugate is positively correlated to the amount of sIgE antibodies in the patient sample. After the substrate is added, the HRP catalyze the chemical reaction to generate a recordable signal in the form of light. The intensities of the light were obtained by using a chemiluminescence quantitative analyzer, and the standard curve was generated according to the 7-point calibration, and then the sIgE antibody concentrations in the serum sample were analyzed. The overall agreement, method comparison, precision, linearity, and stability of our mixed and single assay kits were evaluated using serum samples (n>140) that had been characterized with Phadia.

Results:

The overall agreement for the animal dander mix assay is 91.84%, with the average intra-run and inter-run precision to be 1.97% and 4.21% respectively. The limit of blank (LoB) and limit of detection (LoD) for the animal dander mix assay is 0.019 kU/L and 0.045 kU/L respectively. The sensitivity for our single E1, E3, E4, and E5 assays are 97%, 85.6%, 70.8%, and 80.2%, respectively. All single assays have a specificity >90%, total precision <5% CV, and a linearity with R2 >0.999. The LoB, LoD and recommended LoQ for all single assays are 0.019 kU/L, 0.037 kU/L, and 0.1 kU/L respectively. The average signal drifts after 10 days incubation in 37C is less than 10% indicating the assays are stable.

Conclusion:

Autolumo A2000 Plus is a fully automated chemiluminescence Immunoassay platform that provides accurate and cost-effective screening for allergy diagnostics with excellent overall agreement, precision, and stability.

B-059

Allergen-specific IgE Measurement for Peanut Ara h 2*

T. Chuang[1], N. Ready[2], M. Rowlands[2], J. Chamberlain[2], D. Hammer[1], M. Sommer[1], D. Hovanec-burns[1]. *[1]Siemens Healthcare Diagnostics Inc., Tarrytown, NY, [2]Siemens Healthcare Diagnostics Products Ltd, Llanberis, United Kingdom*

Background:

Legumes such as peanuts are a major source of food allergies among children and adults. Ingestion is the primary route of exposure, although inhalation may also trigger an allergic response in patients with known hypersensitivity. Allergic reactions to peanut can include atopic dermatitis, angioedema, vomiting, diarrhea, and anaphylaxis. Several allergenic peanut proteins have been identified and linked with variable severity of patient symptoms. Ara h 2 is one of the proteins uniquely associated with severe peanut allergy. In this study, we evaluate the analytical performance of the Ara h 2 allergen containing reagent for use in the IMMULITE® 2000 3gAllergy® Specific IgE assay.

Methods:

Serum samples were collected from 13 patient samples with documented histories of tree nut or legume-related allergies. The samples were analyzed for specific IgE reactivity to a commercially available recombinant Ara h 2 (rAra h 2) using the IMMULITE 2000 3gAllergy Specific IgE assay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). Precision, linearity, limit of detection, and endogenous interference were assessed.

Results:

Repeatability and within-lab precision at all levels tested were less than 10% over a 20-day study. Regression analysis was performed following linearity assessment and resulted in a slope 95% CI of 0.9251-1.0492 (range 0.3-24 kU/L). Limit of blank and limit of detection were 0.02 kU/L and 0.08 kU/L, respectively. Endogenous interference testing showed <10% difference when spiked with hemoglobin (1000 mg/dL), intralipid (3000 mg/dL), and unconjugated bilirubin (40 mg/dL).

Conclusion:

rAra h 2 allergen containing reagent for use in the IMMULITE 2000 3gAllergy Specific IgE assay is sensitive and capable of quantitatively measuring IgE in patients with clinical history of nut allergy. *Assay under development. Not available for sale. Future availability cannot be guaranteed.

B-060

Measurement of Infliximab Clearance using a Bayesian forecasting tool and impact of Albumin and immunization status during maintenance treatment of inflammatory bowel disease

O. McLachlan, M. Schwalbe, J. McFarland, D. Pham, P. Hughes, T. Dervieux. *Prometheus Laboratories, San Diego, CA*

Background: Accelerated Clearance of monoclonal antibodies such as Infliximab (IFX) is a known predictive factor of pharmacokinetic origin that associates with poor outcome in patients with Inflammatory bowel diseases (IBD). We have implemented a Bayesian forecasting tool for IFX (PredictrPK® IFX) and calculated its Clearance. Our objective was to evaluate the impact of Albumin (ALB) and antibodies to IFX (ATI) on Clearance and exposure from patient specimens with IBD and submitted for testing in the routine clinical pharmacokinetic laboratory.

Methods: IFX concentration and ATI status were determined from serum using a homogenous mobility shift assay. Serum ALB concentration was determined using nephelometry. Clearance was estimated using inputted IFX concentration, ATI status, ALB, dose and interdose interval, and nonlinear mixed effect modeling with Bayesian priors. Patient specimens were collected anytime 20 days after the infusion and submitted for testing. Median Clearance (expressed as L/day) and estimated IFX trough concentrations by ATI status and lower Albumin levels (< 4.0 g/dL) was calculated. Statistical analysis consisted of nonparametric Mann-Whitney tests.

Results: The prevalence of ATI was 9.1% in this cohort of patient specimens. Median Clearance was 0.246 L/day (IQR: 0.191 - 0.323 L/day) and median IFX Trough concentration was 11.9 µg/mL (IQR: 6.5 - 19.9 µg/mL). ATI status associated with lower IFX Trough concentrations and higher Clearance (p<0.01). Lower ALB concentration regardless of ATI status also associated with lower IFX Trough concentrations (p<0.01). There was twofold higher Clearance and twelvefold lower IFX Trough concentration in the presence of ATI and lower ALB. Results are presented in Table I.

Conclusion: The data are consistent with the notion that IFX immunization and lower ALB impact Clearance and exposure to IFX.

Table I - Median (interquartile range, IQR) for Clearance (L/Day) and IFX Trough (µg/mL) by Albumin Concentration and ATI Status.

	ATI Not Detected	ATI Detected	AllSpecimens
Albumin concentration< 4.0 g/dL	IFX Trough: 11.3 (6.6 - 19.6)Clearance: 0.262 (0.200 - 0.338) N = 379	IFX Trough: 1.2 (0.7 - 2.8)Clearance: 0.463 (0.355 - 0.560) N = 56	IFX Trough: 9.8 (4.7 - 18.0)Clearance: 0.275 (0.208 - 0.366) N = 435
Albumin concentration≥ 4.0 g/dL	IFX Trough: 14.9 (9.7 - 22.3)Clearance: 0.214 (0.180 - 0.262) N = 318	IFX Trough: 3.8 (1.5 - 6.3)Clearance: 0.347 (0.300 - 0.484) N = 14	IFX Trough: 14.3 (9.0 - 21.8)Clearance: 0.217 (0.182 - 0.269) N = 332
AllSpecimens	IFX Trough: 13.2 (8.0 - 21.0)Clearance: 0.238 (0.188 - 0.306) N = 697	IFX Trough: 1.4 (0.7 - 3.4)Clearance: 0.433 (0.342 - 0.558) N = 70	IFX Trough: 11.9 (6.5 - 19.9)Clearance: 0.246 (0.191 - 0.323) N = 767

B-062

A Self-Imposed Gray Area? Analysis of Anti-Tissue Transglutaminase and Anti-Endomysial Antibody Discordance in a Celiac Disease Screening Serology Testing Algorithm

S. Ezra[1], D. Orton[1], J. L. Gifford[2]. *[1]Alberta Precision Laboratories and Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, AB, Canada, [2]DynaLIFE Medical Labs and Department of Pathology and Laboratory Medicine, University of Calgary., Calgary, AB, Canada*

Background: Celiac disease (CD) is an autoimmune enteropathy affecting around 1% of the population. While occasionally asymptomatic, the disease can present in a variety of ways and can be triggered by dietary gluten at any age. CD serology, including anti-tissue transglutaminase (anti-tTG) IgA and/or IgG, anti-endomysial antibody IgA and/or IgG, and anti-deamidated gliadin peptide IgA and/or IgG, is a key tool for CD screening, diagnosis, and monitoring. Of these tests, the clinical utility of anti-EMA testing has been questioned due to its high cost and limited sensitivity: 5-10% of CD patients do not test positive for anti-EMA and a negative anti-EMA does not rule out CD. The objective of this study is to: 1) evaluate the concordance between anti-tTG and anti-EMA serology test results, and 2) assess the clinical validity of our current CD reflex testing algorithm in which every positive anti-tTG test result goes on for anti-EMA testing.

Methods: Using our laboratory's information system, we conducted a retroactive study on patients who underwent CD serology testing at our institution. Query was performed for all patient data [pediatric (<18 years) and adult (>18 years)] collected between April 2020 and August 2022 for anti-tTG IgA performed on the BioPlex 2200 (BioRad Laboratories Inc., Hercules, CA) and anti-EMA IgA (Euroimmun, Germany) (*N*=124,308). Vendor-supplied clinical sensitivity and specificity are as follows: anti-tTG IgA 94.3% sensitivity, 98.8% specificity; anti-EMA IgA 95.3% sensitivity and 98.0% specificity.

Results: From our data, we calculated a mean positive agreement between our anti-tTG IgA and anti-EMA test results of 53.2% (95% confidence interval (CI) 47.5-58.8%) for all anti-tTG IgA positive cases (*N*=6,691, 5.4% of total anti-tTG IgA test results). The agreement between anti-tTG IgA and anti-EMA test results is improved if multiples of the upper limit of normal (ULN) for anti-tTG IgA is applied. The mean positive agreement between anti-tTG IgA and anti-EMA test results when the anti-tTG result is >10x ULN is 98.4% (95% CI 97.7-99.2%). However, if an anti-tTG IgA cutoff of <10x ULN is employed a mean positive agreement of 32.3% (95% CI 25.8-38.7%) is observed. At an anti-tTG IgA cutoff of <3x ULN, the mean positive agreement is just 11.8% (95% CI 8.2-15.4%). These findings are consistent in both pediatric (*N*=29,381) and adult (*N*=94,961) populations and are problematic as 45.4% of our positive anti-tTG IgA test results are accompanied by a negative anti-EMA.

Conclusion: The agreement between anti-tTG IgA and anti-EMA is dependent on the value of anti-tTG IgA with greater agreement observed with increasing anti-tTG IgA values. As all specimens at our institution with positive anti-tTG IgA test results are automatically reflexed for anti-EMA testing, has led to physician confusion as the majority of patients are falling into a gray area with conflicting CD serology test results. Our algorithm is also out of line with most CD screening guidelines that suggest an initial positive anti-tTG test result is followed up with biopsy. Finally, discontinuing anti-EMA reflex testing could result in cost savings of approximately $120,000 CAD per year.

B-063

Towards improving mRNA vaccine effectiveness in immunosuppressed adults: Elucidating the mechanisms and evaluating potential improvement strategies

M. Narasimhan, K. Kim, L. Mahimainathan, R. Zhang, S. Malladi, A. Muthukumar. *UT Southwestern Medical Center, Dallas, TX*

Background: The success of COVID-19 mRNA vaccines (Co-mV) has provided a renewed impetus in developing "onetime universal flu, COVID", and other mRNA-based vaccines to offer broader and long-lasting protection. However, to bring this endeavor to fruition, it is of prime importance to address the mechanism(s) underlying poor vaccine response in some patients and close this gap of inequitable effectiveness through improvement strategies, which is the focus of our current study. To date, there is no 'empirical' evidence to link the perturbation of translation, a rate-limiting step for mRNA vaccine efficiency, to its dampened response.

Methods: This IRB approved study involved 1) an assessment of a total of 1009 immunocompromised (IC) patients and immunocompetent subjects who had received 2 or more doses of Co-mV, 2) *in vitro* cell-culture experiments, and 3) *in vivo* animal studies. Impact of immunosuppressants (ISs), tacrolimus (T), mycophenolate (M), rapamycin/sirolimus (S), and their combinations on Pfizer Co-mV translation were determined by the Spike (Sp) protein expression following Co-mV transfection in HEK293 cells. *In vivo* impact of ISs on SARS-CoV-2 spike specific antigen (SpAg) and associated antibody levels (IgG_{Sp}) in serum were longitudinally assessed (26 days) in Balb/c mice after two doses (2D) of the Pfizer vaccine. Spike Ag and IgG_{Sp} levels were assessed in 259 IC patients and 50 healthy controls (HC) who received 2D of Pfizer or Moderna Co-mV. In addition, 67 immunosuppressed solid organ transplant (SOT) patients and 843 non-transplanted (NT) subjects following three doses (3D) of Co-mV were assessed. Expression of Sp and p70S6K phosphorylation (translation surrogate) were evaluated following higher vaccine doses and transient drug holidays. Statistical analysis was performed using GraphPad software 9.3.1. $p<0.05$ was considered as statistically significant.

Results: The 2D and 3D Co-mV received IC patients showed a significantly lower IgG_{SP} response ($p<0.0001$) relative to their matched controls. M or S profoundly dampened ($p<0.001$) the IgG_{SP} response following Co-mV in the IC patients relative to those that were not on these drugs. M and S, when used individually or in combination in HEK293 cells, significantly ($p<0.05$) attenuated the Co-mV-induced Sp expression concurrently with translation surrogates. In contrast, T did not change these indices. Notably, the cellular uptake of Co-Mv was not altered by these drugs. *In vivo* sirolimus combo pretreatment significantly ($p<0.05$) attenuated the Co-mV induced IgM_{Sp} and IgG_{Sp} production. This correlated with a decreasing trend in the early levels (after day 1) of Co-mV-induced Sp immunogen levels. Neither higher Co-mV concentrations (6μg) nor a 1-day break of S could overcome the repressed Sp protein levels. Interestingly, 3-days of S holiday or using T alone rescued Sp levels *in vitro.*

Conclusion: This is the first study to reveal that ISs, sirolimus and mycophenolate restrain Co-mV-induced Sp protein synthesis via translation suppression. Transient holiday of sirolimus or selective use of tacrolimus at the time of vaccination can be a potential option to rescue translation-dependent Sp protein production. These compelling findings lay a solid foundation for guiding future studies aimed at improving mRNA-based vaccine effectiveness in high-risk IC patients.

B-064

Analytical and Diagnostic Performances of Anti-Integrin Antibody System in Inflammatory Bowel Disease

M. Schwalbe, O. McLachlan, A. Everts-van der Wind, T. Dervieux. *Prometheus Laboratories, San Diego, CA*

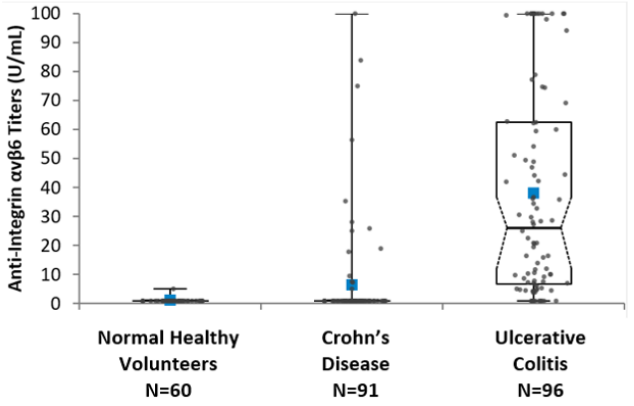
Background: Anti-integrin αvβ6 autoantibodies may have value in the diagnosis of Inflammatory Bowel Disease (IBD) and more specifically Ulcerative Colitis (UC). Our objective was to establish the analytical and diagnostic performance of this novel autoantibody system in distinguishing IBD from a group of Normal Healthy Volunteers (NHV) and UC from Crohn's Disease (CD).

Methods: All testing was performed using a Tecan Evo200 equipped with 8-channel and 96-channel arms, plate washer, and plate reader. The ELISA assay used calibration controls and diluted serum samples added to ELISA plates coated with Integrin αvβ6 antigen. Anti-human IgG horseradish peroxidase complexed with Anti-integrin αvβ6 antibodies was detected using colorimetry. The analytical performance was evaluated by using repeatability and reproducibility with coefficient of variation (CV) calculated. The diagnostic performances were evaluated by testing specimens collected from consented subjects available from a biobank in autoimmune gastrointestinal diseases. This consisted of 60 NHV, and 187 IBD (91 CD and 96 UC). Sensitivity, specificity, diagnostic odds ratio (OR), positive and negative predictive values (PPV and NPV) were estimated.

Results: Intra-assay CVs ranged from 1.5% to 5.2% over dynamic range. Inter-assay CVs were below 10%. Median titers were below 5 U/mL for NHV and CD, and 26 U/mL for UC (Figure 1). Cutoff of 5 U/mL was 100% specific for UC and CD. Sensitivity for UC and CD was 80% (77/96), and 15% (14/91), respectively. Diagnostic Odds Ratio yielded 22.3-fold (95% CI: 10.5-47.5) higher likelihood of UC than CD in the presence of Anti-integrin αvβ6 titers above cutoff, with PPV and NPV values of 84% (76%-90%) and 81% (74%-87%), respectively (50% pre-test).

Conclusion: We have established the analytical performance of an Anti-integrin αvβ6 autoantibody ELISA assay. These preliminary data suggests that the Anti-Integrin αvβ6 autoantibody system is highly specific for UC with high sensitivity and specificity.

Figure 1. Anti-integrin αvβ6 Titers in IBD and Normal Healthy Volunteers



B-066

Evaluation on Effectiveness of Colloidal Gold Immunochromatography Assay for Rapid Detection of Carbapenemase-producing *Enterobacteriaceae*

G. Yang, J. Yan, Z. Zhang, W. Yao, Y. Wang, H. Wang, Y. Su, Z. Zhou. *Tianjin Enterprise Key Laboratory for precision diagnosis technology of invasive fungal diseases, Tianjin, China*

Background: The identification, treatment, and control of carbapenem-resistant *Enterobacteriaceae* (CRE) infections are a major challenge for health care institutions and diagnostic laboratories worldwide. A novel Carbapenemase-producing Bacteria Test Kit (Lateral Flow Assay) from Dynamiker Biotechnology (Tianjin) Co., Ltd. is a qualitative test that was easy to operate and can be widely accepted by clinical and primary medical. Results can be read in just 15 minutes using colloidal gold immunochromatography, which can detect double and multiple carbapenemase types (Fig. 1). We analyzed the effectiveness of Dynamiker carbapenemase-producing bacteria test kit for rapid detection of CRE carbapenemase.

Methods: A total of 120 strains of Enterobacterales in blood culture were collected, including 80 strains of CRE and 40 strains of CSE (carbapenem-susceptibility Enterobacteriaceae). PCR was used to detect KPC, NDM, IMP, VIM and OXA-48 genes as gold standard. Dynamiker carbapenemase-producing bacteria test kit was used to detect the carbapenemase produced by CRE, which was conducted by the performance evaluation combined with PCR results.

Results: The 80 strains of CRE expressing carbapenemase genes were all positive for the detection of carbapenemasstre by Dynamiker carbapenemase-producing bacteria test kit, which includes 39 strains producing KPC, 26 strains producing NDM, 8 strains producing IMP, 5 strains producing VIM and 2 strains producing OXA-48 enzymes; the 40 strains of CSE were negative and no carbapenemase were detected. Compared with the PCR results, the sensitivity and specificity of the four enzymes by Dynamiker carbapenemase-producing bacteria test kit were both 100% (Fig. 2).

Conclusion: Dynamiker carbapenemase-producing bacteria test kit can be applied as a simple, rapid, sensitive and specific diagnostic method for the detection of different CRE carbapenemase types, which is of great value for the accurate use of clinical anti-infective therapy.

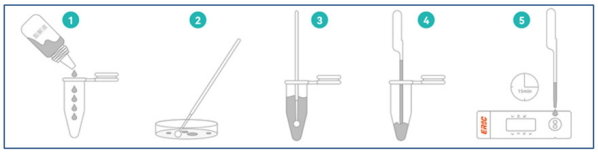


Fig. 1 Operating procedures of colloidal gold immunochromatography

Fig. 2 Results of Dynamiker carbapenemase-producing bacteria test kit

C: Control, K: KPC, N: NMD, I: IMP, V: VIM, O: OXA-48

B-067

Performance Assessment of a Newly Developed Lateral Flow Assay for the Quantification of Faecal Pancreatic Elastase

J. Afonso, M. Schneider, D. Guschin, C. Gerhold, B. Ricken. *BÜHLMANN Laboratories AG, Schönenbuch, Switzerland*

Background:

Pancreatic exocrine insufficiency (PEI) can be defined as an insufficient secretion of pancreatic enzymes and bicarbonate to maintain a normal digestion (1). PEI is associated to fat malabsorption, weight loss and malnutrition-related complications. Due to its important role in the digestive process, as a proteolytic enzyme and the high stability of the enzyme during intestinal transit, fecal pancreatic elastase (fPELA) is an ideal biomarker for the assessment of pancreatic function in patients suffering from PEI. The aim of this work was to develop and optimize a new rapid test for the quantification of fPELA.

Methods:

A quantitative lateral flow assay was designed for the selective measurement of elastase antigen concentrations from stool extracts. The BÜHLMANN Quantum Blue® fPELA is a sandwich immunoassay. A monoclonal detection antibody is conjugated to gold nanoparticles. Elastase present in the sample binds to this gold conjugate. A polyclonal capture antibody, highly specific for elastase, is immobilized on the analytical membrane and captures the complex of elastase bound to the anti-elastase gold conjugate, resulting in a coloring of the test line. The remaining free anti-elastase gold conjugate binds to the control line. The signal intensities of the test line and the control line are measured quantitatively by the Quantum Blue® Reader. Pancreatic elastase is quantified in extracts of human stool samples collected with the BÜHLMANN CALEX® Cap device. Several analytical studies, such as sensitivity, linearity, High Dose Hook Effect (HDHE), were performed during optimization phase to assess the performance of the assay. Moreover, a method comparison with the commercially available high throughput turbidimetric BÜHLMANN fPELA® turbo assay was also performed.

Results:

Both analytical and clinical performance were evaluated. Preliminary results of the assay performance were obtained during the optimization phase. Analytical sensitivity was determined as 12.8 µg/g LoB, 16.6 µg/g LoD and 17.2 µg/g LoQ. The linearity evaluation revealed a linear range of 10 to 472 µg/g. No HDHE was observed in native samples up to 4'000 µg/g. Method comparison of the new lateral flow assay with BÜHLMANN fPELA® turbo assay, with 80 clinical samples, revealed good analytical agreement with a slope of 1.04 (Passing-Bablok) and a bias at cutoff (200 µg/g) of 5.6%. Relative bias (Bland-Altman) of the two assays was 6.9%. Considering the turbidimetric assay as the reference method, the sensitivity and specificity were 92% and 83%, respectively.

Conclusion:

The newly developed quantitative lateral flow assay, BÜHLMANN Quantum Blue® fPELA, is an accurate, non-invasive and rapid test to determine fPELA levels in fecal extracts. The lateral flow assay is an attractive alternative to ELISA with a turn-around time of only 15 minutes enabling patients' management in a more patient near clinical environment.

(1) Dominguez-Muñoz, J. Enrique, Curr Opin Gastroenterol. 2018 Sep;34(5):349-354.

B-068

Reference Intervals for Serum Free Light Chains when Ruling out Monoclonal Gammopathies in Subjects with Normal or Minimally Variant Electrophoretic Findings

L. STEZAR, R. Elhady, H. Zari, G. Winston-McPherson, J. Carey, B. Cook. *Henry Ford Hospital, Detroit, MI*

Background: Serum free light chains (SFLC) analysis for detection and initial diagnosis of monoclonal gammopathies (MC) is a critical tool in the comprehensive assessment of risk and in management of established disease. Our reference intervals (RIs) for SFLC were based on the seminal report by Katzmann. Recent literature indicates with current analytic platforms and reagent formulations, significantly different RIs might be more appropriate.

Methods: The study sought to establish RIs for the population typically evaluated for possible MC and was expected to include acute/chronic inflammation, polyclonal elevations of gamma-globulins, hypogammaglobulinemia and chronic liver disease. The reference population inclusion criteria were: serum submitted for a serum monoclonal protein evaluation and SPE (± IFIX); lacked any direct or indirect evidence of a MC; normal or minimally variant SPE pattern; no prior history of leukemia, lymphoma, myeloma or MGUS; normal eGFR (CKD1-2 or > 60 mL/min). CKD-EPI calculation was based only on IDMS-standardized creatinine measurements without race modifiers. Of the 202 cases encountered over 15 days, 135 met the inclusion criteria. Specimens were tested using a Binding Site Optilite system.

Results: The mean subject age was 62 years. There was a mild female predominance (80 females v. 55 males) and females tended to be younger than males (58 v. 68 years). Two approaches used to establish the central 95% interval (mean ±2 SD and ranked-order analysis for 2.5 and 97.5 percentiles) yielded similar limits for SFLC and SFLC ratio (Table). These results agree well with recent reports from others.

Conclusion: As international guideline prognostic thresholds for SFLCR in plasma cell neoplasia are based upon the original Binding Site free light chain analysis, we will maintain those RIs, adding an appended comment to all SFLC reports to indicate for screening purposes, the HFH laboratory-established SFLC ratio interval is 0.8-1.9:1.

SFLC and Ratio Reference Intervals			
	FLC Ratio	FKLC, mg/L	FLLC, mg/L
Count	135	135	135
Mean	1.33	23.60	18.51
Median	1.31	21.30	16.80
95% RI	0.80-1.86	4.08-43.11	0.45-36.57
2.5th	0.88	12.50	8.44
97.5th	1.91	51.53	38.70

B-069

Performance Evaluation of a Ma2 Autoantibodies ELISA

A. Gorsh, T. Kryzer, A. McKeon, J. R. Mills, S. Pittock. *Mayo Clinic, Rochester, MN*

Background:

Ma-2 is a neuronal nucleolar protein with purported involvement in RNA transcription and apoptosis regulation. Patients with Ma2 antibodies present with autoimmune encephalitis (limbic or brainstem). Ma-2 antibodies are a high-risk marker for paraneoplastic neurologic cause for autoimmune encephalitis (>75% of patients having either testicular cancer or non-small cell lung cancer). In older patients, there is a higher frequency of small cell lung cancer or other cancers especially when Ma 1 antibodies are also detected. Identification of Ma2 antibodies is important to help

diagnose autoimmune encephalitis, guide the cancer search and aid treatment selection. The objective of this study was to develop and document the performance of a qualitative enzymatically amplified sandwich-type Ma2 Antibody ELISA.

Methods:

The Ma2 ELISA was produced in-house utilizing commercially available protein and was performed on a Hamilton Star liquid handling instrument equipped with a plate washer and plate reader. Each assay plate included patient samples along with 3 independent negative serum calibrators and 4 quality controls (Both positive and negative controls per matrix [CSF or serum]) each tested in duplicate. Mean optical densities (OD) for patient specimens were compared to the average OD of the negative serum calibrators to generate a ratio. A ratio cut-off of 8 was selected for reporting positivity based on preliminary studies and was validated during the method performance assessment. To assess assay performance we measured precision (3 positive pools at or above the cut-off ratio for each specimen type were tested 20 times in one assay and in duplicate across 10 independent assays), accuracy comparing to the current reference method at Athena® Diagnostics (N=10 positives / 40 negatives), analytical specificity (20 samples with high titer autoantibodies directed against other proteins), and the impact of common interferences (lipids, bilirubin, hemolysate were spiked into 2 positive and 1 negative sample per interference). Clinical performance of the assay for differentiating healthy donors without disease (N=140) and those with non-Ma2 neurological autoimmunity (N=23) from those with clinical phenotypes consistent with Ma-2 associated encephalitis (N=8) was measured.

Results:

Serum intra-assay imprecision (coefficient of variation; %CV) ranged from 3.0-6.5 %. Inter-assay imprecision ranged from 21-29%. CSF intra-assay imprecision ranged from 3.5-10%. Inter-assay imprecision ranged from 13-15.5%. Accuracy was assessed via qualitative comparison to a reference method [western blot or nanoliter scale immunoassay]. There was perfect agreement between methods. To assess analytical specificity, samples with high concentrations of immunoglobulins (hypergammaglobulinemia [N=10] or Systemic Lupus Erythematosus [N=10]) were tested in serum (and spiked into CSF); all were negative. Interference studies were conducted on 2 positive Ma2 serum and CSF samples, spiked for highly hemolytic (1000 mg/dL), lipemic (2000 mg/dL) and icteric (60 mg/dL) conditions. All samples remained positive under given interferents. A negative sample was tested for each matrix, both remained negative. Clinical specificity was 100% with all samples from patients without Ma-2 encephalitis testing negative (N=183). All ELISA positives identified in this study had phenotypes consistent with Ma-2 autoimmune encephalitis (N=8).

Conclusion:

The performance of the Ma2 ELISA was acceptable for clinical laboratory implementation.

B-070

Performance Assessment of an Immunoassay for Detection of APOE4 Carriers

A. G. Shields[1], M. S. Shields[1], I. Jiménez-González[2], A. Rodríguez-Martín[2], S. Ito[3], D. Verbel[4], J. Snyder[1]. *[1]Siemens Healthcare Laboratory, LLC, Berkeley, CA, [2]Biocross S.L., Valladolid, Spain, [3]Eisai Co., Ltd., Tokyo, Japan, [4]Eisai Inc., Nutley, NJ*

Background The APOE gene corresponds to apolipoprotein E, a glycoprotein involved in lipid metabolism. There are three different versions of the APOE gene, e2, e3, and e4 where e3 is the most common allele. APOE4 has been associated with Alzheimer's Disease (AD) and other neurological diseases. The presence of a single APOE4 allele increases the risk of AD by 3-5 fold and two copies, increase the risk by 8-12 fold. Detection of the APOE4 allele is commonly performed using genetic test methods. Studies were undertaken to evaluate the analytical performance of an immunoassay that detects the presence of APOE4 protein in and assess the discrimination of APOE4 gene carriers from non-APOE4 gene carriers.

Methods The Biocross e4Risk® test is a latex agglutination immunoassay used for the quantitative determination of isoform E4 of the apolipoprotein E (APOE4) in human EDTA plasma samples. The test consists of one reagent with latex particles and another reagent with a mouse anti-ApoE4, which induces the latex particle agglutination when ApoE4 is present in the plasma sample. This agglutination is measured photometrically. The turbidity is proportional to APOE4 content of the sample, which can be used to infer the APOE4 genotype responsible.

The Biocross kit reagents were run on the Siemens Healthineers Atellica® Chemistry Analyzer (open channel). Stored clinical samples from Eisai's elenbecestat Phase 3 MissionAD Studies were used in the analysis.

Results

Characteristic	Performance	Performance Claim
Sensitivity	LoB 0.23 µg/mL LoD 1.63 µg/mL	LoB 0.87 µg/mL LoD 1.22 µg/mL
Precision	The assay positive control (neat and diluted) and an EDTA pool near the assay cutoff of 4.62 µg/mL were used to evaluate assay precision. Samples were run over ten days with two runs per day. A total of 80 replicates were analyzed. Results are as follows: Positive Control (13.6 µg/mL) Repeatability 5.9 %CV Within-Lab 14.2%CV Diluted Positive Control (5 µg/mL) Repeatability 4.5 %CV Within-Lab 14.3% CV EDTA Pool (5 µg/mL) Repeatability 7.2 %CV Within-Lab 14.5% CV	Positive Control (7.12 µg/mL) Repeatability 3.88 %CV Within-Lab 11.57 %CV
Method Comparison	Using the recommended cutoff of 4.62 µg/mL from the manufacturer, the assay's sensitivity and specificity were 100% and 94% respectively.* *Based on a panel of 50 positive and 50 negative samples.	100% sensitivity 99% specificity

Conclusion The Biocross e4Risk® test on the Atellica® Chemistry Analyzer (open channel) demonstrated good analytical performance, with sensitivity and precision comparable to manufacturer's claims. The assay also had very good sensitivity and specificity when compared to known APOE4 genotype results. A simple blood-based robust APOE4 immunoassay test that runs on a routine clinical chemistry instrument may be a more convenient and cost-effective tool to help recruit patients for AD clinical trial programs.

B-071

Comparison of humoral and cellular immune responses between ChAd-BNT heterologous vaccination and BNT-BNT homologous vaccination following the third BNT dose: A prospective cohort study

H. Kang[1], W. Sim[2], J. Jung[1], J. Lee[1], G. Ko[1], H. Park[1], K. Cha[3], J. Choi[4], K. Park[5], E. Oh[1]. *[1]Catholic University of Korea, Seoul, Korea, Republic of, [2]Department of Internal Medicines, The Armed Forces Capital Hospital, Seongnam, Korea, Republic of, [3]Seoul St. Mary's hospital, Seoul, Korea, Republic of, [4]Armed Forces Medical Command, Seongnam, Korea, Republic of, [5]Medical Corps, ROK Army, Seongnam, Korea, Republic of*

Background: The differential immune responses after two additional BNT162b2 (BNT) doses between ChAdOx1 nCoV-10 (ChAd)-primed and BNT-primed groups have not been elucidated. The aim of this study was to compare vaccine-induced humoral and cellular immune responses and evaluate breakthrough infection between the two vaccination strategies. **Methods:** In 221 healthy subjects (111 in the ChAd group), longitudinal immune responses were monitored at 3, 4, and 6 months after the 2nd dose and 1, 3, and 6 months after the 3rd dose. Humoral immunity was measured by two fully automated chemiluminescent immunoassays (Elecsys and Abbott) and a surrogate virus neutralization test (sVNT). Cellular immunity was assessed by two interferon-γ (IFN-γ) release assays (QuantiFERON SARS-CoV-2 and Covi-FERON). **Results:** After the 2nd dose of BNT vaccination, total antibody levels were higher in the ChAd group, but IgG antibody and sVNT results were higher in the BNT group. Following the 3rd dose vaccination, binding antibody titers were significantly elevated in both groups (ChAD-BNT; 15.4 to 17.8-fold, BNT-BNT; 22.2 to 24.6-fold), and the neutralizing capacity was increased by 1.3-fold in both cohorts. The ChAd-BNT group had lower omicron neutralization positivity than the BNT-BNT group ($P = 0.001$) at 6 months after the 3rd dose. Cellular responses to the spike antigen also showed 1.7 to 3.0-fold increases after the 3rd dose, which gradually declined to the levels equivalent to before the 3rd vaccination. The ChAd cohort tended to have higher IFN-γ level than the BNT cohort for 3-6 months after the 2nd and 3rd doses. The frequency of breakthrough infection was higher in the ChAd group (44.8%) than in the BNT group (28.1%) ($P = 0.0219$). Breakthrough infection induced increased humoral responses in both groups, and increase of cellular response was significant in the ChAd group. **Conclusion:** Our study showed differential humoral and cellular immune responses between ChAd-BNT-BNT heterologous and BNT-BNT-BNT homologous vaccination cohorts. The occurrence of low antibody levels in the ChAd-

primed cohort in the humoral immune response may be associated with an increased incidence of breakthrough infections. Further studies are needed on the benefits of enhanced cellular immunity in ChAd-primed cohorts.

B-072

Analysis of Anti-SARS-Cov-2 S1-RBD and N IgG in saliva and serum

K. Shin[1], H. Lee[2], S. Yu[3], S. Lee[2], S. Lee[2], W. Kim[2]. *[1]Pusan National University Hospital, Pusan, Korea, Republic of, [2]Pusan National University Yangsan Hospital, Yangsan, Korea, Republic of, [3]Haeundae Paik Hospital, Inje University College of Medicine, Busan, Korea, Republic of*

Background: Mucosal antibody testing may be a significant and more practical tool for measuring humoral immunity since it is non-invasive. The aim of this study was to evaluate the SARS-CoV-2-specific antibody prevalence in saliva compared to serum in 2022.

Methods: From February to December 2022, the paired serum and saliva specimens were collected twice over a period. The saliva specimens were collected using a Salivette Cortisol (Sarstedt AG & Co. KG, Germany) device according to manufacturers' instructions. Antibody tests were performed using nucleocapsid protein-based Elecsys anti-SARS-CoV-2 (anti-N IgG) and spike protein targeted SARS-CoV-2 IgG II Quant (anti-S1-RBD IgG).

Results: 162 (97.6%) of 166 had anti-S1-RBD IgG in their serum. At the 1st visit and 2nd visit, 79 (47.5%) and 97 (58.4%) patients, respectively, had anti-N IgG in their serum. At 1st visit and 2nd visit, respectively, 14 (8.4%) and 13 (7.8%) patients had anti-S1-RBD IgG in their saliva. Four (2.4%) and five (3.0%) patients had anti-N IgG in their saliva during the 1st visit and 2nd visit, respectively. The correlation coefficient of the results of anti-S1-RBD IgG and anti-N IgG quantitative results between at 1st visit and 2nd visit were low (r=0.1721 to 0.6448). All patients in the anti-S1-RBD IgG seronegative group (N=4) had anti-N IgG in their serum, but neither anti-S1-RBD IgG nor anti-N IgG were detected in their saliva. All anti-N IgG seronegative patients (N=68) had anti-S1-RBD IgG in serum, while 3 patients had anti-S1-RBD IgG and 7 patients had anti-N IgG in saliva.

Conclusion: Mucosal antibodies were detected in certain seronegative patients. The assessment of SARS-CoV-2-specific saliva antibodies should be regarded as a supplemental non-invasive diagnostic to serum to evaluate the prevalence and transmission of COVID-19.

B-073

The Clinical Utility of Reporting AC-2 Pattern With Anti-DFS70 Antibody

H. Choi[1], C. Jeon[2], S. Jeon[1], S. Kee[1]. *[1]Chonnam National University Hospital, Gwangju, Korea, Republic of, [2]Chonnam National University Research Institute of Medical Science, BMSGP, Chonnam National University Medical School, Hwasun, Korea, Republic of*

Background: The dense fine speckled (DFS) pattern (AC-2) is often observed in antinuclear antibody (ANA) indirect immunofluorescence (IIF) assay, and the related anti-DFS70 antibody is known to be negatively associated with a systemic autoimmune rheumatic disease (SARD). Identifying the AC-2 pattern is challenging, as it could be generally confused with the AC-1, AC-4, AC-5, or AC-29 patterns displaying stained metaphase plates or speckled nuclei. Since the anti-DFS70 antibody is not included in routine autoantibody tests, the clinical utility of the AC-2 pattern and anti-DFS70 antibody is not adequately applied in the laboratory and clinical practice. We evaluated the prevalence of anti-DFS70 and other autoantibodies in samples presenting AC-2 pattern in ANA IIF assay. Additionally, we analyzed the association of anti-DFS70 antibodies and clinical features in patients with AC-2 patterns. **Method**: We retrospectively reviewed the ANA IIF images of 1,839 samples made by an automated ANA analyzer classified as AC-1, AC-2, AC-4, AC-5, and AC-29 patterns. AC-1, AC-2, AC-4, AC-5, and AC-29 patterns comprised 60.5% of all ANA positives (1,839/3,039). Among them, the AC-2 pattern was identified in 18.5% (341/1,839) by two experts, and only 197 with residual samples were available for further evaluation. Those 197 samples were tested for anti-DFS70 antibodies with enzyme-linked immunosorbent assay (ELISA) and line immunoblot assay (LIA). They were also tested for 22 other autoantibodies (dsDNA, nucleosomes, histones, SS-A/Ro-52, SS-B, RNP/Sm, Sm, Mi-2α, Mi-2β, Ku, CENP-A, CENP-B, Sp100, PML, Scl-70, PM/Scl100, PM/Scl75, RP11, RP155, gp210, PCNA) with LIA. Clinical information from 197 patients was obtained from a retrospective review of electronic medical records. **Results**: Anti-DFS70 antibody prevalence by ELISA and LIA was 32.0% (63/197) and 19.8% (39/197), respectively. The agreement percentage between the two assays was 81.7%, with a kappa value of 0.53 (95% confidence interval: 0.40 - 0.66). Of the 69 DFS70+ samples (as per ELISA or LIA), the coexistence of other autoantibodies was found in 81.2% (56/69). Of the 128 DFS70- samples, 90.6% (116/128) revealed other autoantibodies. The three most frequent autoantibodies (except anti-DFS70) in 197 patients with AC-2 patterns were anti-SS-A/Ro-52 (30.5%, 60/197), anti-nucleosomes (27.4%, 54/197), and anti-histones (20.3%, 40/197). There was no significant difference in the prevalence of the other 22 autoantibodies between DFS70+ and DFS70- patients. The anti-DFS70 prevalence in the SARD group was 33.3% (50/150), and in the non-SARD was 40.4% (19/47) without significant difference. However, of the 69 DFS70+ patients, the isolated anti-DFS70 was found more frequently in the non-SARD group (36.8%, 7/19) than in the SARD group (12.0%, 6/50) (P = 0.0346). **Conclusion**: The anti-DFS70 positive rate in AC-2 pattern samples is lower than a third, and the prevalence is affected by different detection assays. Along with the AC-2 pattern, just reporting the presence of anti-DFS70 is not valuable for SARD exclusion. Therefore, it is important to co-examine other autoantibodies, such as anti-SS-A, anti-nucleosomes, and anti-histones, to confirm the isolated anti-DFS70.

B-075

Biophysical Changes of Leukocyte Activation (and NETosis) in the Cellular Host Response to Sepsis

M. Sorrells[1], Y. Seo[2], M. Magnen[2], R. Sheybani[1], A. M. Shah[1], H. T. Tse[1], M. R. Looney[2], H. O'Neal[3]. *[1]Cytovale LLC, San Francisco, CA, [2]University of California San Francisco, San Francisco, CA, [3]LSU Health Sciences Center / Our Lady of the Lake Regional Medical Center, Baton Rouge, LA*

Background:

During sepsis, neutrophils, upon activation, undergo biophysical and biochemical changes that may terminate in the release of neutrophil extracellular traps (NETs)[1-3]. The IntelliSep test is a rapid sepsis diagnostic that assesses immune activation by quantifying biophysical properties of leukocytes from whole blood in < 10 minutes. The test results in the IntelliSep Index (ISI), ranging between 0.1-10.0, stratified into three interpretation bands (Band 1, Band 2, and Band 3) based on the probability of the clinical syndrome of sepsis[4,5]. We investigated the correlation between the IntelliSep result and immunological changes observed during leukocyte activation through in-vitro studies and using clinical samples from non-septic and septic patients.

Methods:

In-vitro Studies: Healthy blood samples were collected between April - August 2022 from 18 volunteers at a blood donor center. Aliquots of each blood sample were incubated for 10 minutes with phorbol myristate acetate (PMA) in phosphate-buffered saline at one of 3 concentrations: 0, 200, and 400 nM prior to the IntelliSep test (3 repeats per donor per concentration).

Clinical Studies: Subjects with signs or symptoms of infection were enrolled from Emergency Departments (EDs) in three similar but distinct prospective cohort studies (February 2016 - September 2019)[1,2]. The IntelliSep test was performed on a aliquot of fresh whole blood from each subject, and remnant plasma was prepared and frozen for later analysis. Plasma levels of neutrophil elastase-DNA (NE DNA) and citrullinated histone H3-DNA (Cit-H3 DNA) complexes were quantified using previously reported custom ELISAs 3,6. Subjects were retrospectively adjudicated as sick (adjudicated as septic with SOFA scores peaking on the day of enrollment compared to two subsequent days) and "healthy" (SOFA for day of enrollment < 2, hospital length of stay < 3 days).

Results:

Significant increases in ISI scores were observed with increasing concentration of PMA in healthy blood samples (0 and 200: $p < 10^{-10}$; 0 and 400: $p < 10^{-10}$). The clinical analysis cohort consisted of 39 "sick" and 42 "healthy" subjects. Linear correlation was observed between the ISI and NE DNA and Cit-H3 DNA quantities with significant increases across ISI Interpretation Bands (Band 1 to Band 3: $p < 0.001$).

Conclusion:

Correlation of increasing ISI and PMA concentration supports the hypothesis that the biophysical changes measured by the IntelliSep test result from leukocyte activation. Observed correlation between the ISI and NET quantities suggest that NET formation is one of the activation pathways that result in the biophysical changes measured in the ISI. Together these experiments support that biophysical changes of leukocyte activation measured using a cellular host response test could provide a window into a patient's state of dysregulated immunity and may have the potential to aid ED physicians in timely diagnosis of sepsis.

References: 1.O'Brien, et al. American Journal of Medicine (2007). 2.Cinel, I. & Opal, S. M. Critical Care Medicine (2009). 3.Lefrançais, E., et al. JCI Insight (2018). 4.O'Neal, H. R. et al. PLoS One (2022). 5.Guillou, L. et al. PLoS One (2021). 6.Sayah, D. M. et al. Am J Respir Crit Care Med 191, 455-463 (2015).

B-076

Comparison of Three Methods for the Detection of Antibodies Against Muscle-Specific Kinase

K. Luong[1], B. K. Lozier[1], C. L. Novis[1], T. L. Smith[2], L. K. Peterson[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Neurology University of Utah School of Medicine, Salt Lake City, UT, [3]Department of Pathology University of Utah School of Medicine, Salt Lake City, UT*

Background: Myasthenia gravis (MG) is a neuromuscular disease characterized by skeletal muscle weakness and fatigue. These symptoms are due to a reduction in synaptic signal transduction caused by autoantibodies against post-synaptic proteins in the neuromuscular junction. Approximately 85% of patients with MG have autoantibodies against acetylcholine receptors (AChR); however, autoantibodies against muscle specific kinase (MuSK) and low-density lipoprotein receptor-related protein 4 (LRP4) may also be found in patients with MG. Detection and identification of these antibodies is important in the diagnosis and treatment of patients under evaluation for MG. MuSK, a transmembrane protein expressed on skeletal muscles, is essential to the formation and maintenance of the neuromuscular junction. Approximately 6% of MG patients have MuSK autoantibodies.

Traditionally, radioimmunoprecipitation assays (RIA) have been used to detect MuSK antibodies in patient sera. However, RIA has several limitations, including safety and regulatory concerns associated with using radioactive materials and the short half-life of reagents. Alternatives to RIA, such as enzyme-linked immunosorbent assays (ELISA) and cell-based assays (CBA), do not have the same challenges. Recent studies outside of the USA suggest that cell-based assays may have equal or superior sensitivity than RIA; particularly in the detection of MuSK antibodies in patients previously diagnosed with seronegative MG (MG patients without detectable AChR or MuSK antibodies by RIA). The objective of this study was to compare the performance of two commercially available ELISA and fixed cell-based assay (f-CBA) kits to RIA for the detection of MuSK antibodies.

Methods: A total of 237 sera were evaluated by RIA, ELISA and/or f-CBA. These sera include 47 MuSK RIA positive sera, 55 MuSK RIA negative sera, 70 self-reported healthy control sera, and 65 sera positive for other neural antibodies associated with MG, Lambert-Eaton Myasthenic Syndrome, paraneoplastic neurologic syndromes, or autoimmune encephalitis.

Results: Both f-CBA and ELISA demonstrated 100% specificity for MuSK antibodies in sera from self-reported healthy controls and sera from patients positive for other antibodies. Forty-seven sera tested positive for anti-MuSK antibodies by RIA; of those 47 sera, 7 sera tested negative by ELISA and 5 tested negative by f-CBA kits while the remaining 40 were concordant between ELISA, f-CBA and RIA. The discrepancy between RIA and the commercial kits for ELISA and f-CBA was only observed in low positive sera (0.04-0.21 nmol/L ARUP RIA). Clinical information was not available for discrepant specimens. However, a review of MuSK RIA results for University of Utah patients shows that of 7 patients with an ARUP RIA result of 0.04-0.21 nmol/L, only 1 had symptoms that may possibly be consistent with MG. The remaining 6 low positive patients did not have symptoms consistent with a diagnosis of MG. This may suggest an issue with RIA specificity rather than ELISA and f-CBA sensitivity. However, further studies are needed to confirm this.

Conclusion: Commercially available ELISA and f-CBA kits offer viable alternatives to traditional RIA for detection of MuSK antibodies.

B-077

Comparison of SARS-CoV-2 Antibody Profile and Omicron-Specific Neutralizing Activity Before and After mRNA Booster Vaccines: Bivalent vs. Original Formulation

A. Windau[1], J. Meyers[1], X. Yang[1], R. Pontoni[1], C. Schmotzer[2], J. Noguez[2], F. Dong[3], X. S. Zhang[2]. *[1]University Hospitals Cleveland Medical Center, Cleveland, OH, [2]University Hospitals Cleveland Medical Center, Case Western Reserve University, Cleveland, OH, [3]Northeast Ohio Medical University College of Medicine, Rootstown, OH*

Background: With entering into the era of SARS-CoV-2 vaccine boosters and shifting from pandemic to endemic, understanding how boosters change antibody levels and durability is important for vaccination strategies. This study investigated antibodies against different antigens and neutralizing antibody levels in immunocompetent healthcare workers who have received the primary 2 doses and booster mRNA vaccines. **Method**: The study included two cohorts. Cohort 1 received 3 doses of the original vaccine and cohort 2 received the 3 original doses plus the 4th bivalent booster. Two blood samples were collected from each participant before the 3rd (pre-3) or 4th dose (pre-4) and 14-30 days after the 3rd (post-3) or 4th dose (post-4). SARS-CoV-2 IgG antibodies against the spike protein receptor-binding domain (RBD), S1, S2, and nucleocapsid protein were measured using a semi-quantitative multiplex assay on a Bioplex 2200 analyzer. Neutralizing antibodies against omicron sublineage BA.2 were determined using a surrogate virus neutralization assay. **Results:** Among the 45 participants aged 24 to 66, 53% had natural infection evidenced by positive anti-nucleocapsid IgG or historical PCR results. In cohort 1 (n=24), the median time between the second and third doses was 375 (range 278-439) days. In cohort 2 (n=21), the median time between the third and fourth doses was 353 (range 102-438) days. Both the 3rd original and 4th bivalent vaccines significantly increased neutralizing, anti-RBD, anti-S1 and anti-S2 antibodies when compared to their pre-dose concentrations ($p<0.01$). The median and interquartile range (IQR) for pre-and post-booster antibody levels were: Neutralizing antibody post-3 median: 189 U/mL (IQR 99-781) versus pre-3 median: 44 U/mL (IQR 0-180), post-4 median: 241 U/mL (IQR 148-630) versus pre-4 median: 37 U/mL (IQR 8-167); anti-RBD post-3 median: 13,100 U/mL (IQR 8,001-31,850) versus pre-3 median: 3,437 U/mL (IQR 201-7,976), post-4 median: 16,490 U/mL (IQR 7,691-24,710) versus pre-4 median: 3,270 U/mL (IQR 1,970-4,847); anti-S1 post-3 median: 9,700 U/mL (IQR 7,281-27,425) versus pre-3 median: 2,287 U/mL (IQR 229-7,710), post-4 median: 11,000 U/mL (IQR 6,935-20,665) versus pre-4 median: 2,280 U/mL (IQR 1,419-2,833); and anti-S2 post-3 median: 148 U/mL (IQR 89-266) versus pre-3 median: 25 U/mL (IQR 9-51), post-4 median: 101 U/mL (IQR 73-280) versus pre-4 median: 28 U/mL (IQR 14-43). No difference was found between the post-4-bivalent and post-3-original antibody levels. The difference in post/pre ratios elicited by the 3rd and 4th doses was also not statistically significant ($p>0.05$). Further analysis showed participants with natural COVID infection had much higher pre-3 and pre-4 dose antibody levels than those without natural infection ($p<0.01$). The pre-booster neutralizing antibody levels of infected participants were not significantly different ($p>0.05$) from the post-booster levels of the non-infected participants. This pattern was consistent across antibodies against different antigens. In addition, there was no significant difference in post-booster antibodies between previously infected and non-infected participants. **Conclusion:** The bivalent and original mRNA vaccines are equally effective in enhancing immunity against SARS-CoV-2. Individuals with no previous COVID infection may need boosters annually to raise waning antibodies. Naturally infected individuals demonstrated long-lasting immunity and may not require annual boosters.

B-078

Prevelance of autoimmune factors in patients with seizures of unknown etiology in Western China

S. Guo[1], Z. Meng[2], M. Wang[2], B. Ying[2]. *[1]West China Hospital, Sichuan University, Chengdu, China, [2]West China Hospital, Scihuan University, Chengdu, China*

OBJECTIVE: To delineate the features of autoantibody in patients with seizure of unknown etiology in Western China and to analyze other potential risk factors of an epileptic seizure. **METHODS:** From January 2017 to June 2022, 639 patients with unexplained seizure were assessed retrospectively. Neuronal autoantibodies were assessed in 471 individuals, including 12 common neural-specific autoantibodies, antibodies against four common pathogens associated with central nervous system infections, or neurophagocytosis, and two thyroid-related autoantibodies. **RESULTS:** Sixty-nine patients were positive for neural-specific autoantibodies in CSF and serum. Among the patients, 32 (46.38%) expressed NMDAR-Ab, 14 (20.29%) expressed GABABR-Ab, 10 (14.49%) expressed LGI1-Ab, 7 (10.14%) expressed GFAP-Ab, 3 (4.34%) expressed Yo-Ab, 2 (2.90%) expressed CASP2-Ab, and 1 (1.45%) expressed Hu-Ab. Compared to patients with non-autoimmune epileptic seizure, the positive rates of thyroid-related antibody expression and the neurophagocytosis pathogen levels of HSV-IgG and *T. gondii*-IgG were significantly different in patients with autoimmune epileptic seizure. **CONCLUSION:** This study elucidated the antibody status of autoimmune epilepsy in western China, allowing for better identification and diagnosis of autoimmune epilepsy in the future.

B-079

Identifying Heterogeneity of Systemic Connective Tissue Diseases by Applying Joint Dimension Reduction and Cluster Analysis to Immunomarkers

J. Yu[1], Y. Tseng[2], W. Lin[3], Y. Wang[4], T. Lin[1], C. Chang[2], H. Wang[1]. *[1]Department of Laboratory Medicine, Chang Gung Memorial Hospital, Taoyuan City, Taiwan, [2]Department of Computer Science, National Yang Ming Chiao Tung University, Hsinchu City, Taiwan, [3]Syu Kang Sport Clinic, Taipei City, Taiwan, [4]Department of Medicine, Harvard Medical School, Boston, MA*

Background:

Accurate identification of systemic connective tissue diseases (SCTDs) can be challenging due to the heterogeneity of each disease. This study aims to explore the heterogeneity of different SCTDs by applying non-supervised learning to immunomarker data and proposes an alternative classification. Additionally, the study investigates the clinical implications of these different groups.

Methods:

We utilized multiple correspondence analysis and k-means clustering to analyze the immunomarker data of patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and Sjögren's syndrome (SS) in Taiwan between 2001 and 2016. To investigate the clinical correlations of the classification results, we conducted a comprehensive review of the clinical findings and compared the results across different groups.

Results:

A total of 11,923 patients with the three SCTDs were classified into six distinct clusters based on their immunomarker patterns. In this clustering system, RA patients were predominantly grouped in cluster 1, whereas SLE and SS patients had a more dispersed distribution. Among patients with SLE, renal involvement was more frequent in clusters 3 and 6 (52% and 51%, respectively) than in other clusters, and discoid lupus was more frequent in cluster 3. Among SS patients, typical SS findings such as inflammation or infection of eyes and allergic reactions were more common in cluster 2 (54% and 51%, respectively). In contrast, patients in cluster 3 had more unspecified benign neoplasms (58%) and were more likely to experience immune disorders (63%).

Conclusion:

The immunomarker-driven clustering yields an alternative classification result with new clinical insights. The data-driven approach would provide a more evidence-based classification and aid in a more accurate diagnosis and management for SCTDs.

B-080

Analytical Performance of ESPLINE HTLV-I/II

A. Kin, K. Yamakawa, S. Kojima, A. Kaneko, K. Aoyagi. *Fujirebio inc., Hachioji-shi, Tokyo, Japan*

Background: The human T-cell lymphotropic virus (HTLV) infects a type of white blood cell called T-cell or T-lymphocyte. There are two types of HTLV, HTLV-I and HTLV-II, which are closely related to human C retroviruses. HTLV-I is endemic in the Caribbean, Japan, South America, and parts of Africa. HTLV-I has been recognized as a cause of adult T-cell leukemia (ATL) and HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-II is found among Africa and the Americas, and injecting drug users in many cities of Western Europe and North America. Currently, an effective vaccine against HTLV is not available, and clinical treatment is limited. The diagnosis and identification of patients are very important for controlling the spread of HTLV infection. There are HTLV-infected lymphocytes in the peripheral blood of patients, but little virus in the serum or plasma can be detected. Therefore, screening tests for HTLV that generally detect antibodies using reagents need dedicated instruments. We have developed an anti-HTLV-I/II reagent (ESPLINE HTLV-I/II) based on the immunochromatography principal using an enzyme immunometric assay that can easily detect both anti-HTLV-I and anti-HTLV-II antibodies in serum and plasma. This report evaluates its efficacy.

Methods: This reagent has a test line coated with a recombinant protein and peptides derived from three env gene proteins (HTLV-I gp21, HTLV-I gp46, and HTLV-II gp46) to detect anti-HTLV-I/II antibodies. The antibodies in the sample bind to antigens labelled with alkaline phosphatase (ALP), and then immunocomplexes are formed. These complexes are captured by the antigens on the test line, and the blue line is detected by ALP enzymatic reaction with a colorimetric substrate. It only takes 15 minutes to obtain the results without any instruments. To evaluate the fundamental performance, we tested specificity, limit of detection, and method comparison with Lumipulse HTLV-I/II, Lumipulse Presto HTLV-I/II, and Serodia HTLV-I, following the CLSI protocol. Commercially available serum and/or plasma were used for most evaluations.

Results: Regarding specificity, 1014 anti-HTLV-I/II negative serum samples, which were confirmed by highly sensitive CLEIA Lumipulse Presto HTLV-I/II, were tested by ESPLINE HTLV-I/II. Then, all 1014 samples were shown as nonreactive, demonstrating a specificity of 100.0%. Regarding the limit of detection, by the analysis of serial dilution from 2 positive plasma specimens, ESPLINE HTLV-I/II assay showed high sensitivity compared to conventional Serodia HTLV-I. Regarding the method comparison, 57 anti-HTLV-I and 30 anti-HTLV-II positive plasma samples from different individuals were tested. All samples showed positive results with ELPLINE HTLV-I/II, Lumipulse Presto HTLV-I/II, Lumipulse G HTLV-I/II, and conventional Particle Agglutination Serodia HTLV-I.

Conclusion: The novel anti-HTLV-I and anti-HTLV-II antibodies assay, ESPLINE HTLV-I/II, showed higher sensitivity than Particle Agglutination Serodia HTLV-I and had good concordance with highly sensitive CLEIA Lumipulse Presto HTLV-I/II and Lumipulse G HTLV-I/II. The ESPLINE system can be used easily to complete an assay in 15 min. This procedure is considered useful in situations such as on-site tests at health centers, screening tests at medical institutions with a small number of specimens, and other emergency tests.

B-082

Diagnostic and Clinical Characteristics of Neuroimmune Markers in a Tertiary Care Hospital

B. Das, M. Mandani, M. Bhatt, J. Mani, A. Aggarwal, T. Raut. *Kokilaben Dhirubhai Ambani Hospital and Medical Research Institute, Mumbai, India*

Background: Neuroimmune disorders are a complex group of inflammatory conditions of the nervous system and remarkable discoveries over the last two decades have elucidated the autoimmune basis of several, previously poorly understood, neurological disorders. Autoimmune Encephalitis has been linked to a wide variety of neuronal autoantibodies targeting both intracellular and plasma membrane antigens. Paraneoplastic encephalitis syndromes are often associated with antibodies against intracellular neuronal proteins like anti-Hu, anti-Ri, anti-GAD65 and others and the encephalitis associated with antibodies against neuronal cell surface/synaptic proteins like NMDA and VGKC is referred to as "autoimmune encephalitis." The diagnosis of these neuroimmune disorders can be challenging due to the wide spectrum of clinical presentations, frequent absence of diagnostic abnormalities on conventional brain MR-imaging and non-specific findings on EEG testing. The importance of early diagnosis and appropriate treatment is paramount. The objective of this study is to examine the diagnostic and clinical characteristics of these disorders with the goals of assisting clinicians in differential diagnosis.

Methods:This study took place from January 2016 to January 2023, at Kokilaben Dhirubhai Ambani Hospital and Research Centre, Mumbai, Maharashtra. We reviewed the laboratory records in our hospital to identify patients diagnosed with positive anti-NMDA receptor antibody, VGKC antibodies like LG1, CASPR2, GABBAR B1/B2, AMPA1, and AMPA2, positive NMO and MOG antibodies and also for antibodies against intracellular antigens like anti-Ri, anti-Hu. Inclusion criteria were adults and adolescents (aged 12 - 90 years) and pediatric population was excluded. The cohort was categorized into four groups according to different antibodies: NMDA group, VGKC group, NMO-MOG and paraneoplastic groups. Each cohort was further divided into two groups : Group I (positive patients) and Group II (negative patients) and their clinical characteristics were compared.

Results: A total of 650 patients aged from 12- 90 years for whom NMDA, VGKC, NMO-MOG and paraneoplastic antibody testing was requested were identified for this retrospective study. Prominent differences became apparent when comparing the basic demographic variables, age and gender. Positive rate for autoimmune encephalitis was found to be 6.82% and for NMO-MOG antibodies, it was found to be 11.11 %. Most common synaptic autoimmune encephalitis was associated with LG1 (55%), followed by NMDA (27 %). Most common onco-neuronal paraneoplastic encephalitis series were GAD65, Yo and Zic. Clinical features, MRI and EEG findings have been evaluated in all groups.

Conclusion: Neuroimmune disorders are a complex group of inflammatory conditions and the clinical presentations and features are highly diverse and largely dependent on the particular neuronal antibodies. Focal seizures were the common symptom associated with anti-NMDA and anti-VGKC positive patients, whereas difficulty walking and lower limb weakness was the most common presentation associated with paraneoplastic antibodies. Autonomic stability and neoplastic etiologies were not

noted in synaptic autoimmune encephalitis but were seen in paraneoplastic encephalitis. Patients with MOG antibodies also were found to have neoplastic etiologies and blurring of vision was the most prevalent symptom noted. Earlier recognition and treatment of these disorders can lead to improved outcomes and reduced disability.

B-083

Novel High Affinity Monoclonal Antibodies for Specific Detection of Neurofilament Light Protein in Serum and Cerebrospinal Fluid

K. Grönholm, E. Vesilahti, S. Eklin, T. Hämäläinen, M. Mattila, P. Tuomiranta. *Medix Biochemica, Espoo, Finland*

Background: Neurofilaments are very important protein components of axons, and they maintain the function of nervous system. Cerebrospinal fluid (CSF) and serum neurofilament levels elevate due to damages in nervous system making neurofilaments promising biomarkers for neural diseases and injuries, such as Alzheimer's disease, traumatic brain injury and Multiple sclerosis. Based on their size, neurofilaments are divided in groups: Neurofilament light (NfL), neurofilament medium (NfM), neurofilament heavy (NfH), α-internexin, and peripherin. Of these, NfL is considered to be the most promising biomarker since it is the most abundant form of neurofilaments.

Methods: We have developed four mouse monoclonal antibodies against human NfL. These antibodies, designated as Anti-h NfL 12601, 12603, 12604 or 12605, were tested as pairs in sandwich fluorescence-based immunoassay (FIA) with NfL spiked serum and buffer to determine linear measuring ranges and the matrix effect. Furthermore, the ability of the antibodies to measure NfL in clinical samples was tested in sandwich FIA by testing 10 CSF samples in the range of 0 to 5 083 pg/mL. The antibodies were also compared against commercially available reference antibodies in FIA. Kinetic parameters were determined with bio-layer interferometry (BLI).

Results: In sandwich FIA, the widest linear measuring range of 10 to 40 960 pg/mL of recombinant human NfL spiked in normal serum was achieved with antibody pair 12603 and 12604. In contrast, the commercial reference antibody pair achieved a linear measuring range of 2 560 to 40 960 pg/mL. Matrix effect caused by serum was negligible. With clinical samples, the best correlation with the reference method (NF-light CSF ELISA from Uman Diagnostics) with a coefficient of correlation of 0.986 was achieved with antibody pair 12604 and 12601. This pair also showed a good correlation with the commercial reference pair with a coefficient of correlation of 0.984. In kinetics measurements, none of four antibodies showed dissociation indicating very high affinity. The fastest association of 4.0 x 10^5 1/Ms was achieved with antibody 12605.

Conclusion: These results demonstrate that the newly developed mouse monoclonal NfL antibodies are promising tools for development of diagnostic immunoassays measuring NfL levels in human serum and CSF samples. Comparison against commercial reference antibodies indicates that higher NfL detection sensitivity and wider detection ranges can be achieved with antibody pairs developed in this study. Additionally, the kinetic measurement results forecast these antibodies to be suitable for fast reaction kinetic assay formats, such as lateral flow assays, which might become a key resolution in acute neural injury diagnostics.

B-085

Sars-cov-2 nt-chip: a multiplex eia platform for detect humoral response and neutralizing antibodies against Sars-cov-2

J. R. Siqueira[1], M. L. Moreira[1], E. M. Carvalho[1], F. Moruzzi[2], D. D. Luche[3], M. Kintrup[4]. *[1]FIOCRUZ, Rio de Janeiro, Brazil, [2]NL Diagnóstica, São Paulo, Brazil, [3]University of São Paulo, São Paulo, Brazil, [4]Viramed, Munich, Germany*

Background: COVID-19 pandemic is a current health crisis that began in late 2019 and is caused by the pathogen SARS-CoV-2. This virus causes respiratory illness and has led to numerous infections and deaths worldwide. SARS-CoV-2 has an essential protein, the spike protein (S) containing a receptor-binding domain (RBD) binding to the angiotensin-converting enzyme 2 (ACE2) receptor on host cells. Studies have shown that mutations in the RBD can affect its ability to bind to the ACE2 receptor, and some of these mutations have been associated with increased transmissibility or disease severity. During infection, the host immune response can generate a variety of antibodies against SARS-CoV-2 proteins, including neutralizing antibodies that can target RBD. Serological tests could detect the presence of these antibodies in blood samples and are being used to determine whether a person has been infected with SARS-CoV-2 in the past or has developed immunity against the virus after vaccination. These tests also can help to better understand the spreading of new VOCs and epidemiological profiles of COVID-19. However, it is essential to note that the presence of antibodies does not guarantee immunity.

Methods: SARS-CoV-2 VOC Virachip IgG test was designed for the multiplex detection of binding antibodies to multiple SARS-CoV-2 proteins, including nucleocapsid (N), spike fragment S1 (S1), spike fragment S2 (S2), and the RBDs of Wuhan strain (RBD-w), Delta VOC (RBD-d), and Omicron VOC (RBD-o). This test was modified and further developed to also measure the neutralization effectiveness of antibodies binding against different RBDs. The percentage of inhibition of neutralizing antibodies against Wuhan and Omicron RBDs was determined. To achieve this, a purified ACE-2 Alkaline-Phosphatase conjugate was incorporated into the assay, and its ability to bind RBD spots in the microarray wells was measured after preincubation with sera or plasma. The presence of neutralizing antibodies was assessed for its capability of preventing such interaction, hence allowing us to detect neutralizing antibodies indirectly. Furthermore, it was also possible to generally detect binding antibodies against RBDs and the N protein. Assessing past infections in people immunized with mRNA vaccines, and in the same assay, detecting the subset of antibodies that promote functional neutralization against SARS-CoV-2 infection was feasible.

Results: A cohort containing 50 pre-pandemic and 61 post-infection sera the SARS-CoV-2 NT-CHIP yielded 100% specificity and 96.7% sensitivity regarding neutralization antibodies against RBD-w. In case of RBD-o analysis of 111 serum samples from patients before the emergence of Omicron and 69 serum samples from patients after infection resulted in 99.1% specifictiy and 95.7% sensitivity. These tests were performed in comparison with PRNT, the gold standard test for measuring neutralization.

Conclusion: This innovative microarray platform allows for quick upgrades to incorporate new RBDs from currently important VOCs. Given the status of the pandemic, the emergence of new variant strains, and vaccine adjustments, the SARS-COV-2 NT-CHIP could be a very valuable tool for assessing the humoral immunity generated against future VOCs. Thus, it contributes to the decision-making processes in the public health care system and is well suited for new vaccine studies.

B-086

High Precision T Cell Testing Using the T-SPOT Technology: Improved Cell Yields From Lower Volumes of Blood When the T-SPOT Technology is Used With the T-Cell Select Reagent Kit for Cell Isolation

T. Jackson-Soutter, R. Brignall, M. H. Quinn, R. S. Ahmed, M. M. Dudek. *Oxford Immunotec, Abingdon, United Kingdom*

Background:

T cells play a critical role in protecting humans from disease, and are a powerful diagnostic tool when used to measure immune responses to infection. However, the use of T cells in a clinical setting has been limited due to challenges associated with T cell testing methodologies. One of the key challenges has been the ability to scale up T cell testing to meet the needs of clinical laboratories. The T-SPOT technology is most widely known for the T-SPOT.*TB* test, an Interferon-Gamma Release Assay (IGRA) diagnostic test based on the detection of *Mycobacterium tuberculosis*-specific effector T cells using a single cell resolution platform (ELISPOT). The conventional method of running the T-SPOT.*TB* test was to use density gradient isolation on 6 mL of whole blood to isolate peripheral blood mononuclear cells (PBMCs) for high precision T cell testing. However, this testing was not accessible to some clinical laboratories due to the hands-on time associated with isolating PBMCs from whole-blood. The T-Cell *Select* reagent kit was developed to positively select mononuclear cells and enhance cell separation efficiency for the T-SPOT technology. The T-Cell *Select* reagent kit offers the opportunity to automate the T-SPOT technology which improves scalability and reliability, but it also means that an improved cell yield can be achieved from a lower volume of blood. This makes high precision T cell testing more accessible, whilst maintaining the high sensitivity and specificity of the T-SPOT technology.

Methods:

PBMCs from 242 samples were isolated from 6 mL of whole blood, and were processed according to the T-SPOT.*TB* test package insert for density gradient isolation. Matched samples were used to isolate PBMCs from 3.5 mL of whole blood using magnetic bead-based positive selection according to the T-Cell *Select* reagent kit package insert.

Results:

Cell yields were compared using matched samples for density gradient and the T-Cell *Select* reagent kit isolated cells. Cell count means were 6.6 vs 6.7 x 10^6 cell/mL for the T-Cell *Select* reagent kit and density gradient isolation methods, respectively. Of the 242 samples tested, two samples had insufficient yields to run the T-SPOT.*TB* test with the density gradient method, whilst the T-Cell *Select* reagent kit isolated

sufficient numbers from all samples. Results of the T-SPOT.*TB* test had an overall agreement between the two methods of 97%, negative agreement 99% and positive agreement 94%.

Conclusion:

When used with the T-SPOT technology, the T-Cell *Select* reagent kit provides an improved cell isolation methodology, requiring up to 50% lower blood volumes, and reducing the hands-on time associated with T cell testing. This makes T cell testing more accessible to labs, meaning that fewer blood samples need to be redrawn due to low cell numbers, providing an advantage in the immunosuppressed.

B-087

The Antifouling Properties of BSA on Magnetic Beads are Affected by BSA Grade, Bead Type, and pH

C. N. Kunkler, C. D. Warner. *Proliant Health and Biologicals, Ankeny, IA*

Background:

BSA (bovine serum albumin) is a protein that is widely used for its antifouling properties in a variety of immunoassays. One such immunoassay is a bead-based ELISA (enzyme-linked immunosorbent assay), which can provide quantitative detection of many diverse analytes. BSA increases the sensitivity of a bead-based ELISA by coating the beads and blocking nonspecific interactions, thereby lowering background noise. However, the BSA grade chosen for the assay is frequently based on experience, as there is a lack of understanding of how BSA grades interact with different bead surfaces under various assay conditions. The objective of this study is to examine if different BSA grades work better with particular bead chemistries and how pH influences those interactions. Specifically, the aim is to quantify the amount of BSA on ELISA beads and assess the blocking of nonspecific antibody signal with different bead types, BSA grades, and pH.

Methods:

The mass of BSA binding to beads was measured by heating BSA-coated beads in 1x SDS loading dye, then running on a denaturing PAGE gel. Total protein was measured by densitometry against a standard curve using ImageJ. The amount of blocking was measured using a modified bead-based ELISA, comparing the amount of antibody-HRP binding to the beads in the presence and absence of BSA. The amount of antibody-HRP was measured using a SpectraMax Plus 384 plate reader against a standard curve. All data were analyzed using GraphPad Prism and presented as the average of at least three independent replicates with error bars representing standard deviation. Conditions tested include different Dynabeads (hydrophilic vs. hydrophobic - Dynabeads M-270 Carboxylic Acid and M-280 Tosylactivated), BSA grade (fatty acid (FA) vs. fatty acid-free (FAF) - Proliant SKUs 68100 and 68700), and pH (7 vs. 5.2).

Results:

The choice of pH is more influential than the BSA grade on bead surface coating and blocking, regardless of bead type. Lowering the pH from 7 to 5.2 increases the mass of BSA on the beads ~1.7-fold for hydrophobic beads and ~10-fold for hydrophilic beads. However, the mass of BSA on the beads is reduced after the pH returns to 7, indicating the increased BSA binding at pH 5.2 is reversible. We also observed BSA decreases nonspecific antibody-bead interactions by up to 90%, depending on assay conditions. Though the amount of BSA coating the beads between FA and FAF grade BSA is not significant, FAF grade BSA blocks the antibody-bead interactions ~10% more than FA grade BSA, suggesting FAF grade BSA has better antifouling properties.

Conclusion:

Herein, we show the presence of BSA reduces the noise within bead-based ELISAs by binding to the bead and blocking nonspecific antibody-bead interactions, regardless of BSA grade, bead type, and pH. FAF grade BSA demonstrates slightly better blocking than FA grade BSA, but both BSA grades decreased nonspecific antibody-bead interactions. Further, BSA shows antifouling properties at the typical assay pH of 7, but lowering the pH to 5.2 can significantly increase BSA bead coating.

B-088

Estimation of Antibodies to Hbsag and Liver Function Markers From a Large Laboratory Database in São Paulo, Brazil

G. S. Fernandes, G. C. Carvalho, D. R. Ramadan, S. Tufik, M. C. Feres. *Associação Fundo de Incentivo a Pesquisa, São Paulo, Brazil*

Background: The Hepatitis B virus (HBV) infection can lead to chronic hepatitis B and severe hepatic disease. The Gamma Glutamyl Transferase (GGT), Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT) tests are commonly used to assess liver function. Despite having an effective vaccine schedule, many people in Brazil are still susceptible to HBV. Vaccines use the surface protein (AgHBs) of the virus to induce immunity, and patients with antibody levels above 10 mUL/mL to this protein are considered to have vaccine-induced immunity. The study aims to analyze the pattern of hepatitis B markers and liver function in HBV-positive populations.

Methods: A retrospective analysis from 2020 to 2022 was conducted from a large laboratory database in São Paulo, Brazil selecting all individuals from whom the following serological diagnostic markers for Hepatitis B were requested: HBsAg, total Anti-HBc, IgM Anti-HBc, and Anti-HBs. HBV groups were classified based upon results for the following serological diagnostic markers: HBsAg, total Anti-HBc, IgM Anti-HBc, and Anti-HBs. The HBV susceptible group was identified as negative for all markers, the naturally immune group with Anti-HBc and Anti-HBs markers, and the vaccinated group with Anti-HBs only. Liver function was evaluated in two conditions: naturally immune and vaccinated, using liver cell injury markers: GGT, AST, and ALT. The Mann-Whitney U and Kruskal-Wallis tests were used for statistical analysis with $p<0.05$ considered significant.

Results: Results from a total of 57,311 (32%) men and 121,404 (68%) women were evaluated. The study observed a lower frequency of individuals in the male vaccinated group (36%) compared to the susceptible group (56%). Conversely, the female group showed a higher frequency of individuals immunized by vaccination (51%) in relation to the susceptible group (45%). The rate of individuals exposed to natural infection was higher in the male group (9%) compared to the female group (4%). Regarding Anti-Hbs antibody levels, higher levels were observed in the population with natural infection markers compared to those immunized by HBV vaccination (527.5 ± 4.0 SEM vs 332.3 ± 1.1 mUI/mL, $p <0.001$). Analyzing the age ranges with the greatest susceptibility (31-40 and 41-50 years), a gradual decrease in antibodies was observed over the years when compared: Naturally, $p = 0.03$, and immunized, $p < 0.001$). Indicator exams of liver function in both conditions showed average GGT levels above the reference value for the naturally infected groups: female 62.4 ± 5.34 SEM U/L; male 86.7 ± 5.4 SEM U/L, $p < 0.001$. No significant values of ALT and AST activity levels were seen.

Conclusion: The study results showed that this specific male population was more susceptible to HBV infection, while the female population has higher concentrations of Anti-HBs antibodies. Individuals who had a natural Hepatitis B infection exhibit GGT values above the standard.

B-089

Development of a secondary standard for quantitation of IgG antibodies to SARS-CoV-2 proteins in a multiplex assay

N. Venkateswaran, J. Sarwar, W. M. Nelson, K. S. Venkateswaran. *Tetracore, Inc., Rockville, MD*

Background: Serological surveillance is essential for monitoring disease burden and vaccine uptake at an individual and population level. During the pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), several serological assays were developed and obtained emergency use authorization to detect antibodies after infection. At that time, the need was to differentiate the infected from the naïve population, and these qualitative assays played a critical role in disease management. In the wake of vaccinations, there is a need for the development of quantitative assays. The world health organization (WHO) developed an international standard for quantitation. We performed this study to develop and validate an in-house secondary standard for our multiplex assay based on this WHO standard.

Methods: We developed a novel multiplex assay for quantifying human IgG antibodies to four different SARS-CoV-2 antigens in human serum or plasma. We have used Luminex® xMAP® technology for this assay and immobilized four recombinant proteins, namely, the receptor-binding domain of S1(RBD), nucleocapsid protein (NP), S1 protein (S1), and trimeric Spike protein (trimer) on internally coded magnetic microspheres. We procured lyophilized WHO quantification standard 20/136 from the National Institute for Biological Standards and Control (NIBSC), which has the arbitrary assignment of 1000 BAU/mL after reconstitution in 0.25mL. We tested this standard in the range of 10 to 0.01 BAU/mL for the standard curve. We tested NIBSC 20/162 control reagent using this assay for the linearity of dilution. Thirty-seven samples from the NIBSC verification panel were also tested for this evaluation. We also quantified IgG antibodies in 25 samples from vaccinated individuals, ten samples from vaccine breakthrough individuals, 25 samples from PCR-positive individuals, and 75 samples collected before 2019.

Results:The secondary standard was calibrated using the 20/136 standard. The NIBSC 20/136 and the secondary standard displayed a wide dynamic range of quantitation. The difference in the expected and actual concentrations was less than 20%.

Multiple dilutions of the serum or plasma samples were used to get readings within the standard curve range. Excellent linearity was noted at various sample dilutions to get the correct quantification. We observed that vaccinated individuals had a higher concentration of binding antibodies to S1, Trimer, and RBD and were negative for NP antibodies. Conversely, the acute samples from infected individuals collected less than ten days post-positive PCR showed higher antibodies to NP than different spike proteins. The vaccine break-through infection samples showed lower NP antibodies than unvaccinated but infected individuals.

Conclusion: Our development of a secondary standard calibrated against WHO international standard is vital for quantitatively measuring different SARS-CoV-2 antibodies. A secondary standard can help manufacturers and users to normalize results from various assays and lots over a prolonged period.

B-090

Evaluation of clinical serology assays through the development of linearity materials for aHBs IgG, Rubella IgG, and Toxoplasma IgG

D. Avery[1], J. Herod[2], J. Pawlak[2]. *[1]LGC Clinical Diagnostics, Cumberland FOreside, ME, [2]LGC Clinical Diagnostics, Cumberland Foreside, ME*

Background: The human body produces immunoglobulin antibodies as a response to various viruses, bacteria, and pathogens. Automated antibody assays, developed to detect immune response or infectious state, are typically qualitative or semi-quantitative where a positive or negative designation is reported out based on a comparison of signal intensity from the chemical reaction and a predetermined signal to cut-off ratio. In these assays the result is not suggestive of a concentration but rather the strength of the antibody antigen avidity. Use of daily QC, in conjunction with linearity materials, can be used to monitor the performance of these assays. Although the signal response may be linear, serological assays can show variability due to the assay's antigen specificity to the antibodies present in the sample and the antibody heterogeneity within the samples. Understanding these variables is crucial in interpreting patient and control results, as well as developing controls and linearity material to meet the needs of regulatory requirements for clinical testing.

Methods: Evaluation of high and low titer patient samples was carried out across multiple platforms including the Roche cobas®, Abbott Alinity, Ortho Vitros, and Siemens Centaur, where applicable, for analyte recovery of aHBs IgG, Toxoplasma IgG and Rubella IgG. These patient samples were then used to evaluate the linear response across the analyzer measuring range using equal delta admixtures, as well as serial dilution studies.

Results: The serology assays tested here, for Rubella IgG, show a significant difference in recovery of approximately 50% when comparing a 60% diluted patient sample run across multiple platforms. Results vary at both the low and high end of the assays. The linearity of aHBs IgG assays and linear variability in the Rubella IgG and Toxoplasma IgG assays is demonstrated via equal delta analysis. These results demonstrate the incongruity in results within a patient sample, likely due to the heterogeneity of antibodies in the patient samples and the non-linear dilution effect this can cause.

Conclusion: Since serology assays are based on antigen antibody avidity, patient pools taken from recently infected patients, while ideal low positives, can be variable lot to lot and assay to assay as the antibodies are not matured and contain a mix of IgG and IgM, decreasing avidity. Lot to lot variability is decreased in third party controls and linearity materials such as VALIDATE by diluting high titer patient samples or by using recombinant antibodies. Laboratories must set their own acceptance criteria for daily QC and calibration verification linearity testing, keeping in mind inherent assay variability and variability in the controls themselves. It is important to keep in mind that in-kit controls and linearity materials are typically optimized for that specific lot of reagent and calibrators and that although they may be required as part of the manufacture's IFUs, use of a third-party quality control and linearity material in conjunction with the in-kit controls can help to ensure laboratories are monitoring the assay for changes over time and allows for comparison against peer data when determining clinical significance of assay performance.

B-091

Assessing Postvaccination SARS-CoV-2 Neutralizing Antibodies at Different Timepoints: a Laboratory-Based Study

G. M. Souza, G. C. Carvalho, R. A. Nunes, F. M. Cristaldi, D. R. Ramadan, S. Tufik. *Associação Fundo de Incentivo a Pesquisa, São Paulo, Brazil*

Background: Serum-neutralizing antibodies (NAbs) against SARS-CoV-2 may be produced as a result of either viral infection or vaccination. NAbs can disrupt the connection between the RBD (Receptor-Binding Domain) of the Spike protein and the ACE2 receptor, hindering the virus's entry into cells. However, only a portion of these antibodies are able to neutralize the virus and prevent it from infecting new cells. While the presence of NAbs should not be considered a guarantee of neither immunity nor vaccine effectiveness, it can serve as an indicator for seroprevalence studies in healthcare settings. The purpose of this study was to compare results for NAbs from a group of healthcare individuals that received either CoronaVac/Sinovac (CVC/S) or Oxford-AstraZeneca (O/AZ) in São Paulo, Brazil. **Methods:** Study Population and Characteristics: The current study evaluated a total of 33 and 34 healthcare professionals that received, respectively, O/AZ and CVC/S in the year 2021. Blood samples were collected 15 days (15D1) after the first dose of vaccination and at 30 (30D2), 60 (60D2), or 90 (90D2) days after the second dose. The presence of neutralizing antibodies was assessed with the cPassTM SARS-CoV-2 Neutralization Antibody Detection Kit GenScript. Results were classified into three categories based on reference values: not reactive (0-20%), undetermined (20-30%), and reactive (greater than 30%). **Results:** Among the 67 health professionals, mostly were women in both vaccination groups: CVC/S (85.3%) and O/AZ (90%). At 15 days after receiving the first dose, the presence of neutralizing antibodies was detected in individuals who received both vaccines. However, a total of 32.3% (11/34) and 15.1% (5/33) individuals for CVC/S or O/AZ respectively do not show reactive antibodies at this first period evaluated. NAbs values obtained at 15D1, 30D2, 60D2, and 90D2 for CVC/S and O/AZ were 53.9%, 79.3%, 74.7%, 66.7% (for CVC/S) and 68.2%, 87.7%, 82.9%, 69.6% (for O/AZ). For both vaccination groups, higher levels for neutralizing antibodies were detected at 30 days after the second dose, and decreased at 60 and 90 days. **Conclusion:** Oxford-AstraZeneca induced higher levels of neutralizing antibodies in this group of healthcare individuals. Serum follow-up samples taken at 30D2 elicited increased NAbs compared to other timepoints.

B-092

Limited Clinical Utility of N-type Antibody Testing in Lambert-Eaton Myasthenic Syndrome

J. L. Wu, M. Majed, A. Cepress, A. Zekeridou, V. A. Lennon, S. J. Pittock, A. McKeon, C. J. Klein, D. Dubey, J. R. Mills. *Mayo Clinic, Rochester, MN*

Background:

Lambert-Eaton myasthenic syndrome (LEMS) is caused by IgG-mediated disruption of certain voltage-gated Ca^{2+}-channel (VGCC) functions and diminished peripheral motor nerve-stimulated release of acetylcholine by presynaptic terminals at the neuromuscular junction and selected autonomic synapses. Both P/Q-type (Cav2.1) and N-type (Cav.2) channels are the targets of IgG-mediated autoimmunity in LEMS. Functional presynaptic VGCCs at the healthy mature mammalian neuromuscular junction are P/Q-type. About 60% of LEMS cases are paraneoplastic, of which a majority are associated with small-cell lung cancer (SCLC-LEMS). LEMS can also have a non-paraneoplastic etiology (idiopathic, non-tumor LEMS [NT-LEMS]). It is important to have reliable autoantibody biomarkers to aid the serological diagnosis of LEMS and to distinguish between SCLC-LEMS and NT-LEMS, because the treatment and prognosis differ. Previous studies have reported seropositivity for SOX1/AGNA-1-IgG in ~ 64% of SCLC-LEMS cases, but in no NT-LEMS cases. When coexisting with P/Q-type VGCC IgG in LEMS patients, IgGs of both N-type VGCC and SOX1/AGNA-1 IgG specificities have been suggested as useful biomarkers for predicting SCLC. Here we compared the clinical utility of serological tests for P/Q-type and N-type VGCCs and SOX1/AGNA-1 IgGs as diagnostic aids for LEMS and for differentiating paraneoplastic from idiopathic LEMS.

Methods:

This is a retrospective study of 102 patients with a clinical (electrophysiologically confirmed) diagnosis of LEMS who were tested for both P/Q-type and N-type VGCC IgGs by radioimmunoprecipitation assays performed at the Mayo Clinic Neuroimmunology Laboratory. 95 patients had comprehensive clinical screening for cancer; 66 had NT-LEMS, 21 had SCLC-LEMS and 8 had LEMS with non-SCLC cancers. Historical data were reviewed retrospectively. Of the NT-LEMS and SCLC-LEMS patients, 76 were tested for SOX1/AGNA-1 IgG. 122 healthy sex and age-matched controls were tested.

Results:

Among the 102 LEMS patients, 92 (90%) were P/Q-VGCC IgG positive (P/Q+) and 26 (26%) were N-VGCC IgG positive (N+); 67 (66%) were P/Q+ only, 1 (0.9%) was N+ only, 25 (25%) were P/Q+N+, and 9 (9%) were seronegative for both. Three of the 122 healthy control subjects (2.5%) were N+ only, the rest were seronegative. The frequency of isolated N-type VGCC seropositivity did not differ significantly for LEMS patients and healthy controls (p=0.69). All 21 SCLC-LEMS cases (100%) were P/Q-VGCC IgG positive, compared with 58 of the 66 NT-LEMS cases (88%). The rate of N-type VGCC IgG positivity in P/Q+ LEMS patients did not differ signifi-

cantly for SCLC-LEMS (5 of 21) and NT-LEMS (13 of 58) diagnoses (24% vs. 22%, *p*=0.90). The frequency of SOX1/AGNA-1 IgG was 35% for the SCLC-LEMS cases (7 of 20), but none of the NT-LEMS cases (n=56) was SOX1/AGNA-1 IgG positive.

Conclusion:

N-type VGCC improved neither sensitivity for LEMS nor positive predictive value for cancer and has limited diagnostic utility in assessing patients with suspected LEMS. PQ-type VGCC IgG negativity does not exclude a diagnosis of LEMS. SOX1/AGNA-1 positivity has high specificity but low sensitivity for SCLC. Therefore, while antibody testing confirms a diagnosis of autoimmune LEMS, electrophysiology (to confirm neurological diagnosis) and body imaging (for cancer detection) remain important for the diagnostic evaluation.

B-093

Evaluation of the Association of Non-criteria Antiphospholipid Antibodies with the Presence of Lupus Anticoagulant

A. Saadalla, V. Nandakumar. *ARUP laboratories, University of Utah, Salt Lake City, UT*

Backrground Laboratory diagnosis of the antiphospholipid antibody syndrome (APS) requires evidence ofpersistently positive lupus anticoagulant (LAC), detected using clot-based assays and/or thepresence of high-titer serum anti-cardiolipin (aCL) and/or anti-beta-2 glycoprotein-1 (aB2GPI)antibodies, measured by semi-quantitative solid-phase immunoassays. Several non-criteria anti-phospholipid antibodies (NC-APLA) have been proposed as candidates for APS diagnosis:phosphatidyl-serine/prothrombin (aPS/PT; IgM and IgG isotypes), anti-phosphatidyl serine (aPS;IgM and IgG), anti-prothrombin (aPT-IgG). However, their diagnostic relevance in routine APSevaluation is still unclear. The objectives of this study were 1) to determine the prevalence andassociation of NC-APLA in relation to LAC, aCL and aB2GPI antibodies' positivity,independently and in combination and, 2) to assess the ability of NC-APLA to predict LACactivity, as an indirect measure of their function. **Methods** Results from 492 patients submitted for antiphospholipid antibody (APLA) panel or standaloneserology testing between January 2016 to January 2023 were retrieved in accordance withinstitutional guidelines. Patients were grouped according to LAC status and serology positivity(using manufacturer-suggested cutoff) into three groups: Single-positives (SP) for LAC, aCL oraB2GPI; Double-positives (DP) for aCL and aB2GPI; Triple-positives (TP) for LAC, aCL andaB2GPI. NC-ALPA titers were compared between LAC-positive and negative patients. Statisticalsignificance was established using unpaired Student's t-test. Receiver operating curve (ROC)analysis was employed to assess the ability of NC-APLA to predict LAC status. **Results** Out of the total 492 patients, 1.8% (9/492) were TP, 2.4% (12/492) were DP and 24.7% (122/492)were SP. All NC-APLA titers were significantly higher in TP than in SP patients (P<0.001).aPS/PT IgG (Mean: 76.3 vs 12.8 Units) and aPS IgG (Mean: 64.8 vs 5.5 GPS) antibodies weresignificantly higher in TP than DP patients (P=0.008 and <0.0001, respectively). aPS/PT IgM(Mean: 62.5 *vs* 19.4 Units) and aPS IgM titers (Mean 31.9 *vs* 7.7 MPS) were significantly higherin DP compared to SP patients (P<0.0001). When classified by LAC status, 40 patients werepositive and 452 were negative. Levels of all NC-APLA antibody titers (P<0.0001) followed byaCL (Mean IgG 21.9 vs 7.8 GPL; IgM 20 vs 12.6 MPL; P<0.01) and aB2GPI (Mean IgG 20.1 vs4.9 SGU; IgM 13.8 vs 7.6 SMU, P<0.001) antibodies were significantly higher in LAC-positivepatients compared to the LAC-negative individuals. ROC analyses based on the LAC statusresulted in the highest area under the curve (AUC, 95% CI) for aPS/PT IgM (0.72, 0.62 to 0.81)Private Information and aPS/PT IgG (0.74, 0.65 to 0.83) antibodies relative to aCL (IgM:0.53, 0.43 to 0.64; IgG:0.66,0.56 to 0.77) or aB2GPI (IgM:0.5, 0.51 to 0.72 and IgG:0.6, 0.51 to 0.72) antibodies; aPS IgMand aPT IgG displayed similar performances (0.65, 0.54 to 0.75 and 0.66, 0.57 to 0.74,respectively). Whereas aPS IgG was of lower AUC (0.58, 0.48 to 0.68). **Conclusion** Our data demonstrated that at high titers, NC-APLA, display increased association with LACpositivity in comparison to aCL or aB2GPI antibody levels. Particularly, aPS/PT antibodiesdisplayed the superior ability to determine the LAC presence.Private Information

B-094

Vaccination Anti-SARS-Cov2 Scheme Effect on Humoral Responses on Brazilian Cohort

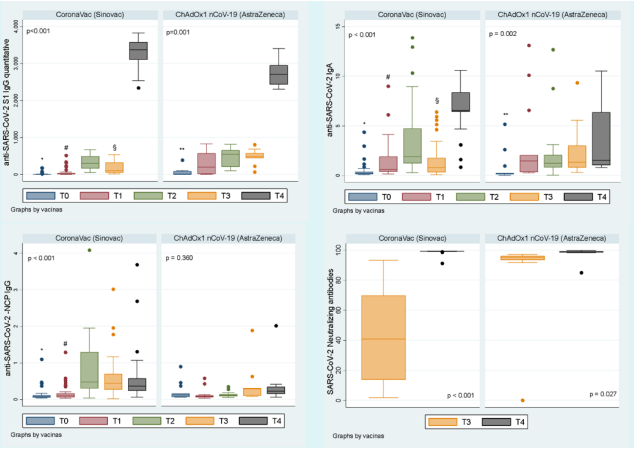
N. B. Bacarov[1], D. Christofolini[2], C. L. Sano[2], R. H. Dias[1], F. T. Bellucco[1], M. M. Soane[1], B. Bianco[2], C. P. Barbosa[2]. [1]*EUROIMMUN Brazil, São Caetano do Sul, Brazil,* [2]*Centro Universitário Faculdade de Medicina do ABC (FMABC), Santo André, Brazil*

Background: Due to high spread of SARS-CoV-2, a global emergency vaccination program using diverse and new vaccines was implemented, providing protection mortality of COVID-19. In this way, populational investigation before and after vaccination is essential to understand and monitoring the effectiveness of these efforts.

Methods: Observational non-randomized study was performed using samples of 95 workers from healthcare institutions. They were vaccinated with two homologous doses of Sinovac, ChAdOx1 and Pfizer booster dose. Serum levels of anti-S1 IgA and IgG, anti-NCP IgG and neutralizing antibodies (Nabs) were measured immediately before (T0), two weeks after each dose (T1, T2), 21 days after T2 (T3) and two weeks after boost (T4). Tests were performed through commercial kits of EUROIMMUN.

Results: Results are summarized in Figure 1. Anti-S1 IgG and IgA titles of CoronaVac samples showed a statistically significant increase after each vaccine dose (T1, T2 and T4). For ChAdOx1, statistically significant difference (SSD) was noticed between T1 and T4. Comparing anti- S1 IgG titles between both vaccines, SSD was observed between all collections, above 2500 BAU/mL on both.Anti-NCP titles increased after each CoronaVac dose, as expected since is an inactivated virus vaccine and SSD was observed from T1 to T4. NAbs at T3 and T4 showed SSD between collection times for each vaccine and between the vaccines. At T3 NAbs titles were higher for ChAdOx1 in comparison to Coronavac vaccinated. However, after the booster dose there was no difference of titles between vaccines and all participants were considered responsive.

Conclusion: The vaccine antibodies titles increased after each vaccine dose. Although there was difference of the diverse antibody titles between vaccines after 1st and 2nd doses, after booster dose all vaccinated were equally highly responsive. Such results reinforce the importance of the booster dose in the vaccination strategy against SARS-CoV-2.



B-095

Determination of Error Rates with Duplicate Analysis in Anti-Drug Antibody Testing

P. M. Vanderboom, K. T. Herbstritt, M. V. Willrich, A. E. Tebo, M. R. Snyder. *Mayo Clinic, Rochester, MN*

Background:

Duplicate measurements can be used to characterize assay imprecision, identify outliers, and estimate error rates. The benefits of duplicate analysis, however, need to be balanced against the increased financial cost. The objective of this study was to evaluate the need for continued replicate measurement in a laboratory developed assay measuring anti-drug antibodies to infliximab (ATI). To this end, duplicate patient sample measurements from the ATI assay over a two-year period were analyzed to calculate outlier and error frequencies.

Methods:

ATI were measured by electrochemiluminescence (ECL) on the MesoScale Discovery (MSD) platform, with a standard curve from 20-1250 U/mL using commercially available monoclonal ATIs. All patient samples tested for ATI over a 27-month period of time (n = 9,582) were subject to inclusion in this study. Only patient samples with ATI levels within the analytical measuring range (AMR) of the assay, however, were selected for further analysis. Samples were divided equally into 7 bins across the measuring range based on mean replicate concentration. Within-run imprecision was determined for each bin using the standard deviation (combined from all replicates using sum of squares) and mean concentration derived from all replicates. Outliers were defined as replicate measurements with a percent difference greater than ±3 SD of the calculated assay imprecision. Errors were defined as samples which had replicate values on opposite sides of the clinical cutoff of positive ATI (≥50 U/mL) and a percent difference greater than 3SD of the assay imprecision.

Results:

Of the 9582 total patient samples analyzed, 2467 had ATI values within the AMR and 1,655 had levels over the clinical cut-off of 50 U/mL (17.3% positivity rate). Within-run imprecision determined from the mean and combined SD of the duplicate measurements ranged from 15.7% at the lowest concentration bin (20 - 27.5 U/mL) to 10.0% at the highest concentration bin (358 - 1,243 U/mL). The mean percentage of outliers in each bin ranged from 0.8% - 3.4% resulting in an overall outlier frequency of 2.1% when considering all samples within the AMR. There were 66 samples within the AMR which demonstrated qualitative discrepancies between the replicates. However, only 21 (31.8%) of these samples, had a percent agreement greater than 3SD of the assay imprecision resulting in an overall error rate of 0.2% when considering all 9582 samples.

Conclusion:

In this study, the analysis of replicate patient samples enabled the establishment of a precision profile and estimation of error rates for a laboratory developed test measuring ATI. The calculated error rate of 0.2%, however, is well within the reported range of most clinical assays indicating that the continued practice of duplicate measurement may not be necessary. Additionally, updating the laboratory protocol to eliminate replicate testing would have positive impact in laboratory workflow, cost, and turn-around-time for results.

B-096

Are we truly Choosing Wisely? An Assessment of non-specific testing, time to diagnosis and costs related to the diagnosis of Rheumatoid Arthritis

T. Borunda Duque[1], V. Joy[2]. *[1]Rhodes Group, Albuquerque, NM, [2]Thermo Fisher Scientific, Portage, PA*

Background:

Treatment of Rheumatoid Arthritis (RA) early in the disease course can significantly impact patient quality of life by preventing the development of joint erosion and slowing down disease progression. Timely diagnosis is a critical aspect of early treatment. Most patients initially present within a primary care (PC) setting where a variety of non-specific tests/test algorithms are used. We reviewed 8 years' worth of RA real-world laboratory data to assess time to diagnosis (TTD) between different tests/test combinations.

Methods:

Data extraction spanning 2014 - 2021 was pulled from a US laboratory, using Rheumatoid Factor (RF) and Anti-Cyclic Citrullinated Peptide (CCP) positivity as the primary inclusion criteria. Additionally, sex, age, ordering physician specialty, ICD-10 codes, test order date and test codes (ESR, CRP, ANA screen, RF isotypes) were pulled longitudinally.

Results:

This data set includes 3,024 patients with RA (RA ICD code). Of these, 45% were first suspected and evaluated within PC. TTD between patients initially tested with only CRP or ESR had a median TTD of 920 days and those tested with ANA only had a median TTD of 685 days. Those tested with RF+CCP or RF+CCP+ANA had a median TTD of 367 days and 290 days, respectively.

Conclusion:

Our data set highlights the impact of test methods on TTD of patients with RA, with tests combinations more specific for RA significantly decreasing TTD. Further analysis will be conducted to determine the downstream implications resulting between these diagnostic delays. Standardizing and implementing initial testing algorithms could potentially decrease RA TTD, and directly impact burden of care.

B-097

Storage stability of HbA1c in whole blood tubes at room temperature on the Abbott Alinity c clinical chemistry system

M. Berman[1], X. Wang[2], P. Cheng[2], V. Kulasingam[2]. *[1]Abbott Labs, North Chicago, IL, [2]University Health Network, Toronto, ON, Canada*

Background: Hospital laboratories frequently receive requests to order additional tests on an existing specimen. For this reason, laboratories routinely save specimens after initial testing has been completed for a defined period of time. The current literature lacks specific information regarding HbA1c stability in whole blood and the effects of red blood cell settling. In addition, literature references are not inclusive of various conditions which can cause gaps in data collection such as samples in queue for prolonged periods. To obtain information on prolonged storage of whole blood tubes, HbA1c was assessed for storage at room temperature (RT) (20-25 °C) for up to 4 hours on the Alinity c system. In addition, potential carryover from a whole blood to a serum sample was assessed.

Methods: A minimum of 40 whole blood sample pools (approx. 8 mLs) were prepared from remnant samples. Sample pools were mixed, aliquoted, and stored at RT on the Alinity c system. The samples were tested as a single replicate continuously for up to 4 hours without mixing (minimum of 20 cycles) and compared to the baseline sample values. Carryover from a whole blood to a glucose serum sample was assessed after 4 hours continuous testing of whole blood to mimic a high volume testing laboratory. The stability of each HbA1c sample and carryover into a glucose sample were evaluated by comparing the mean absolute or percent difference from the respective sample baseline measurement. The allowed deviation from baseline was 5%.

Results: After 4 hours at RT, the whole blood cells had settled to approximately 50% from the top of the tube. The red blood cell settling had no significant impact on the results. Within sample precision for the 20 cycles of repetitive testing was </=1%. The 48 individual samples showed insignificant difference from the baseline value, with a mean change of 1.0% (Range 0-3.2%). Sample carryover from the whole blood samples to a serum glucose sample was not significant (2.71 Baseline vs 2.64 first replicate).

Conclusion: This study demonstrated that the storage stability of HbA1c is stable for up to 4 hours at RT on the Alinity c system.

B-098

Analytical performance evaluation of sigma strong clinical chemistry assays on the Alinity c system

M. Berman[1], V. Bhartia[2], X. Wang[3], P. Cheng[4], V. Kulasingam[3]. *[1]Abbott Labs, North Chicago, IL, [2]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada, Toronto, ON, Canada, [3]Division of Clinical Biochemistry, University Health Network, Toronto, Canada, Toronto, ON, Canada, [4]Division of Clinical Biochemistry, University Health Network, Toronto, Canada, North Chicago, IL*

Background: The aim of this study was to evaluate the analytical performance of 5 Abbott next generation (Sigma Strong) clinical chemistry assays on the Abbott Alinity c system. Method comparison, precision, linearity, accuracy, and sigma metrics were assessed for Albumin BCG2 (Bromocresol green), Albumin BCP2 (Bromocresol purple), Cholesterol2, Total Protein2 and Amylase2.

Methods: Method comparison between new (second generation) and current on market Alinity assays was performed by measuring 126-138 serum/plasma samples in duplicate. Assessment of imprecision was performed by running 2 levels of quality control material (BioRad; Chemistry UA) and 3 pooled patient samples 5x twice per day for 5 days. 10 replicates of ERM-DA470 k IFCC material for albumin, ERM-AD456 k IFCC material for amylase, NIST SRM 927 material for total protein, and NIST 1951cL1 for cholesterol were run to determine the accuracy and calculated sigma value for each analyte. Linearity testing was performed by running 5-6 levels of commercially available linearity materials in replicates of 3. Bias, acceptable imprecision, and total allowable error were based on Clinical Laboratory Improvement Amendments and Accreditation Canada Diagnostics guidelines. Statistical analysis was performed using EP evaluator.

Results: Data for method comparison (Passing Bablok), precision (Range of total %CV across 5 concentrations), accuracy (% Bias from target value), and linearity (Range of % recovery) for 5 Abbott second generation clinical chemistry assays are shown in Table 1. All assays demonstrated ≥6 Sigma performance.

Conclusion: The Abbott second generation (Sigma Strong) clinical chemistry assays on the Alinity c system showed acceptable performance for precision, accuracy, linearity, and agreement with the on-market Alinity c clinical chemistry assays.

Table 1. Results for the analytical performance of next generation clinical chemistry assays

Assay	Precision (%CV Range)	Accuracy (% Bias)	Linearity (% Recovery)	Method comparison (r)
Albumin BCG2	0.9 - 1.8%	0.2%	92.1 - 103.7%	0.996
Albumin BCP2	0.6 - 1.7%	1.2%	91.1 - 103.3%	0.995
Cholesterol2	0.7 - 2.1%	0.5% (Level 1) 0.7% (Level 2)	92.6-102.5%	0.998
Total Protein2	0.76 - 1.04%	2.2%	90.9-102.5%	0.997
Amylase2	0.4 - 2.8%	1.6%	98.8-105.1%	0.998

B-099

Development and Improvement of Novel MAS™ Infectious Controls for Serology Diagnostic Tests

D. Crandall, H. Wang, N. Krishnaswami, R. Hu. *Thermo Fisher Scientific, Fremont, CA*

Background: Human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) remain major infections around the world. As not all viral infections are preventable, serological assays play a critical role in diagnoses, therapeutic monitoring, and treatment of these infections. The Thermo Scientific™ MAS™ Omni™ Infectious Controls are a family of controls containing single or multiple analytes in human plasma-based matrix designed to mimic patient samples in the assessment of serological assay performance. One of the greatest challenges within the field of Infectious Serology testing is method variation amongst the semi-quantitative assays. As an example, a result that is considered a low positive on one analyzer, may be considered a high positive on a different analyzer. Therefore, platform agnostic Quality Control material will provide evaluation among different methods. Quality Controls may also be developed to focus on certain platforms based on "fit-for-purpose" applications. We report here the development and performance evaluation of the MAS™ Omni Infectious Controls - For Use with Siemens Assays.

Methods: Two new MAS™ Omni Infectious Controls were developed and characterized to be added to the existing catalog of MAS™ Omni Infectious Control Products. MAS™ Omni Infectious BSI (Bloodstream Infectious) Positive Control Panel - For Use with Siemens Assays* is a multi-constituent IVD quality control containing anti-HIV-1/2, anti-HBc, anti-HCV, and anti-HTLV I/II antibodies, as well as Hepatitis B surface antigen (HBsAg) that was developed for the Siemens Assays platform. The new product has been adapted from the existing BSI Positive Control Panel* by increasing the titer of HBsAg while decreasing the titer of anti-HBc and anti-HCV. MAS™ anti-HBs Positive Control - For Use with Siemens Assays* was developed for the assessment of assay performance detecting anti-HBs on the Siemens Assays Platform. The new product has been adapted from the existing anti-HBs Positive Control by increasing the titer of anti-HBs. Development and performance evaluations were performed on SIEMENS™ and non- SIEMENS™ platforms. Shelf life of the products are determined by accelerated, real-time stability, open vial, and in-use monitoring.

Results: Two new MAS™ Omni Infectious Controls were developed and evaluated to be specifically used on the Siemens Assays Platform. Both products demonstrated acceptable performance on the SIEMENS™ Centaur™ and Abbott™ Architect™. Stability studies demonstrated a minimum of 24- or 12-months shelf life, 30 or 60 days of open vial stability, and 8 hours of in-use stability when stored at 2-8°c, respectively. Additionally, the studies demonstrate a basic understanding of method variation within Infectious Serology test methods.

Conclusions: Two new MAS™ Omni Infectious Control products that mimic patient samples have been developed for the assessment of assay performance on the Siemens Assay Platform in the clinical laboratory for anti-HBc, anti-HCV, and Hepatitis B surface antigen (HBsAg). These reliable and stable control materials will better support the assessment of serological assay performance on the Siemens platforms. *Availability of product in each country depends on local regulatory marketing authorization status

B-100

Hbsag Performance Improvement of Novel MAS™ Infectious Controls for Serology Diagnostic Tests

D. Crandall, N. Krishnaswami, R. Hu. *Thermo Fisher Scientific, Fremont, CA*

Background: Hepatitis B is a liver infection caused by the Hepatitis B virus. While preventable by vaccination, Hepatitis B remains prevalent across the globe. While prevalence is low in many areas of the world, there are still regions in which the prevalence is considered to be high. Hepatitis B surface antigen (HBsAg) can be one of the first indicators of Hepatitis B infection, and it is a commonly screened diagnostic test to determine if a patient is immune, susceptible, or has acute or chronic HBV infection. While HBsAg is a critical marker in diagnosing a patient, it presents unique challenges. The Thermo Scientific™ MAS™ Omni™ Infectious BSI Positive Control Panel* is a multi-constituent IVD control containing anti-HIV-1/2, anti-HBc, anti-HCV, and anti-HTLV I/II antibodies, as well as Hepatitis B surface antigen (HBsAg) in a human plasma-based matrix. Development and Stability Studies were conducted to evaluate and ensure optimal analyte performance throughout the shelf-life duration. As this is a multi-constituent control, the HBsAg challenges must be adequately addressed to ensure the integrity and reliability of the results of all analytes. Two specific areas of focus are HBsAg instability at increased temperature and HBsAg-Fibrinogen interaction in plasma products. We report here the continued development and assessment of HBsAg in the MAS™ Omni Infectious Controls family.

Methods: The BSI Positive Control Panel was evaluated with IVD assays to assess Shelf-Life and demonstrate the effect of elevated temperature and fibrinogen on HBsAg. Shelf life of the products was determined by real-time, accelerated, open vial, and in-use stability monitoring. Additionally, open-vial stability monitoring demonstrates the relationship between HBsAg and fibrinogen within the control products. Between Month 0 and 12, samples were run neat without processing. At Month 12, samples were centrifuged to verify the validity of the open-vial stability claim.

Results: The evaluation of the BSI Positive Control Panel demonstrates the effects of temperature and fibrinogen on HBsAg. The Real-Time and Accelerated stability studies combine to demonstrate the effect of elevated temperatures on HBsAg within the multi-analyte control. The Open-Vial stability studies completed at 0 and 12 months combine to demonstrate the effect of fibrinogen on HBsAg within the multi-analyte control.

Conclusions: The MAS™ Omni Infectious control products will perform optimally provided the proper sample processing and storage conditions are implemented. Improper storage at elevated temperatures will result in degradation at an increased rate for the HBsAg present in the sample. Additionally, it is imperative that the proper raw materials are chosen in the production of the multi-analyte controls. When using plasma products, it is best to use defibrinated plasma. For extra precaution, raw material should be centrifuged and filtered prior to production to avoid degradation due to fibrinogen. Reliable, high-quality controls are vital in ensuring that clinical diagnostic assays are functioning properly.

*Availability of product in each country depends on local regulatory marketing authorization status

B-101

Aquaporin 4 and neuromyelitis optica spectrum disorders

T. Gonzalez Cejudo, T. de Haro Romero, L. Papay Ramirez, M. Lopez Velez, T. de Haro Muñoz. *Hospital Universitario Clínico San Cecilio, Granada, Spain*

Background:Neuromyelitis óptica (NMO) is a rare demyelinating inflammatory disease of the central nervous system, characterized by acute optic neuritis (ON) and transverse myelitis (TM). Previously, it was considered as a variant of multiple sclerosis (MS), however, there are now several clinical, laboratory, neuroimaging and pathological anatomical features that distinguish it from MS as the presence of a specific autoantibody in blood called IgG-NMO or anti-AQP4 that binds to water channels named aquaporin-4 (AQP4) expressed on astrocytes and plays a key role in the pathogenesis of this disease. Some patients remain seronegative for AQP4-IgG despite a definite diagnosis of NMO and the use of the finest methods for antibody detection. In anti-AQP4 seronegative forms, antibodies against myelin oligodendrocyte glycoprotein (MOG) may be identified. We have carried out a review of those patients with anti-AQP4-IgG detected in the autoimmunity laboratory during the last 3 years.
Methods: Each year we receive an average of 260 samples from patients with suspected demyelinating pathology with or without visual impairment. For the detection of antibodies against AQP-4 specifically HEK293 cells transfected were used as the

standard substrate. Anti-MOG antibody can be detected in the same test.Measurement range: The dilution starting point for this measurement system is 1:10 (serum/plasma) and 1:1 (CSF, undiluted).

Results: A total of 5 patients were positive for anti AQP-4 antibody in serum samples, all women aged between 23 and 78 years. All patients except the youngest, who presented to the neurology department for loss of visual acuity, had other autoimmune diseases: systemic lupus erythematosus (SLE) in conjunction with rheumatoid arthritis, SLE in conjunction with Graves' disease and hypothyroidism, the two oldest (59 and 78 years), respectively. The oldest patient was admitted to the internal medicine department for persistent nausea, vomiting and hiccups and was diagnosed as NMO spectrum disorder. In all cases, the oligoclonal immunoglobulin G bands study in CSF was negative. None of the CSF samples tested were positive for anti AQP-4 antibody. All patients were treated with methylprednisolone, but treatment with azathioprine or rituximab was necessary in 3 patients.

Conclusion:Titers of antibodies against AQP-4 are significantly higher in serum than in CSF, with serum being the most sensitive simple. NMO is more prevalent in women than men, with a female predominance usually higher than observed in MS. The median age of onset is also higher than MS, with a median of 35-45 years. Since the discovery of AQ-4 antibodies, there has been an increase in the number of clinical and radiological manifestations of NMO beyond involvement of the optic nerve and the spinal cord, including manifestations in the brain. It is important to recognise them in order to make an early diagnosis, to avoid unnecessary complementary tests and to establish the most suitable treatment. Persistent and intractable hiccups and nausea may occur in 17-43% as in our oldest patient's case. Associations with organ-specific autoimmune diseases such as autoimmune thyroiditis, and non-organ-specific diseases such as SLE and Sjögren's syndrome have been described.

B-102

Screening food allergies in our health primary care area

T. De Haro Romero, C. Aldana, T. González Cejudo, T. de Haro Muñoz. *Hospital Universitario Clínico San Cecilio, Granada, Spain*

Background: Allergy is the immunological disease with the greatest impact in the world. Food allergy typically manifests with cutaneous, digestive and/or respiratory symptoms, of immediate onset, usually immunoglobulin E-mediated. Doctors from primary health-care providers are often challenged with these patients, becoming the first-line for the recognition and management of food allergies. As a laboratory, our duty is to provide the health care system with the most effective diagnostic tools.

Methods:

We recollected all the results from Primary care of our health area analyzed in our laboratory for one year. Screening assay included a panel of specific IgE antibodies to the most common food allergens: egg yolk, egg white, milk, cod, wheat, peanut and soybean. They were detected semi-quantitatively by enzyme-labeled chemiluminescent immunoassay using the Immulite Allergy Food Panel FP5 kit (Siemens Diagnostic Products Corporation, Los Angeles, USA), according to the test procedure. If the assay was positive, all specific IgE allergens contained in the mixture were analyzed separately to determine their responsibility for the positive screening test.

Results:

Our laboratory processed 24125 samples from primary care patients. Of these, 1251 were positive (greater than 0.35 Ku/L), which represented 5.19% positive of the total analyzed. Once all the specific IgE allergens contained in the mixture were analyzed, the results were: 608 positive for egg yolk, 753 positive for egg white, 523 positive for milk, 109 positive for cod, 405 positive for wheat, 597 positive for peanut and 480 positive for soybean.

Conclusion:

As with most medical disorders, a thorough medical history is the initial step in diagnosing adverse food reactions. The medical history is extremely useful, especially in acute reactions, but it can be unreliable in chronic disorders such as atopic dermatitis and asthma. In vitro assays help in the diagnosis, but they must be considered as a screening and no diagnosis tool, especially when no symptoms are associated with the positive food allergen result. That's why in vitro results must be confirmed by prick test, which is considered gold standard for allergy diagnosis. In our study, egg white is the most prevalent allergen detected (60.19%), followed in order by egg yolk (48.60%), peanut (47.72%), milk (41.81%), soybean (38.37%), wheat (32.37%) and being cod the least allergen detected (8.71%).

B-103

A Single Centre Experience of Anti-carbamylated Protein Antibody Testing in the Routine Evaluation of Rheumatoid Arthritis

C. L. Novis[1], H. A. Nelson[2], D. L. Lebiedz-Odrobina[2], V. L. Nandakumar[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT*

Background: Rheumatoid arthritis (RA) early diagnosis and treatment can slow disease progression and potentially prevent bone erosion. Anticyclic citrullinated peptide (CCP) IgG and rheumatoid factors (RF) serological testing supports the diagnosis of RA; however, these markers are not detected in about 20% of RA patients. Recently, IgG antibodies against carbamylated proteins (CarP) emerged with implications on preclinical RA diagnosis and disease severity. CarP testing is offered as part of RA panel (CarP, RF and CCP) and as a standalone test in our institution. In this study, we evaluated the contribution of CarP testing for RA diagnosis in our routine clinical practice. Our objectives were 1) to assess the clinical performance of CarP in combination with CCP and/or RF antibodies 2) to understand the association of CarP with disease severity. **Methods:** Our cohort included 331 subjects submitted for RA panel serology. Retrospective chart-review revealed 136 clinically defined RA-positive and 195 RA-negative patients. Patients' sera were tested for CCP, CarP (Inova Diagnostics, San Diego, US) and RF (Roche Diagnostics, Indianapolis, US) antibodies. Clinical performance characteristics were evaluated for CarP individually and in combination with CCP and RF. Disease manifestations (bone damage and synovitis), were correlated with CarP testing.**Results:** Single antibody testing for CarP differentiated RA patients from disease-controls with a 27.2% sensitivity, 93.3% specificity, 74.0% positive predictive value and 64.8% negative predictive value. When CarP testing was combined with RF, specificity increased to 95.4%, in combination with CCP it increased to 98.5% and in a triple combination with CCP and RF, specificity increased to 99.5%. The specificity observed in CCP and RF combination (98.5%) was equivalent to CCP and CarP combination or CCP, CarP and RF triple combination. Amongst the RA patients, frequency of bone erosions was slightly greater (P=0.76) in the presence of CarP positivity (19/37, 51.4%) compared to the presence of CCP (51/107, 47.7%) or RF (50/112, 44.6%) positivity in isolation and/or in combination. Frequency of synovitis was higher in RA patients that tested positive for CarP (20/37, 54.1%) compared to patients who tested positive for CCP (44/107, 41.1%) or RF (43/112, 38.4%). In the seronegative subgroup, 17.2% (5/29) of CCP negative and 14.3% (3/21) of RF negative patients were positive for CarP. Six out of 8 patients in this subgroup displayed bone erosions and/or synovitis. Of the early RA- diagnosed patients (< 6 months of symptom onset), 22.2% (2/9) were CarP positive, of which one patient (11.1%) was seronegative for CCP but positive for RF. **Conclusion:** Despite low sensitivity, individual CarP testing offered a specificity comparable to CCP. In combination with CCP and/or RF, CarP displayed a superior specificity compared to any individual testing, however, was inferior to the CCP and RF combination. CarP positivity independently and/or in combination was associated with a severe disease course. These data support the use of CarP in combination with CCP and RF for assessing disease severity but may not be advantageous for initial diagnosis. In CCP or RF seronegative patients, CarP testing may be beneficial in establishing a diagnosis of RA.

B-104

Comparison of the Performance Characteristics of the Third Generation Anti-Cyclic Citrullinated Peptide CCP3.1 IgG/IgA and CCP3 IgG Assays

C. L. Novis[1], V. Nandakumar[2]. *[1]ARUP Laboratories - University of Utah, Salt Lake City, UT, [2]Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT*

Background: Rheumatoid arthritis (RA) is one of the most common systemic autoimmune diseases. Anti-citrullinated protein antibody (ACPA) is the most specific serological biomarker for RA and is associated with significantly more disease activity compared to rheumatoid factor (RF). Improved autoantibody detection relied in the generation of tests against cyclic citrullinated peptide (CCP), a subset of ACPA. The 3rd generation anti-CCP IgG antibody (CCP3) emerged with increased sensitivity compared to the earlier versions. More recently, CCP3.1 with conjugate to detect IgA antibodies in addition to the usual IgG antibodies became available. This is described to exhibit enhanced sensitivity due to early RA patients producing IgA antibodies to CCP3 in the absence of IgG. In this study, we evaluated the diagnostic characteristics of CCP3.1 and its association with disease severity in comparison to CCP3. **Methods:** Our cohort included a total of 331 subjects submitted for RA panel or independent

antibody testing. Chart review revealed 136 clinically defined RA- positive and 195 RA- negative patients. Residual sera from these patients were tested for RF (Roche Diagnostics, Indianapolis, US), CCP3 and CCP3.1 (Inova Diagnostics, San Diego, US) antibodies. Clinical performance characteristics at the manufacture-suggested cut-off and association with disease severity were assessed and compared between the two assays. **Results:** The overall diagnostic accuracy was comparable between the two assays with CCP3 exhibiting an area under the curve (AUC) of 0.879, 95% CI: 0.836 to 0.922, and CCP3.1 showed an AUC of 0.890, 95% CI: 0.849 to 0.931. CCP3 displayed a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 78.7%, 90.8%, 85.6% and 85.9% respectively. CCP 3.1 displayed an equivalent performance with 77.9% sensitivity, 93.3% specificity, 89.1% PPV and 85.8% NPV. Both assays showed an excellent agreement with a positive percent agreement of 92.0% and negative percent agreement of 98.7%. There was a strong quantitative correlation between the two assays (Spearman correlation coefficient, r=0.864, P<0.001). Paired t test showed significantly higher titers of CCP3 (mean:118, median:145) compared to CCP3.1 (mean:110, median: 108) in RA patients. RF and CCP3.1 combination yielded a slightly greater specificity (99.0%), positive likelihood ratio (65.9) and odds ratio (201.7) compared to the RF and CCP3 combination (specificity: 98.5%, positive likelihood ratio: 44.5, odds ratio: 138.4). Nine early-RA patients (with symptom onset <6 months of diagnosis) were detected by both assays and displayed comparable titers (CCP3.1, mean:138, median:149; CCP3, mean:149, median:161). Out of 60 RA patients exhibiting more severe disease course with the presence of bone erosions, 50 of them were detected by CCP3.1 (mean:125, median:146) and 51 were detected by CCP3 (mean:139, median:151) and the titers were comparable. **Conclusion:** CCP3 and CCP3.1 assays displayed comparable performance for detection of RA and correlated similarly with the disease severity. In our study cohort that mainly comprised of RA patients diagnosed >6 months after symptoms onset, addition of IgA isotype showed no added value but did not compromise performance.

B-107

Serodiagnosis of anti-glomerular basement membrane disease using a newly developed chemiluminescence immunoassay

M. Sabalza[1], Y. Bestmann[2], L. Hartwig[2], A. Kühnl[2], C. Dähnrich[2], W. Schlumberger[2]. *[1]EUROIMMUN US, Mountain Lakes, NJ, [2]Institute for Experimental Immunology, affiliated to EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany*

Background:

Autoantibodies against the glomerular basement membrane (GBM) are important markers in the diagnosis and monitoring of autoimmune glomerulonephritides. Fast and reliable detection of these autoantibodies is crucial as anti-GBM disease can progress rapidly with fatal outcome. Here, we investigated the diagnostic performance of a newly developed, standardized anti-GBM chemiluminescence immunoassay (ChLIA).

Methods:

The diagnostic performance of the EUROIMMUN Anti-GBM ChLIA (IgG), processed on the EUROIMMUN RA Analyzer 10, was assessed using sera from 67 clinically characterized anti-GBM disease patients and 221 disease controls. Results were compared with those obtained by the EUROIMMUN Anti-GBM ELISA (IgG). Inter-assay concordance, measurement range and interference were determined in a subset of samples.

Results:

The ChLIA reached 100% sensitivity at a specificity of 98.6%, while the ELISA was less sensitive (89.6%) and more specific (100%). High qualitative concordance between both assays was evidenced by positive and negative agreement rates of 100% and 95.6%, respectively, and a kappa score of 0.901. The ChLIA showed linearity within a measurement range of 3.8-517.3 CU/ml. Coefficients of variation were calculated as 1.2-3.3% (intra-lot) and 1.6-4.2% (inter-lot). No interference was observed for hemolyzed, lipemic or icteric samples.

Conclusion:

These validation results demonstrate a high quality of the novel Anti-GBM ChLIA. Given its excellent performance compared to the corresponding ELISA, it represents a promising alternative tool for accurate anti-GBM assessment in routine diagnostic settings with the advantage of rapid turnaround time and fully automated random-access processing. Future studies will address the assay's suitability for monitoring anti-GBM levels during follow-up.

B-108

T-cell-mediated immunity against SARS-CoV-2 in health care workers:Head-to-head comparison of two interferon-gamma release assays and associations between cellular and humoral immunity

M. Sabalza[1], J. Martinek[2], H. Tomášková[2], A. Lochmanová[2], H. Zelená[2], J. Motlochová[2], K. Dieckmann[3], E. Grage-Griebenow[3], S. Saschenbrecker[3], D. Zapf[3], V. Herbst[3], E. Ježo[2], J. Janošek[4]. *[1]EUROIMMUN US, Mountain Lakes, NJ, [2]Public Health Institute Ostrava, Ostrava, Czech Republic, [3]Institute for Experimental Immunology, affiliated to EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany, [4]Center for Health Research, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic*

Background:

T-cell responses are a crucial constituent in the adaptive immunity to SARS-CoV-2 and can be quantified using interferon-gamma (IFN-γ) release assays (IGRAs). This study aimed at comparing the results of two commercial IGRA systems, the EUROIMMUN Quan-T-Cell SARS-CoV-2 and the Oxford Immunotec T-SPOT.COVID, presenting the first head-to-head comparison of these two assays for the evaluation of SARS-CoV-2-specific T-cell activity.

Methods:

Blood samples were collected at the Public Health Institute Ostrava from 90 health care professionals with a history of COVID-19 infection or vaccination. All samples were tested for T-cell-mediated immunity using the two IGRAs as well as for humoral immunity using the EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA IgG and an in-house virus neutralization test.

Results:

Similar results were obtained by both IGRAs, yet the sensitivity of the Quan-T-Cell appeared to be insignificantly higher than that of the T-SPOT.COVID.

Conclusion:

Both assays demonstrated excellent overall agreement with virus neutralization activity and anti-S IgG positivity, with the only exception that 4/6 subjects in the subgroup of unvaccinated Omicron convalescents were devoid of IgG. In these cases, however, T-cell responses were at least borderline positive, indicating that IGRAs provide higher sensitivity in detecting immune responses to SARS-CoV-2 than do IgG-specific assays. This applies particularly to unvaccinated persons who contracted only SARS-CoV-2 Omicron variant infection but is also likely to be relevant to other patient groups.

B-109

Outcome of a Large Multicenter Prospective Clinical Study Aimed at Validating a Newly Developed VlsE1/pepC10 IgG/IgM Chemiluminescent Immunoassay (CLIA) in Conjunction with a New Automated Analyzer

Z. Polston, K. Cichonski, D. Accardi, J. Torres, A. Torregroza, D. Ryan, M. Kopnitsky, R. Cruver, D. R. Zweitzig. *ZEUS Scientific, Branchburg, NJ*

Background: Lyme borreliosis is the fastest growing vector-borne illness in North America, with recent estimates as high as 476,000 new cases annually in the U.S. alone. Measurement of *Borrelia*-specific antibodies via serologic testing remains an important diagnostic aid. Since 1994, serologic testing for Lyme disease in the United States has consisted of a two-stage algorithm, termed standard two-tiered testing (STTT). In the STTT algorithm, specimens equivocal/positive by first tier screening methodologies are subsequently tested via western blots. In recent years, the FDA has cleared several alternatives for replacing western blots with more sensitive second-tier tests, termed modified two-tiered testing (MTTT). We recently developed a VlsE1/pepC10 IgG/IgM chemiluminescent immunoassay (CLIA), to be used in conjunction with a new automated analyzer. The goal of these studies was to conduct a multi-site validation for this new assay and analyzer as a first-tier screening test within an STTT algorithm. **Methods:** Retrospective cohort:280 clinically characterized samples were provided from the U.S. Centers for Disease Control and Prevention (90 cases, 190 controls). The CDC provided VlsE1/pepC10 IgG/IgM ELISA data derived from their own testing. VlsE1/pepC10 IgG/IgM CLIA and western blotting data were also generated for this panel at ZEUS Scientific. Sensitivity and specificity values were calculated for the retrospective cohort as follows:VlsE1/pepC10-CLIA versus existing VlsE1/pepC10 IgG/IgM-ELISA, and VlsE1/pepC10-CLIA + Western blots, versus VlsE1/pepC10 IgG/IgM-ELISA + Western blots, interpreted as STTT algorithms. All testing of the retrospective cohort was performed at ZEUS Scientific. Prospective Cohort: 600 routine Lyme serology specimens were prospectively collected at three locations

(1800 total): Mayo Clinic (MOC), Rochester MN.; Marshfield Clinic (MC), Stevens Point, WI; Health Network Laboratories (HNL), Allentown PA. The samples were assayed and analyzed in the same combinations as described above for the retrospective cohort; however, instead of sensitivity and specificity, the results were reported as positive percent agreement (PPA) and negative percent agreement (NPA) since the prospective samples were not clinically characterized. Testing of the prospective cohort was evenly split across three different laboratories, one of which was ZEUS Scientific. **Results:** Retrospective cohort:[VlsE1/pepC10 CLIA, Sensitivity = (77/90) = 85.56%, Specificity = (177/190) = 93.16%; VlsE1/pepC10 ELISA, Sensitivity = (80/90) = 88.89%, Specificity = (179/190) = 94.21%], [STTT-VlsE1/pepC10-CLIA + Western blots, Sensitivity = (72/90) = 80.00%, Specificity = (190/190) = 100.00%; STTT-VlsE1/pepC10-ELISA + Western blots, Sensitivity = (70/90) = 77.78%, Specificity = (190/190) = 100.00%]. Prospective Cohort (1 sample out of the 1800 collected was omitted due to an invalid western blot result):[VlsE1/pepC10-CLIA versus VlsE1/pepC10-ELISA, PPA = (196/235) = 86.51%, NPA = (1452/1564) = 92.84%; STTT-VlsE1/pepC10-ELISA versus STTT-VlsE1/pepC10-ELISA, PPA = (160/167) = 95.81%, NPA = (1607/11632) = 98.47%]. **Conclusion:** These studies demonstrate that the newly developed VlsE1/pepC10 IgG/IgM CLIA, in conjunction with a new automated analyzer, yielded accurate results as a first-tier screening assay for both the retrospective and prospective multi-site validation cohorts. Future studies, utilizing the same sample cohorts, will be aimed at validating additional second tier CLIA tests as part of an MTTT algorithm.

B-110

Validation of Digital and Automated anti-dsDNA IFA Interpretations Using the dIFine Automated Fluorescent Microscope System

D. Accardi, D. Ryan, K. Cichonski, A. Torregroza, J. Torres, Z. Polston, M. Kopnitsky, D. R. Zweitzig. *ZEUS Scientific, Branchburg, NJ*

Background: Detection of anti-double stranded DNA antibodies (anti-dsDNA) by the indirect fluorescent antibody (IFA) assay is a well-established tool for aiding in the diagnosis of systemic lupus erythematosus (SLE). ZEUS Scientific has manufactured an FDA-cleared anti-dsDNA IFA test system for decades, which relies upon manual interpretation of qualitative results using a standard fluorescent microscope. This same anti-dsDNA IFA test system was recently utilized to train software, in conjunction with a specialized fluorescent microscope (collectively termed dIFine), towards automating qualitative result interpretation. The objective of this study was to validate the reproducibility, sensitivity, and specificity of the ZEUS anti-dsDNA IFA test system, in conjunction with the dIFine automated microscope.

Methods: Multi-site reproducibility study– 2 low-positive, 2 mid-positive, 2 high-positive, and 2 negative serum samples were assembled to create a reproducibility panel. The sample identities of the panel members were scrambled prior to testing. The panel was assayed via the ZEUS anti-dsDNA-IFA test system at a 1:10 screening dilution, in triplicate, twice per day, on five days, at three different laboratories (including ZEUS Scientific). At each laboratory, the processed slides were interpreted manually by two operators, then scanned by dIFine to generate digital images of the wells, as well as an automated qualitative result interpretation. The digital images of each slide well were also interpreted by two operators, resulting in 3 independent interpretations per sample, for each operator and instrument (Manual, Digital, Automated). The number of slide wells processed for interpretation at each laboratory was: 8 samples x 30 replicates each = 240 total. Clinical study–300 serum samples derived from donors previously diagnosed with SLE were assembled. A control cohort consisting of 360 samples from donors previously diagnosed with other rheumatic and non-rheumatic diseases was also assembled. The sample identities were scrambled for both cohorts prior to testing. The 660 samples were assayed at a 1:10 screening dilution using the ZEUS anti-dsDNA IFA test system at ZEUS Scientific. Manual, digital, and automated interpretations were carried out at ZEUS Scientific as described above.

Results: Reproducibility (Actual/Expected): [Site 1 - Operator A - Manual (240/240), Digital (240/240); Site 1 - Operator B - Manual (240/240), Digital (240/240); Site 1 - Automated - (231/240)]. [Site 2 - Operator A - Manual (240/240), Digital (240/240); [Site 2 - Operator B - Manual (240/240), Digital (240/240); Site 2 - Automated - (236/240)]. [Site 3 - Operator A - Manual (240/240), Digital (238/240); Site 3 - Operator B - Manual (240/240), Digital (238/240); Site 3 - Automated - (231/240)]. Sensitivity for SLE cohort (Positive/Total, %): [Operator A - Manual, (79/300, 26.3%), Digital (79/300, 26.3%)]; [Operator B - Manual, (79/300, 26.3%), Digital (79/300, 26.3%), Automated (81/300, 27.0%)]. Specificity for control cohort (Negative/Total): [Operator A - Manual, (358/360, 99.4%), Digital (358/360, 99.4%)]; [Operator B - Manual, (358/360, 99.4%), Digital (358/360, 99.4%), Automated (358/360, 99.4%)].

Conclusions: These validation studies demonstrate that the ZEUS anti-dsDNA-IFA test system, in conjunction with the automated dIFine microscope, yields highly reproducible and accurate results derived from digital and automated interpretations.

B-111

Parallel detection of antibodies against CDR2L and CDR2 increases the diagnostic specificity in serological diagnostics of paraneoplastic cerebellar degeneration

I. Venkataraman[1], A. Ott[2], M. Scharf[2], R. Miske[2], D. Jäger[2], J. Honnorat[3], L. Komorowski[2], T. Scheper[2], W. Meyer[2]. *[1]EUROIMMUN US, Mountain Lakes, NJ, [2]Institute for Experimental Immunology, affiliated to EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany, [3]French Reference Center for Paraneoplastic Neurological Syndrome, Hospices Civils de Lyon, Institut NeuroMyoGene INSERM U1217/CNRS UMR 5310, Université de Lyon - Université Claude Bernard Lyon 1, F-69372, Lyon, France*

Background:

Onconeuronal anti-Yo antibodies are key diagnostic markers for paraneoplastic cerebellar degeneration (PCD) and often associated with gynaecologic/breast cancer. Cerebellar degeneration-related protein 2-like (CDR2L), but not cerebellar degeneration-related protein 2 (CDR2), has recently been shown to be the major anti-Yo target [1,2]. This study investigated if inclusion of CDR2L in diagnostic tests results in increased specificity for PCD.

Methods:

Three panels were examined for reactivity against CDR2L and CDR2 by line blot analysis (EUROLINE). Panel A comprised 14 preselected sera from women with Yo-specific reactivity by indirect immunofluorescence assay (IFA) on rat cerebellum, presenting with cerebellar ataxia (n=13) or peripheral neuropathy (n=1) and underlying gynaecologic/breast cancers (n=14). Panel B included 2,132 non-selected consecutive serum/CSF samples from patients with neurological symptoms. Panel C consisted of 150 samples from healthy blood donors.

Results:

In Panel A, all samples (100%) from patients with typical clinical characteristics for Yo-associated PCD showed strong positive reactivity to both CDR2L and CDR2. In Panel B, seven (0.3%) sera were anti-CDR2 negative, but anti-CDR2L positive, all of them from patients (6M/1F) without PCD and two with cancer or pre-cancerous condition. Eleven (0.5%) patients (9M/2F) were found to be anti-CDR2 positive, but anti-CDR2L negative, including two with cerebellar ataxia and three with cancer or pre-cancerous condition. In all 18 cases, IFA on rat cerebellum was negative, suggesting non-specific line blot reactivity to CDR2L or CDR2. In Panel C, 0/150 sera were anti-CDR2L and 1/150 anti-CDR2 positive, corresponding to specificities of 100% and 99.3%, respectively. The anti-CDR2 positive blood donor was male with negative IFA result.

Conclusion:

Using CDR2L in addition to CDR2 increases the specificity in diagnosing anti-Yo-associated PCD. Positive line blot reactivity against both parameters seems to confirm the diagnosis. In contrast, results showing isolated reactivity against either CDR2L or CDR2 have to be interpreted carefully as they may apply to non-PCD cases. **References** [1] Kråkenes et al. Ann Neurol 2019; 86(2):316-321. [2] Herdlevær et al. Neurol Neuroimmunol Neuroinflamm 2021;8(2):e963.

B-112

Routine application of PR3- and MPO-ANCA chemiluminescence immunoassays demonstrates enhanced detection rates

I. Venkataraman[1], J. van Beers[2], J. Damoiseaux[2], C. Dähnrich[3], A. Kühnl[3], W. Schlumberger[3]. *[1]EUROIMMUN US, Mountain Lakes, NJ, [2]Central Diagnostic Laboratory, Maastricht University Medical Center, Maastricht, Netherlands, [3]Institute for Experimental Immunology, affiliated to EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany*

Background:

Sensitive and accurate PR3- and MPO-ANCA detection are crucial in the diagnosis and monitoring of patients with ANCA-associated vasculitides (AAV) and the specific diseases categorised thereof, including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA). Chemiluminescence immunoassays (ChLIA) and bead technology have emerged as promising tools for fast, sensitive and accurate autoantibody detection. Applied to PR3- and MPO-ANCA, they could improve limitations in sensitivity, narrow measuring range and level of quantification seen in other assay formats. Here, we examined the routine performance of ChLIAs using human native MPO as well as human recombinant PR3 antigens.

Methods:

The Anti-PR3 ChLIA (IgG) and prototype Anti-Myeloperoxidase ChLIA (IgG) were processed on the RA Analyzer 10 (EUROIMMUN) and the EliA PR3^S and EliA MPO^S on the Phadia250 (Thermo-Fisher Scientific), both in accordance to the recommendations by the manufacturers. For a comparative analysis of the routine performance, 675 sera from 615 individuals requested for PR3-ANCA, MPO-ANCA or both at the Central Diagnostic Laboratory at Maastricht University Medical Center were measured with the respective assays from both automation systems. The results were compared in respect to the qualitative outcome and numerical correlation.

Results:

Of the total 675 samples, 585 and 556 were subjected to PR3-ANCA and MPO-ANCA analysis, respectively. For both ANCA types, all samples found positive with EliAs were also positive in the corresponding ChLIAs. 24 and 26 samples resulted positive for PR3- and MPO-ANCA by ChLIA, respectively, but were below cut-off in the relevant EliA test. Of those, 50.0% (n=12 for PR3) and 96.2% (n=25 for MPO) were from individuals in follow-up after AAV diagnosis, in the remaining cases an ANCA involvement was suspected based on symptoms of the patients. The overall concordance between the two assay systems was 96.0% for PR3-ANCA and 95.4% for MPO-ANCA detection.

Conclusion:

In a routine scenario, the ChLIAs fully covered the PR3- and MPO-ANCA detection by the compared reference test system. Moreover, further positive results in samples found negative by EliA demonstrated enhanced detection rates by both ChLIAs, predominantly in samples from individuals with a known history of AAV. The clinical relevance will be subject to further studies.

B-113

Diagnostic value of autoantibodies against D-3-phosphoglycerate dehydrogenase (PGDH) among patients with liver diseases

I. Venkataraman[1], X. Feng[2], Y. Liu[3], I. Karl[4], A. Ott[5], T. Scheper[5], W. Meyer[5], H. Yan[2]. *[1]EUROIMMUN US, Mountain Lakes, NJ, [2]Central Laboratory for Infection & Immunity, Beijing YouAn Hospital, Capital Medical University, Beijing, China, [3]Central Laboratory for Infection & Immunity, Beijing YouAn Hospital, Capital Medical University, Beijing, China, [4]2Institute for Experimental Immunology, affiliated to EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany, [5]Institute for Experimental Immunology, affiliated to EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany*

Background:

We determined serum autoantibodies against D-3-phosphoglycerate dehydrogenase (anti-PGDH) in patients with liver diseases using a well-validated line immunoblot assay, and analysed their diagnostic significance.

Methods:

The studied panel comprised serum samples from 383 patients with the following liver diseases: 87 autoimmune hepatitis (AIH), 88 primary biliary cholangitis (PBC), 76 chronic hepatitis B, 79 hepatitis C, 53 drug-induced liver injury as well as sera from 69 patients with autoimmune diseases (systemic lupus erythematosus or rheumatoid arthritis) and 30 healthy blood donors. The line immunoblot EUROLINE Autoimmune Liver Diseases 14 Ag (EUROIMMUN) was used to test for anti-PGDH. The intensities of the bands were automatically evaluated using the EUROLineScan software (EUROIMMUN). Indirect immunofluorescence was used to detect other autoantibodies, including anti-nuclear autoantibodies (ANA) and anti-smooth muscle antibodies (ASMA).

Results:

The prevalence of anti-PGDH was 18.3% across all patients with liver diseases (36.8% in AIH; 10.2% in PBC), 1.5% in patients with autoimmune diseases, and 3.3% in blood donors. ANA but not ASMA were detectable in all anti-PGDH positive patients with liver diseases. Patients with anti-PGDH displayed no significant differences in age, liver function, IgG serum level and ANA patterns compared to those without anti-PGDH. Anti-PGDH positivity was observed at various stages of liver disease, including acute and chronic liver injury, liver cirrhosis, and liver cancer.

Conclusion:

The anti-PGDH prevalence in AIH patients was highest among patients with liver or autoimmune diseases. Presence of anti-PGDH seems to be a specific marker for AIH and may support the diagnosis of AIH.

Data Analytics, Informatics, and Statistics

B-118

Evaluation of Thirteen Commercially Available Clinical Chemistry Assays on the Alinity® *c* System

H. Li, M. Li, J. Wang, H. Guo. *Abbott Core Diagnostics, Shanghai, China*

Background: Expansion of instrument analyte menus is often needed to accommodate country specific needs. To address specific needs in China, thirteen clinical chemistry assays have been modified for evaluation on the Alinity *c* system. These analytes are used in the investigation of several diseases including liver disease, hepatobiliary disease, cardiovascular and cerebrovascular disease, kidney disease, inflammation, infectious disease, alcoholism and glucose metabolism disease. **Methods:** The following analytes were evaluated: a1-Microglobulin (a1-MG), Ischemia modified albumin (IMA), Glutamate Dehydrogenase (GLDH), Fibrin Degradation Products (FDP), Myeloperoxidase (MPO), Fibronectin (FN), Pyruvate (PYR), Monoamine Oxidase (MAO), Heart-type Fatty Acid-Binding Protein (H-FABP), Cholyglycine (CG), Serum Amyloid A Protein (SAA), Ethanol (EtOH) and Ferritin (Fer). Reagents from Beijing Strong Biotechnologies Inc. (BSBE) were evaluated on the ABBOTT Alinity c system. Precision, LoQ and linearity range were evaluated with guidance from CLSI documents EP15-A3, EP17-A2 and EP06-Ed2. Correlation to commercially available assays were performed using human serum/plasma/urine samples across the measuring range. **Results:** The table below summarizes key performance of assays.

Assay	5-Day Precision (Total %CV)	Sensitivity (LoQ)	Linearity Correlation	Method Comparison
a1-MG (serum)	2.0%	0.47 mg/dL	0.9999	r = 1.000, slope = 1.023
a1-MG (urine)	1.7%	0.15 mg/dL	0.9994	r = 0.999, slope = 0.9874
IMA	1.0%	0.53 U/mL	0.9993	r = 0.999, slope = 1.0000
GLDH	4.2%	0.81 U/L	0.9998	r = 1.000, slope = 0.9518
FDP	1.7%	1.47 µg/mL	0.9989	r = 0.999, slope = 0.9838
MPO	2.0%	11.77 ng/mL	0.9999	r = 1.000, slope = 0.9886
FN	6.0%	1.59 mg/L	0.9999	r = 1.000, slope = 0.9992
PYR	3.0%	8.79 µmol/L	1.0000	r = 1.000, slope = 1.020
MAO	4.8%	1.18 IU/L	0.9997	r = 1.000, slope = 0.9967
H-FABP	9.9%	2.27 ng/mL	0.9993	r = 0.998, slope = 0.9783
CG	2.2%	0.13 µg/mL	0.9986	r = 0.999, slope = 0.9816
SAA	1.5%	0.56 mg/L	0.9999	r = 0.999, slope = 0.9750
EtOH	3.2%	0.0026 g/L	0.9999	r = 1.000, slope = 1.011
Fer	1.1%	4.27 ng/mL	0.9999	r = 0.998, slope = 0.9948

Conclusions: These initial results are very promising. All the assays tested exhibit good precision, linearity, anti-interference, and LOQ performances on Alinity *c* system. In addition, they also have excellent correlation with Jinya assays from BSBE on ARCHITECT system except SAA assay, and SAA has excellent correlation with Siemens BNII SAA assay.

B-120

Atellica® Ci 1900 assay sigma metrics evaluation

K. W. Kolewe[1], H. Leipold[2], S. A. Lewisch[1], J. T. Snyder[1]. *[1]Siemens Healthineers, Newark, DE, [2]Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is a stand-alone, integrated clinical chemistry and immunoassay analyzer. Offering the same assay portfolio as the Atellica® Solution, the Atellica® CI 1900 analyzer is designed to complement the Atellica® Solution in hub-and-spoke settings, enabling labs of every size to offer state-of-the-art patient testing. Six Sigma is synonymous with process performance, and Sigma metrics have become the analytical quality benchmark for the medical device industry. In this study, Sigma metrics were calculated for a total of 71 Atellica® assays to provide a simple, translatable value to evaluate a given assay's performance on the Atellica® CI 1900 Analyzer. **Methods:** Allowable total error (ATE) for each assay was defined from the Clinical Laboratory Improvement Amendment (CLIA) table. When an analyte was not present in the CLIA table, alternative sources were consulted, including the RiliBÄK table, Royal College of Pathologists of Australasia (RCPA)

table, and Ricos Biological Variability. Precision studies were conducted in accordance with Clinical and Laboratory Standards Institute (CLSI) document EP05-A3 over 20 days (n = 80 replicates) using the Atellica® CI 1900 Analyzer. Method comparison studies conducted on the Atellica® CI and Atellica® CH/IM Analyzers, with at least 100 patient samples spanning the assay's measuring interval in accordance with CLSI document EP09A3, were used to establish bias at the selected target precision concentration. Sigma metrics were determined for each assay using the equation Sigma metric = (ATE – %bias)/(Within-lab %coefficient of variation [CV]). **Results:** Sigma metrics were determined for a total of 71 Atellica® assays, resulting in 93% of assays performing above Five or Six Sigma and 0 assays performing below 4 Sigma. Of these, 44 of 52 clinical chemistry and 15 of 19 immunoassays performed above the 6 Sigma level. **Conclusions:** Sigma metrics that provide a benchmark for laboratory quality classify over 90% of assays on the Atellica® CI 1900 Analyzer with scores of 5 or 6, corresponding to the classification of excellent or world-class performance. Therefore, laboratories can expect high quality assay performance with strong comparability between the Atellica® CI 1900 Analyzer and the Atellica® Solution.**Not available for sale in the US. Product availability varies by country.

B-122

Estimated Improvement of Sigma Error Metrics associated with Manual Secondary Result Review, and Subsequent Artificial Intelligence Driven Quality Assurance.

J. Bornhorst[1], D. Rokke[1], P. Day[1], S. Erdahl[1], M. Wieczorek[2], R. Carter[2], P. Jannetto[1]. *[1]Mayo Clinic, Rochester, MN, [2]Mayo Clinic, Jacksonville, FL*

Background: Analytical and post-analytical errors are a persistent problem, which can be benchmarked using sigma metrics. Manual processes are associated with error rates of 3-4 sigma. Secondary review of manual reporting is meant to reduce/eliminate errors and is widely used, yet the true efficacy of manual secondary review is difficult to assess. Kidney stone analysis is a manually intensive process, requiring personnel to identify stone composition from spectra and to transcribe stone percent composition into an LIS system. Recently, we developed an AI program that allows for quantitative determination of kidney stone composition from spectra. This allows for a unique opportunity to estimate error rates associated with manual reporting and those following secondary review by means of subsequent AI quality assurance analysis.

Methods: Experienced technologists assessed stone composition and estimated the percent of each component (+/- 10%), based on Fourier transform infrared (FTIR) spectroscopy. These manual spectral composition assessments are secondarily reviewed by other experienced technologists for identity and percentage composition accuracy. The recently described Kidney stone AI spectral analysis (RokkStar) was implemented to assess reported stone composition identity and percent composition for agreement following secondary review in 159,334 stone reports. AI flagged discrepant reports were reviewed by personnel and reports were revised when appropriate. Secondary reviewer error detection rate was obtained by monitoring detection in a subset of 7440 kidney stones, and was that rate was extrapolated to establish secondary review error detection rate over the entire data set. The sum (total error) of estimated secondary reviewer error detection, and well as AI-mediated error detection, and all client reported errors were used to determine error rates in sigma following manual reporting, secondary review, and AI quality assurance.

Results: Manual entry led to an estimated 2060 errors in 159,334 stone reports, yielding an initial sigma value of 3.73. Secondary manual review resulted in an estimated 1909 (error detection rate of 92.5%) of these errors being detected over the entire report set, improving the sigma value to 4.61. Post-verification AI caught an additional 138 errors, leading to an improved post-AI sigma value of 5.27. Post-AI quality assurance, there were 13 remaining errors, 9 of which were manual reporting process deviations subsequently caught by laboratory staff, and 4 were reporting errors observed by client physicians. All 13 of these errors involved usual stone constituents or typographical errors that were not assessed by the current AI quality assurance version.

Conclusion: This study represents a unique large-scale estimate of efficacy of secondary result review by means of AI quality assurance review. While the presence of undetected errors cannot be completely ruled out, in this case the relative paucity of client reported errors, as well the alignment of the estimated rate of manual errors with previous reports may indicate that most errors were detected in this system. Importantly, manual secondary review improved errors rates by ~0.9 sigma, and the use of AI further improved estimated overall sigma metric by ~0.7, leading to a "world class" 5+ sigma level for this complex reporting process.

B-123

Utilization of five data mining algorithms combined with simplified preprocessing to establish reference intervals of thyroid related hormones for nonelderly adults

J. Zhong, C. Ma, L. Hou, Y. Yin, F. Zhao, Y. Hu, A. Song, D. Wang, L. Li, X. Cheng, L. Qiu. *Department of Laboratory Medicine; Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing, China*

Background: Despite the extensive research on data mining algorithms, there is still a lack of a standard protocol to evaluate the performance of the existing algorithms. Therefore, the study aims to provide a novel procedure that combines data mining algorithms and simplified preprocessing to establish reference intervals (RIs), with the performance of five algorithms assessed objectively as well. **Methods:** The Test data set and the Reference data set are the two data sets derived from the population undergoing a physical examination. After the thyroid-related hormone including thyroid stimulating hormone (TSH), free triiodo-thyronine (FT3), total triiodo-thyronine (TT3), free thyroxine(FT4), and total thyroxine (TT4) were measured by an ADVIA Centaur XP chemiluminescence immunoassay analyzer, five data algorithms were used to calculated RIs. Hoffmann, Bhattacharya, Expectation Maximum (EM), kosmic, and refineR algorithms combined with two-step data preprocessing respectively were implemented in the Test data set to establish RIs for thyroid-related hormones. The first step is to conduct a random sampling strategy to balance the ratio of sex and age, and the second step is to identify the outliers of variables in each subgroup by the Tukey method. Algorithm-calculated RIs were compared with the standard RIs calculated by transformed parametric method from the Reference data set in which reference individuals were selected following strict inclusion and exclusion criteria. RIs partition were comprehensively determined by the multiple linear regression and variance component analysis. Objective assessment of the methods is implemented by the bias ratio (BR) matrix, of which the BR threshold was set to 0.375. **Results:** The levels of the all five thyroid-related hormones are significantly different in sex, with the male having lower TSH and higher FT3, FT4, TT3, and TT4 compared to the female. Further analysis indicates the establishment of sex-specific RIs for FT3 and FT4. Standard RIs derived from the Reference data set by transformed parametric method are 0.801-4.221μIU/L for TSH, 2.58-3.82 pg/mL for FT3, 0.98-1.53ng/dL for FT4, 0.80-1.38ng/mL for TT3, 5.46-10.05g/dL for TT4, respectively. There is a high consistency between TSH RIs established by the EM algorithm and the standard TSH RIs (BR=0.063), although EM algorithms seems to perform poor on other hormones with the BR higher than 0.375. RIs calculated by Hoffmann, Bhattacharya, and refineR methods for free and total triiodo-thyronine, free and total thyroxine respectively are close and matched the standard RIs. **Conclusion:** An effective approach for objectively evaluating the performance of the algorithm based on the BR matrix is established. EM algorithm combined with simplified preprocessing can handle data with significant skewness, but its performance is limited in other scenarios. The other four algorithms perform well for data with Gaussian or near-Gaussian distribution. Using the appropriate algorithm based on the data distribution characteristics is recommended.**Keywords:** Algorithms; Data mining; Reference interval; Thyroid-related hormones**;**

B-124

Applicability of measurement uncertainty in the validity of results in the clinical laboratory

O. Martinez Nieto[1], A. Garzón[2], V. Barrera[3], A. Romero[4], A. Garzón[4], N. Hernández[4]. *[1]Fundacion Santa Fe de Bogotá, Bogota, Colombia, [2]Scientific Director of Auditoria Calidad y Gestión Ltda., Bogota, Colombia, [3]Fundacion Santa Fe de Bogota, Bogota, Colombia, [4]Auditoria Calidad y Gestión Ltda., Bogota, Colombia*

Background:

The results of the clinical laboratory must comply with attributes of precision, veracity, and accuracy to guarantee the validity of the result; since the assurance of analytical quality, different performance evaluation strategies have been applied with the use of tools such as total permissible error (ETa) and sigma metric. However, based on the requirements to qualify for the accreditation process by standards such as ISO 15189 and ISO 17025, the concept of the "measurement uncertainty" of the measured magnitude values was introduced, giving special relevance in the interpretation of the values obtained, therefore it is important to consider its estimation and evaluation as a tool that contributes to patient safety in the interpretation of clinical decision limits.

Methods:

Uncertainty estimation was performed for forty-one measurands that were previously selected considering the variables of clinical risk and processing volume. The estimation of uncertainty, ETa and sigma metric were made with the RANDOX Acusera software 24/7 online from the information obtained from the internal quality control data and the scheme of the external quality evaluation. The uncertainty obtained was compared against four criteria of acceptability of uncertainty.

Results:

After comparing the uncertainty obtained against the four criteria of acceptability of uncertainty, it was found that the most demanding criterion is that derived from the reference biological interval. The implementation of this criterion would allow objective comparability of performance between laboratories. Conclusions: The estimation of uncertainty as an indicator of the validity of the results provides relevant information in the interpretation of the results that are within the limits of clinical decision, while the ET and sigma metric contribute to the monitoring and control of the performance of the analysis or measurement procedure.

Conclusion:

We observed that the efforts of the laboratory for minimizing the variation sources that can affect the analytical accuracy have been prolific. Total Error quantification, Sigma 6 and MU have a correlation, favorably impacting the tests performance. Aiming to guarantee a constant improvement of the results' quality, the Total Error and MU goals must be periodically revised, ensuring their utility throughout time.

B-125

Artificial intelligence application to improve the algorithm for autoverification of results of biochemical markers that assess thyroid function

G. Dip[1], V. Colombo[2], S. Lejona[2], A. Lombardo[2]. *[1]TURNER Laboratory, Rosario, Argentina, [2]Laboratorios TURNER, Rosario, Argentina*

Background: The results verification process is the most important control step of the post-analytical phase. It can be done in two ways, manually by a professional, or using autoverification systems. Appropriate results are verified and submitted to the Laboratory Information System (LIS). Corrective actions are initiated for discordant results prior to their submission to the LIS. Autoverification is a process where the laboratory tests results are published without any professional's intervention. It is recommended that each laboratory creates the necessary cut-off values for autoverification, according to its population and its biochemical-medical criteria. In our laboratory, an autoverification algorithm is applied to evaluate thyroid function, which involves the measurement of 2 parameters: Thyrotropin (TSH) and Free Thyroxine (FT4). These parameters were chosen because they are the most used for characterizing thyroid function. The autoverification algorithm was defined for euthyroid, hypothyroid and hyperthyroid patients. Our professionals proposed a "safe range" for both parameters and we relate those parameters results for autoverification. We study the usefulness of artificial intelligence models by clustering non-supervised machine learning algorithms, to find behavioral patterns of not autoverified sample results, to support and even broaden the "safe ranges" established by our professionals. The goal was to improve the current autoverification algorithm by using artificial intelligence for thyroid function test results and to increase the autoverification percentage.

Methods: The autoverification process was carried out at TURNER Laboratories, Rosario, Argentina. The autoverification algorithm was designed according to the Laboratory and Clinical Standards Institute Guide AUTO10-A. The Atellica Data Manager (ADM) middleware was used to provide the connection between the Atellica Solution Immunoassay & Clinical Chemistry Analyzers and the LIS. ADM receives the results from the analyzers and processes them in consideration of the pre-analytical (age, sex, etc.), analytical (QC, flags, etc.) and post-analytical (Delta Check, etc.) conditions. It then applies the autoverification algorithm to send the results to the LIS. In the first semester of 2022, we used the autoverification algorithm with the existing "safe ranges" in 18,626 samples of outpatients between the ages of 16 and 60. On the other hand, we applied different Clustering algorithms to the samples that were not autoverified, to find behavior patterns. The ones we used are: KMeans, Fuzzy C-Means, DBSCAN, Agglomerative Clustering, Dendrogram and Affinity Propagation. We chose KMeans with k=4. Our professionals thoroughly analyzed each of the clusters and defined "new safe ranges" for autoverification. In the second semester of 2022, 18,947 samples were analyzed and the autoverification algorithm was applied with the "safe ranges" and the "new safe ranges".

Results: In the first semester of 2022, by applying the existing "safe ranges" we obtained an autoverification of 19.0%. We then simulated an autoverification with "safe ranges" and "new safe ranges" and obtained an autoverification of 71.5%. In the second semester, we applied autoverification with "safe ranges" and "new safe ranges" obtaining an autoverification of 72.7%.

Conclusion: The application of machine learning algorithms has significantly improved the level of autoverification of biochemical markers that evaluate thyroid function.

B-126

Comparison of Liftover Tools for Conversion to Genome Reference Consortium Human Build 38 from Human Genome 19

K. Park[1], J. Park[2]. *[1]Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea, Republic of, [2]Samsung Medical Center, Seoul, Korea, Republic of*

Background: Genome Reference Consortium Human Build 38 (GRCh38) was released with improvements such as accuracy and completeness over human genome 19 (hg19). However, GRCh38 has not been widely adopted in clinical laboratories because re-alignment is time-consuming and computationally-expensive. To address this issue, faster and more convenient liftover tools have been developed to convert genome coordinates between assemblies. The aim of this study was to compare the performance of liftover tools for conversion to GRCh38 from hg19.

Methods: Two variant call format (VCF) files aligned to hg19 (No. of clinical variants = 1,570,644) and GRCh38 were downloaded from ClinVar (clinvar_20221217.vcf.gz). The liftover tools such as CrossMap, NCBIRemap, and UCSCliftOver were used to convert genome coordinates from hg19 to GRCh38. We investigated the conversion failure of clinical variants and evaluated classification of variants provided by multiple submitters with assertion criteria and evidence according to ClinVar review status, to ensure the authenticity of the variants.

Results: The conversion rates of CrossMap, NCBIRemap, and UCSCliftOver were 99.64% (1,567,848/1,570,644), 99.52% (1,565,963/1,570,644) and 99.81% (1,570,560/1,570,644), respectively. The variants which failed conversion were identified in *ABCC9* (n=2), *ACACA* (n=1), *CNOT3* (n=3), *CYFIP1* (n=1), *DGAT1* (n=9), *EMG1* (n=1), *GAS2L2* (n=5), *GDF2* (n=38), *GPR179* (n=96), *HNF1B* (n=132), *HYOU1* (n=2), *MBOAT7* (n=5), *MYO19* (n=7), *NSDHL* (n=15), *ODAD3* (n=1), *ORAI1* (n=2), *PCGF2* (n=3), *PQBP1* (n=19), *PRPF31* (n=56), *PUF60* (n=9), *RBP3* (n=52), *RPS17* (n=1), *SARM1* (n=1), *SCARF2* (n=1), *SLC35A2* (n=27), *SLC39A4* (n=25), *SLC52A2* (n=86), *SURF1* (n=65), *TAF15* (n=8), *TFE3* (n=2), *TSEN34* (n=15), *WDR45* (n=56), *ZNHIT3* (n=1) genes. The numbers of pathogenic or likely pathogenic variants among variants which failed conversion by CrossMap, NCBIRemap, and UCSCliftOver were 67, 115, and 0, respectively. Classification of converted variants from GRCh37 to GRCh38 remained unchanged.

Conclusions: This study demonstrated that UCSCliftOver generates most reliable results. This study suggests that liftover tools might be one of the practical alternatives for genome conversion in case that re-alignment approaches were not possible.

B-127

An Automated Approach to Obtain Delta Check Limits

t. kampfrath[1], S. Roach[2]. *[1]siemens, walpole, MA, [2]Siemens, Walpole, MA*

Delta checking as a systematic approach to quality control specimens was first described by Nosanchuk and Gottmann in 1974. The purpose is to detect specimen errors due to short-sample aspiration, mislabeled cup or sample, incorrect patient draw, problems in analysis, and/or clerical errors. Once inaccuracy is detected, rejection and specimen recollection are the most suitable approach. The delta check procedure compares a patient's current test results to the previous test results from the same individual using a specified time interval and delta check limit (DCL). The DCL can be expressed as absolute change, percentage change, rate of change, or rate of percentage change. This procedure is typically applied to several analytes, comparing the absolute change with a specified DCL. DCLs are commonly derived from the literature and may be optimized over time based on local patient populations. Creating and modifying DCLs poses a problem, because changes in the DCL can result in flagging too little or too many specimens, and often DCLs don't take into consideration patient location and therefore lack specificity. Here, we describe a software tool that allows the determination of DCLs based on the individual laboratory's or ordering unit's patient results derived from the LIS. The software functionality was tested on a patient dataset from a large county hospital in California, USA. The software allows import of patient data for analysis and creation of population-specific DCLs. Typically, inpatient data is used, as consecutive data points within a common delta check timeframe (hours to days) are less expected for outpatients. The tool allows selection of an unlimited number of analytes of interest to be included in the DCL assessment. Furthermore, the underlying DCL timeframe that compares the two different results is customizable. Lastly, the tool provides the expected number of DCL flags based on the

chosen cutoffs. This allows for proper resource allocation or adjustment of the DCL according to available resources. After the import of raw LIS data, an automatic data cleanup is performed. Then the assays of interest and, if available, the associated patient location are directly selected in the software. The dashboard provides each assay test code that is part of the delta checking process, the desired DCL, and the chosen delta check time period (either in hours or days). The resulting expected number of DCL flags is provided specifically for each test, per hour and per day. Any change to the DCL is displayed on the dashboard in real time. Delta checks are a tool to detect specimen inaccuracies resulting from pre-analytical or analytical errors. This software tool provides customized DCLs based on patient population and the individual laboratory instead of deriving them from the literature. This novel customization enables simple derivation of DCLs that are patient population-specific and optimized for each laboratory's resources. This allows for improved detection of specimen inaccuracies and ultimately leads to improved patient care. *The product is currently under development. Its future availability cannot be guaranteed. Please contact your local Siemens Healthineers organization for further details.

B-129

Uncertainty Calculations for Reference Measurement Procedures

E. Albrecht[1], A. Geistanger[1], A. Hilger[1], F. Bauland[2], J. Taibon[1]. *[1]Roche Diagnostics GmbH, Penzberg, Germany, [2]Chrestos Concept GmbH & Co. KG, Essen, Germany*

Background: Quantitative results based on reference measurement procedures (RMP) require the calculation of the uncertainty of the result. In order to take the uncertainty of the full traceability chain into account a modularisation of the different execution steps is needed, starting from the primary reference material until the final concentration value. Different strategies have to be applied and combined to obtain the final uncertainty.We combine different ways for calculating the uncertainty of an isotope dilution -liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) based reference measurement procedure for Cortisol, and explain the differences between Type A and Type B uncertainties and best strategies to choose from them. In addition, combinations of results from subsequent steps to a final overall uncertainty are presented.

Methods: An ID-LC-MS/MS based reference measurement procedure requires as a starting point a primary reference material with known purity of the analyte under consideration. Nowadays this purity assessment is often done with quantitative nuclear magnetic resonance (qNMR) spectroscopy. The qNMR uncertainty calculation is a Type A approach, based on replicate sample preparations and measurements. With this primary material a first set of primary calibrators is produced, often through multiple steps of weighings, preparation of stock solutions and dilutions. For these steps balances, volumetric flasks, or pipettes are used, all of them coming with an error budget which has to enter the uncertainty budget of the primary calibrator preparation. We will show how this can be done through Type B uncertainty calculation.Based on the primary calibrators the LC/MS instrument is calibrated and measurements of individual samples are carried out. We will introduce a hierarchical variability experiment, which takes into account the most important error sources and results in a Type A uncertainty for the LC/MS measurements.Finally, the errors of the LC/MS measurements have to be combined with those of the primary calibrator preparation to obtain the overall uncertainty of an LC/MS reference measurement procedure.

Results: We show the approaches and calculation strategies based on the Cortisol candidate reference measurement procedure, developed within the Roche LC/MS Reference Measurement Procedure project. The purity assessment by qNMR carries an uncertainty of 0.18%. This is incorporated into the uncertainty calculations of the primary calibrators, where in addition uncertainties from weighing and dilutions are incorporated resulting in uncertainties of the primary calibrator values between 0.6% - 1.1%. Combining these uncertainties with the variabilities of the LC/MS measurement procedure results in final uncertainties between 1.6% - 2.8 %.

Conclusion: We have developed a sound framework for uncertainty calculation of reference measurement procedures, taking into account the different procedure steps, and included this into a process for the management of the different input variables up to comprehensive reports of the calculations.

B-131

The prevalence and clinical relevance of the DFS immunofluorescent staining pattern in a large ANA-positive cohort.

Y. Zhang[1], L. V. Rao[2]. *[1]UMass Chan Medical School, UMass Memorial Medical center, Worcester, MA, [2]UMass Chan Medical School, UMass Memorial Medical center, Quest diagnositics, Worcester, MA*

Background: The dense fine-speckled (DFS) nuclear pattern is one of the most common indirect immunofluorescence (IIF) patterns detected during routine antinuclear antibody (ANA) screening. Although various researchers have studied the DFS pattern in recent years, it presents a clinical puzzle since its significance remains elusive. Anti-DFS-70 antibodies occur in healthy individuals with different medical conditions. Unlike the other ANA patterns, they are not associated with systemic autoimmune disease. The present study examined the association of a DFS ANA pattern with the medical conditions of patients who presented to a tertiary care medical center.

Methods: Retrospective chart review of all laboratory values (January 2020 through June 2022) with ANA- and DFS-positive patterns in patients with ANA tests ordered by various clinicians and compared to the patient diagnosis reported. UMass Human IRB approves this study.

Results: Of 8,885 ANA samples analyzed, 67.8% (6022) were reported negative. Of total positive ANA (2863) samples, 78.6% (2257) had nuclear patterns, 9.1% (260) had mitotic patterns, and 12.1% (346) had cytoplasmic patterns. Of the positive ANA samples, 14.6% (417) had DFS patterns. For samples with DFS pattern, titers ranged from 1:40 to 1:1280; 62.5% were between1:40, and 1:80. Of samples positive for ANA DFS patterns, 76% (317) were from female patients, and 90% (375) were from patients >21 years of age. Among patients with DFS patterns, the most commonly reported diseases were neurological, followed by respiratory, digestive, circulatory, and musculoskeletal (Table 1).

Conclusion: Clinicians should use a positive ANA with a DFS pattern to indicate pathological phenomena other than autoimmune diseases. Only 3.1% of the patients with DFS patterns have a systemic rheumatic disorder. Clinicians should pay close attention to ANA Patterns, especially DFS staining when using ANA testing.

Table 1: Association of Positive ANA-DFS patterns with various disease conditions.

ANA DFS Pattern and Titer	Neurological (arthralgia, myalgia, back pain, neuropathy, migraine, headache)	Respiratory (asthma, COPD, pulmonary HTN, CF, cough)	Digestive (Crohn's, NAFLD, obesity)	Circulatory (Raynaud's, HTN)	Musculo/ Autoimmune (SLE, myositis, urticaria, eczema)	Others (fatigue, fibrocystic breast disease, donor, transplant, endometriosis, esophagitis, anxiety)
1:40	67	8	4	3	2	0
1:80	134	18	6	5	5	9
1:160	27	6	5	0	3	2
1:320	68	8	4	4	1	3
1:640	13	2	0	0	1	1
1;1280	5	0	1	0	1	1
Total	314	42	20	12	13	16
% Association	75.30	10.07	4.80	2.88	3.12	3.84

B-133

Beckman Coulter's DxC 500 AU Clinical Chemistry Analyzer, assessed by Six Sigma metrics, meets and exceeds the demands of CLIA 2024 performance specifications

T. Rice[1], M. Cavalleri[2], S. Frost[2], S. O'Mahony[2], S. Medina[3], S. Westgard[4]. *[1]Beckman Coulter Ireland, O'Callaghans Mills, Ireland, [2]Beckman Coulter Ireland, O'Callaghan's Mills, Ireland, [3]Beckman Coukter, Brea, CA, [4]Westgard QC, Madison, WI*

Background: The analytical Sigma-metric is a quality assessment tool, derived from the well-known Six Sigma management approach, and has been employed in Clinical Chemistry Assay performance assessments for over two decades. The analytical Sigma-metric, for an analyte or test in the clinical laboratory, is calculated using three

variables that are routinely available; imprecision (CV), inaccuracy (bias) and total allowable error (TEa), all expressed as percentages or all in units. A range of assays were assessed for Sigma-metric performance including ISE chemistries for urine and serum, IgG serum and CSF, CRP serum and Glucose serum, urine and CSF, on the Beckman Coulter DxC 500 AU. The Beckman Coulter DxC 500 AU clinical chemistry analyzer* is the latest system from Beckman Coulter. It is a fully automated, random-access analyzer, designed for low to medium volume laboratories, with a throughput of 800 tests/hour including ISEs.

Methods: To assess the 6-sigma performance of the assays on the DxC 500 AU, the standard analytical equation was used; Sigma metric = (TEa-|Bias|)/CV. Precision data was generated following the CLSI EP05 guideline, utilizing the 20-day protocol with test samples at various levels tested twice a day with a minimum of 2 hours between runs. Relative bias was determined through a method comparison study tested, following the CLSI EP09-A3 guideline, between the DxC 500 AU and the DxC 700 AU, and evaluated a minimum of 100 samples completed over a minimum of 3 days. Bias was calculated at various levels across the dynamic range utilizing both Passing-Bablok and Deming regression models. The 6-sigma performance was assessed against total error (TE) goals from various sources including the new CLIA 2024 performance criteria for serum, Royal College of Pathologists of Austral-Asia (RCPA) ALP for urine assays, and in one case, the Wisconsin State Lab of Hygiene (WSLH) goal for a CSF analyte.

Results: Urine applications for ISEs and Glucose all demonstrated > 6-sigma performance. The glucose CSF demonstrated >5-sigma performance while the IgG CSF assay demonstrated > 3-sigma performance. For the serum applications, all assays demonstrated > 4-sigma performance, with CRP, glucose and chloride demonstrating > 6-Sigma, IgG > 4-sigma and potassium and sodium > 5-sigma. Through the use of Westgard Sigma Rules, this indicates that most assays do not require extensive multi-rule monitoring, but instead can rely on fewer rules with wider control limits.

Conclusion: Excellent performance has been demonstrated for the assays evaluated, with all urine applications performing at >6-sigma and serum assay at ≥ 4-sigma, and CSF assays performing at ≥ 3-sigma. Sigma-metric analysis confirms the instrument will meet or exceed adherence to the coming, tighter CLIA 2024 performance specifications. Further, QC for these assays can be optimized to reduce the number of rules, levels, and runs implemented for routine monitoring. The resulting optimized process enables the laboratory to produce quality results efficiently with increased confidence for proper test interpretation.

*Product In development. Pending clearance by the United States Food and Drug Administration and achievement of CE compliance. Not currently available for *in vitro* diagnostic use.

B-134

Artificial Intelligence (AI)-Driven Clinical Decision Support: Potential to Predict the Risk for Multiple Sclerosis

R. Gopalan. *Siemens Healthineers, Tarrytown, NY*

Background: Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by inflammation, demyelination, gliosis, and neuronal loss. Globally, over 2.8 million people have MS, and 300 people receive a diagnosis of MS every day. It is 3 times more common in females than males. Autoimmunity, environmental factors, Epstein-Barr virus, and genetics are implicated in the etiology of MS. The pathophysiology of MS is limited to the central nervous system, resulting in focal inflammation of the blood-brain barrier and neurodegeneration of the axons, neurons, and synapse. Diagnosis is made based on clinical history, physical examination, imaging, and cerebrospinal fluid (CSF) studies. Early diagnosis is key to controlling the progression of the disease using disease-modifying therapies. **Methods:** We trained machine learning models with over 3000 sets of patient data with and without MS. The data was sourced from MIMIC-IV, a hospital-wide electronic health record (EHR) from Beth Israel Deaconess Medical Center, Boston, MA. A random forest model with 300 trees with a maximal depth of 30 layers and gain ratio was used as the criterion for selecting the attributes for splitting. The model was tested with 10-fold cross-validation. This model produced optimal performance. The input parameters consisted of age, gender, and the results of routine blood markers, such as complete blood counts, differential counts, comprehensive metabolic panels, and lipid panels recorded up to 3 years before the diagnosis of MS was identified. An evaluation of the performance was conducted using the area under the receiver operating characteristic curve (AUC). **Results:** We were able to show that the model could predict the risk for MS, with an AUC of 0.98 and accuracy of 0.92. This gave the model 0.91 sensitivity, 0.92 specificity, 0.92 positive predictive value, and 0.91 negative predictive value. Neutrophils, lymphocytes, monocytes, eosinophils, red blood cell counts, hemoglobin, and hematocrit seemed predominantly to contribute to the identification of risk for MS. Prediction accuracy was consistent up to 3 years prior to diagnosis. **Conclusion:** Studies have shown that five types of immune cells (plasma cells, monocytes, macrophage M2, neutrophils, and eosinophils) in cerebrospinal fluid (CSF) were significantly altered in MS cases compared to the control group. Brain shrinkage in patients with MS may be linked to hemoglobin protein in the blood leaked through the blood-brain barrier. Albumin, the most abundant protein in plasma, gains access to CNS tissue, where it is exposed to an inflammatory milieu and tissue damage, e.g., demyelination. Our model identified the pattern of the combined values of these markers, contributing to the prediction. Thus AI/ML-based prediction models may be able to help identify the risk for MS years before neurological symptoms appear. This may help to prompt close monitoring of these patients for periodic neurological and cognitive exams as soon as the first symptoms appear. Early confirmation of the diagnosis with imaging and CSF studies may ensure prompt consideration for disease-modifying therapies.

B-135

Research trends and hotspots of glial fibrillary acidic protein in body fluid for Alzheimer's disease: A bibliometric analysis

Y. Zou, L. Qiu. *Peking Union Medical College Hospital, Beijing, China*

Background:

As the most common form of dementia, Alzheimer's disease (AD) is prevalent worldwide. An increasing number of studies have focused on glial fibrillary acidic protein (GFAP), an astrocytic cytoskeletal protein, for the development, diagnosis and prognosis of AD. Therefore, this study aimed to analyze trends and hotspots of GFAP in body fluid for AD by using a bibliometric method, which is missing in current publications.

Methods:

We systematically searched the Web of Science Core Collection for published papers on GFAP in body fluid and AD. All articles and reviews from inception to December 31, 2022, were included in this study. Full records and cited references of all publications were derived, imported into Microsoft Excel, and analyzed by BIBLIOMETRC Online analysis platform and VOSviewer software.

Results:

A total of 2,269 publications including 2,166 articles were ultimately included based on the inclusion criteria. The data showed that the number of published papers on GFAP in body fluid and AD is annually increasing, which was published in 81 countries/regions and 527 academic journals. Separately, the top 3 prolific countries/regions and institutions were USA, China and England, Univ Gothenburg (Sweden), Univ Fed Rio Grande do Sul (Brasilia) and UCL Queen Square Institute of Neurology (England). Journal of Alzheimer's Disease, Brain Research, and Neuroscience contributed the most publications; Zetterberg, Henrik from Univ Gothenburg, Blennow, Kaj from Univ Gothenburg and Verkhratsky, Alexei from the University of Manchester were the top 3 prolific and cited authors. The most published and linked meaningful keywords included oxidative stress, inflammation, microglia, hippocampus and amyloid, which also annually increased in recent publications.

Conclusion:

Based on a bibliometric analysis, the number of publications on GFAP in body fluid and AD are rapidly increasing, especially in the past several years. Oxidative stress and inflammation are research hotspots, and the diagnostic performance of GFAP in body fluid need to be further summarized and verified.

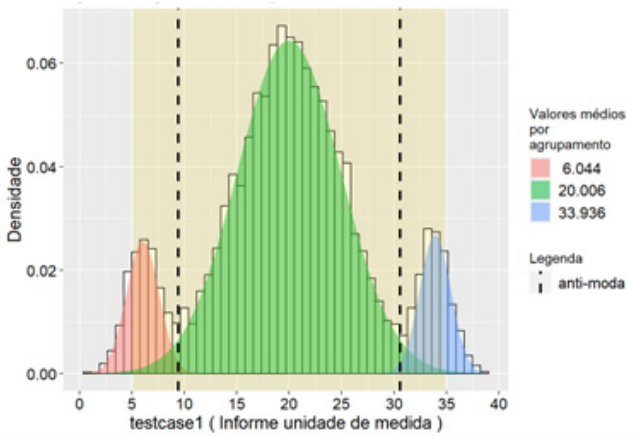
B-136

Indirect estimation and verification tool for reference intervals using unsupervised machine learning

L. M. VIEIRA[1], A. C. Dias[2], D. d. Oliveira[3], C. M. Dias[4], T. Almeida[5], J. R. Campos[6]. *[1]Dasa, Belo Horizonte, Brazil, [2]Sabin, Brasilia, Brazil, [3]Promed, Panama, Panama, [4]Dasa, São Paulo, Brazil, [5]Einstein, São Paulo, Brazil, [6]Lustosa, Belo Horizonte, Brazil*

Background: Determining the Reference Intervals (RI) of quantitative laboratory tests is crucial. This aspect is also part of the accreditation process, which can be challenging. The goal of our study was to create and validate a model using the R programming language that can estimate and verify Reference Intervals using an indirect method by mining data from the Laboratory Information System. **Methods:** The new model integrates algorithms that carry out preliminary processing, grouping, and statistical reduction of results. The algorithm identifies unusual outcomes by analyzing asymmetry and kurtosis and applying the Expectation-Maximization (EM) algorithm. During the grouping and reduction phases, the EM algorithm computes

the mean, standard deviation, and weight of the distribution for each group. Additionally, the model integrates algorithms that determine the global mean/median, modes, antimodes, kurtosis, and skewness, to find values that best represent the reference population's distribution. The distribution is then truncated to the defined IR partition through the combination of algorithms (Figure). The Bias Ratio was used to evaluate the difference between the RI determined by our model and those obtained from the Kosmic and Refine R models. The model's validity was tested using simulated and real data from anonymous reference individuals.**Figure.** Histogram: clustering, estimation of antimodes and truncation. **Results:** The effectiveness of the model was evaluated using was assessed for each test under varying levels of contamination. The overall performance showed excellent results with an accuracy of 0.996, kappa of 0.882, sensitivity of 0.945, specificity of 0.979, positive predictive value of 0.864, negative predictive value of 0.992, and F1-score of 0.896. **Conclusions:** The indirect estimation and verification tool for Reference Intervals is a cost-free and valuable resource for clinical laboratories. It supports the accurate interpretation of patient´s results.



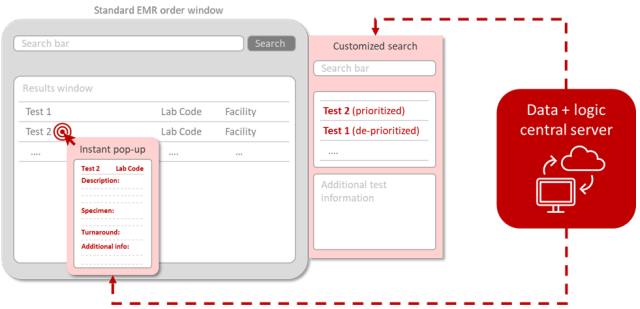
B-137

Development of a Novel Software System for Providing Real-Time Laboratory Test Information to Clinicians at Point of Order Entry

C. S. Douglas, M. Zaydman. *Washington University School of Medicine in St. Louis, St. Louis, MO*

Background: Clinicians depend on an increasingly complex set of laboratory tests to aid in the diagnosis and monitoring of patients. Staying informed about the proper usage of these tests requires active effort, especially as test offerings continually evolve. To aid in test decision making, institutions often implement some form of decision support, including: grouping of common 'order sets', 'best practice alert' popups, and web-based 'lab catalogs'. For many institutions, lab catalogs provide the most comprehensive information on available tests, but typically exist separate from the interface used to place test orders, and thus suffer from underutilization in actual clinical practice.

Methods: We developed a novel, open-source software to closely integrate our institution's lab catalog at the point of order entry within our electronic medical record (EMR) system. The software provides an immediate pop-up window anytime a clinician needs additional information while placing orders. The software also provides an independent, customizable search function that helps steer clinicians away from commonly mis-ordered tests. Both functions are immediately available at the point of order entry, without requiring the user to leave the EMR or otherwise interrupt their normal workflow. We conducted a small usability pilot with a focus group of clinicians, using the System Usability Scale (SUS).[1]



Results: Our software reduced the number of clicks and keystrokes needed to access detailed lab information from 27 to 2. The focus group (n=6) rated the product favorably on the SUS, with average rating 89±9 out of 100.

Conclusion: This study demonstrates proof-of-concept for a novel software decision support tool that provides instant access to lab information at the point of order entry. Future work will focus on evaluating utility in a real-world clinical pilot.

[1] Brooke, J. (1996). SUS-A quick and dirty usability scale. *Usability evaluation in industry*, *189*(194), 4-7.

B-138

Skewed demographics of patient population contributes to selection biases in clinical study population

S. Vettleson-Trutza, Y. Yang, M. Snyder, D. Jazdzewski. *Mayo Clinic, Rochester, MN*

Background: The racial, ethnic and sex compositions of a study population may impact the clinical performance of a biomarker. In a prospective clinical study to evaluate biomarkers for asthmatic and allergic conditions in a large medical center in the upper Midwest, a largely White-alone patient population was encountered. Using several outreach strategies, potential eligible patients were contacted to enroll in a biomarker clinical study and to provide blood specimens. The patient demographics between the eligible candidates and the enrolled participants were compared. The analysis was carried out to understand the likelihood of enrollment in different race, ethnicity, and sex groups; and to identify factors that contribute to selection biases in enrolled cohorts. **Methods:** Patients with eligible asthma and allergic conditions were selected based on relevant diagnostic codes in their electronic medical records, verified by clinical research coordinator. In accordance with Institutional Review Board approval, eligible patients were contacted via their electronic healthcare portal or with a phone call. The study information was also posted in an internal classified ad as an additional outreach strategy. Interested participants were then contacted a second time to complete consent. Upon consent, participants were considered as "enrolled". Demographics information including sex, race, ethnicity, and age were de-identified for comparison analysis. **Results:** Among all eligible patients, a disproportionally low representation of Blacks, Asians, and Hispanics ("non-White" groups) was observed, relative to regional demographics (22.2% non-White groups, 2021 census) and higher asthma and allergy prevalence in the non-White groups. In eligible adult patients (>18 years old) with asthma, only 13.4% were from non-White racial and ethnic groups. In eligible pediatric asthma patients, a larger proportion at 24.1% were from American Indian, Asian, Black, or Hispanic groups. In patients with allergic conditions, 10.3% of the adult patients were from non-White only groups, compared to 21.9% in pediatric patients. Among all eligible patients, White-alone patients were more likely to enroll in the study compared to their counterparts from non-White groups, further skewing the racial and ethnic distribution in the enrolled cohorts. As a result, less than 10% of enrolled adult patients were from non-White background in both allergy and asthma cohorts. Among the enrolled pediatric asthma and allergy patients, 16.9% and 10.3% respectively were from non-White groups. We also identified sex and age dependent enrollment behavior across different study cohorts. In adult groups, there were more female eligible patients (allergy: 66.7% female; asthma: 66.7% female) than male patients. Female adult patients were significantly more likely to participate, thus constituting 79.6% and 75.8% of the enrolled allergy and asthma cohorts. In the pediatric groups, male patients represented a larger share of eligible patients (allergy: 58.6%; asthma 64.7%). Female pediatric patients were still more likely to participate in the study. Hence, male and female enrolled patients were closer to equal distribution (allergy: 50.0% male; asthma 59.7% male). **Conclusion:** The non-even distribution of demographics in eligible patients contributed to biased distribution in enrolled patients. Existing outreach approaches were not effective in reversing enrollment rates in underrepresented racial and ethnic groups.

B-141

Surprisingly good agreement of Siemens and Roche ALT Reference Change Values in Ottawa Hospital outpatients distilled from 5 years and 3 years of Siemens and Roche ALT, respectively

G. Cembrowski[1], J. Qiu[1], C. McCudden[2]. [1]*University of Alberta, Edmonton, AB, Canada,* [2]*Ottawa Hospital, Ottawa, ON, Canada*

Background: Sequential patient data can be transformed into the variable component of the reference change value (RCV) calculation: $(CV_A^2 + CV_I^2)^{1/2}$ (PMID 35137000). We have analyzed sequential healthy and abnormal outpatient ALT to derive RCVs of Ottawa Hospital outpatients (OHOP) who had sequential ALT measured by Siemens or Roche assays.

Methods: We used the American College of Gastroenterology definition of normal ALT with supplementation of P5P as <25U/L and <33U/L in females and males, respectively. We defined abnormal as between the upper limit of normal (ULN) and 3xULN. 5 years of Siemens ALT measurements and subsequently 3 years of Roche ALT were abstracted from the Ottawa Hospital EMR. For the 8 patient cohorts, we tabulated consecutive pairs of intrapatient ALT by time intervals of separation: 0-1 weeks, 1-2 weeks, 2-3 weeks . . ., up to 51-52 weeks. For each interval, we determined the standard deviation of duplicates (SDD) between the paired intrapatient results. SDD was graphed against the midpoints of the weekly interval. Linear regression was used to determine the y-intercept (yo). The 95% RCVs of the male and female, normal and abnormal populations were calculated for the Siemens (with P5P) and Roche (non P5P) assays.

Results: The Table describes each cohort, yo and RCV. According to the Roche/Siemens cross-over study, Roche ALT values were 10% less than Siemens. This Roche bias is reflected in the normal outpatient ALT means and less so in those with elevated ALT. Also presented are the RCV of low ALT (normal) patients as documented by Ricos and Carobene.

Conclusion: Our RCVs are realistic: normal ALT patients demonstrate lower RCVs, close to Ricos' but much larger than Carobene's which are unachievable due to diurnal variation (over 40% of OHOP ALT are drawn at different times of the day). Furthermore, Carobene's CV_A is tiny.

Sex	ALT, U/L	ALT Assay	n patients	yo	StdErr yo	Mean ALT,U/L	100yo/Mean	RCV,%
Female	ALT<25	Roche	9844	3.25	0.043	15.0	21.7	60
Female	ALT<25	Siemens	7962	3.53	0.051	18.8	18.8	52
Male	ALT<33	Roche	9509	4.29	0.056	18.4	23.3	65
Male	ALT<33	Siemens	11082	4.91	0.059	22.2	22.1	61
	Ricos Normal (2004)						26.0	72
	Carobene Normal (M&F, 2022)					22.3	11.5	32
Female	25<ALT<75	Roche	3242	10.34	0.16	38.7	26.7	74
Female	25<ALT<75	Siemens	11308	10.28	0.09	39.4	26.1	72
Male	33<ALT<100	Roche	2786	15.49	0.24	50.6	30.6	85
Male	33<ALT<100	Siemens	5459	15.5	0.22	51.4	30.2	84
	Ricos Liver Disease, 2007							<75

B-142

Development and clinical validation of artificial intelligence assisted flow cytometry diagnosis for acuteleukemia

Z. Lu[1], Y. E. Lyu[2], A. Gao[2], T. S. Yeager[2], M. Morita[2], S. Wang[3], A. Wang[3], G. Fan[2]. [1]*Oregon Health & Science Univeristy, Portland, OR,* [2]*Oregon Health & Science University, Portland, OR,* [3]*DeepCyto, LLC, Portland, OR*

Background:

Flow cytometry is one of the most commonly used diagnostic tools in hematopathology. Manual flow cytometry data analysis is vulnerable to substantial inter-operator variation and typically takes a technologist 20 minutes to analyze a case. In the last decade, artificial intelligence (AI) technology has advanced dramatically and become widely used in image processing. Previously, we created an AI-assisted workflow for classifying white blood cell subsets in peripheral blood. The goal of this study is to evaluate the automated flow cytometry analysis system for diagnosing acute leukemia.

Methods:

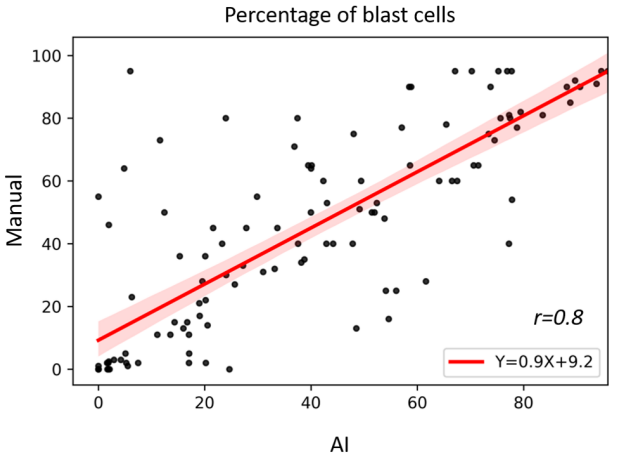
A total of 117 cases were retrieved from the archived diagnostic cases, consisting of three diagnostic categories: 20 normal cases, 37 acute lymphoblastic leukemia and 60 acute myeloid leukemia. Flow cytometry data manually analyzed by a pathologist is the gold standard. The same flow cytometry data was processed by the flow cytometry analysis software DeepFlow® using proprietary AI algorithm. The final diagnosis and blast cell count of AI and manual analysis are compared by Pearson correlation coefficient.

Results:

The AI-assisted blast cell enumeration showed moderate correlation as compared to manual evaluation (r =0.8, Fig 1). Manual adjustment for enumeration of CD34 negative blast population with uncommon immunophenotype is required for 7 cases, including 4 CD34-/CD117+ acute myeloid leukemia and 3 CD34-/TDT+ lymphoblastic leukemia. The AI analysis effectively recognized cell populations and provided correct diagnoses on 94% of cases (110/117). With pathologists' review, all cases were classified into the correct diagnostic categories.

Conclusion:

Our study demonstrated that AI can accurately recognize the flow cytometry pattern of leukemia and make the proper diagnosis. Additional training for CD34 negative blast gating and other uncommon blast immunophenotypes is being conducted. Ultimately, AI will decrease the demand on manual analysis by technologists and pathologists, standardize the analysis process, and provide accurate diagnosis.



B-143

Distribution of uricemia levels in the population of a third-level Hospital. Study of the prevalence of hypouricemia.

A. I. Corominas[1], G. H. Casal[1], F. M. Vommaro[1], G. B. Riquelme[1], S. M. Balconi[1], A. E. Damiano[2]. [1]*Hospital Nacional Prof A Posadas, Buenos Aires, Argentina,* [2]*Laboratorio de Biología de la Reproducción, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO)- CONICET, Buenos Aires, Argentina*

Hypouricemia is a serum uric acid concentration ≤2.0 mg/dl. The causes may be congenital or acquired. Despite not being recognized as a relevant biomarker, the knowledge of the importance of uric acid in redox homeostasis and the hierarchization of hyperuricemia as an independent risk factor in metabolic diseases justifies a reassessment of the significance of the state of acquired hypouricemia. We aimed to study the prevalence of hypouricemia and its relationship with the referred patients' ages, genders, and morbidities. A descriptive, quantitative study was carried out, through the survey and analysis of biochemical records and clinical records. We worked with the database of the laboratory's system between January and October 2019, with the data groups of adult patients (≥16 years) that included serum uric acid dosage. The concomitant biochemical parameters (Urea, creatinine, white blood cell count, glucose) were evaluated, and the diagnoses and treatments were collected in the electronic medical record. To define the prevalence, we worked with the first analysis group of each patient. All statistical analyzes were carried out with the Statistix™ software version 7. The criteria for statistical significance were $P < 0.05$. We found that of the total 159,971 data groups from January to October 2019, 17,983 were selected. There were 234 patients with hypouricemia analyzed. The prevalence of patients with hypouricemia was 2.22%. In our population, postmenopausal women did not match the distribution of uricemia levels to that of men, nor did the mean values of each distribution. Men had higher levels of uric acid than women in any age situation. The prevalence of hypouricemia agrees with what is described in the literature. Of women with hypouricemia, 39% were in the process of pregnancy. Besides that, most patients had a pathology, less than 10% were present for health control, or did not have data in the clinical record. The prevalence of oncological pathology (46%) as a comorbidity in patients with hypouricemia (oncohaematological tumors more frequent than solid ones) and diabetes (22%) was striking. Hypouricemia values were more frequent in outpatients (70%) than in hospitalized patients, however, the last ones showed lower values (1.46±0.4 mg/dl vs 1.52±0.35 mg/dl). The white blood

cell count values (0.1-26*103cells/ul) and glucose (0.3-3.76 g/l) that accompany the hypouricemia situation behold pathological values, and the urea and creatinine values were normal. Because this is a retrospective work, we do not have studies of fractional excretion of uric acid, which could delve into the causal research of hypouricemia. Further studies are needed to elucidate the causes of hypouricemia and to clarify its value in diagnosing and treating diseases.

B-144

A Comparative Analysis of Unsupervised Machine Learning Algorithms for Polyploidy Analysis Using Flow Cytometry

S. Lee, K. Kim, N. Kim, J. Park, J. Lee, Y. Cho, D. Kim. *Jeonbuk National University Medical School and Hospital, Jeonju, Korea, Republic of*

Background: Recently, there has been a growing interest in using cell differentiation inducers to produce platelets in vitro by inducing megakaryocytic differentiation in cell lines. During this process, cells undergo polyploidization through endomitosis. To measure the degree of polyploidy, cell cycle analysis by flow cytometry is commonly used. However, manual gating can be time-consuming and prone to human error, especially when analyzing large datasets. To address this issue, this study aimed to compare the performance of unsupervised machine learning algorithms for polyploidy analysis of flow cytometry data.

Methods: The K562 cell line (Korea Cell Line Bank, Korea) was cultured under four conditions, including 1 nM and 2.5 nM of PMA (phorbol 12-myristate 13-acetate; Sigma Aldrich, USA) and 5 μg/mL of phytosphingosine (TCI, Japan), for four days. Propidium iodide (Sigma Aldrich, USA) was used to stain the cells for ploidy analysis, and flow cytometry (Navios, Beckman Coulter Inc, USA) was used to obtain the intensity values. Twelve flow cytometry measurements were obtained for K562 cells cultured under four different conditions, with each condition tested in triplicate. Unsupervised machine learning algorithms, including K-Means, Bisecting K-Means (BKM), Mini Batch K-Means (MBKM), Agglomerative Clustering (AC), Gaussian Mixture Models (GMM), K-Medoids, and Partitioning Around Medoids (PAM), were used to generate boundaries among three clusters (2n, 4n, and ≥8n). The boundary values generated by each algorithm were comparatively analyzed by calculating the standard derivation index (SDI) on each boundary. The concordance of each algorithm with manual gating of flow cytometry data was evaluated by calculating residual errors.

Results: A total of 168 SDI values were obtained from seven different unsupervised machine learning algorithms for two boundaries among 2n, 4n, and ≥8n clusters of four culture conditions in triplicate. When comparing the average (±95% confidence interval) of SDI for each algorithm for the entire dataset, K-Means showed the most acceptable average SDI value of -0.07 (±0.17) with the narrowest confidence interval followed by -0.13 (±0.54) of AC, -0.26 (±0.18) of MBKM, -0.53 (±0.2) of K-Medoids, -0.58 (±0.2) of PAM, 0.74 (±0.3) of GMM, and 0.84 (±0.35) of BKM. In addition, the average (±95% confidence interval) of the absolute value of the residual errors of each algorithm was calculated, with K-Means showing the lowest value of 6.67 (±2.3), indicating higher concordance with manual gating. AC, MBKM, K-Medoids, PAM, BKM, and GMM had higher average residual errors of 10.04 (±2.5), 10.46 (±4.07), 11.13 (±4.65), 12.04 (±4.83), 12.54 (±3.0), and 12.75 (±3.5), respectively.

Conclusion: K-means demonstrated the best performance for clustering each ploidy among the seven unsupervised machine learning algorithms tested for the flow cytometry data. In addition, the algorithms achieved acceptable concordance with manual gating in most cases in this study. Therefore, unsupervised machine learning algorithms show promise in automating the gating of flow cytometry data for polyploidy analysis and measuring megakaryocytic differentiation in cell lines.

B-145

An R Shiny App for Automated Peak Deconvolution, Interpretation, and Quantitation of Monoclonal Proteins Using Capillary Electrophoresis Immunotyping Data

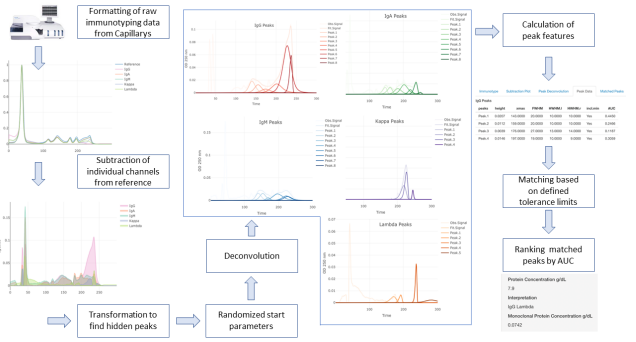
S. Cotten. *UNC, Chapel Hill, NC*

Background: Machine learning methods have been utilized to train algorithms for interpretation of immunofixation electrophoresis. This work developed an R Shiny app for immunotyping (IT) data capable of deconvoluting discrete peaks in overlapping backgrounds and automatic quantitation of monoclonal proteins. Unlike machine learning which obfuscates decision making, each step of this deconvolution and quantitation process is displayed to the end user.

Methods: Individual traces for 1315 previously interpreted IT results were used in the study. A modified Levenberg-Marquardt algorithm using 50 sets of randomized start parameters was used for deconvolution. Primitive peak features such as height, FWHM, AUC and retention time were used to match heavy and light chain deconvoluted peaks for interpretation. The matched peak with the largest AUC was compared to reviewer interpretation and peak quantitation. Repeatability of quantitation was evaluated through 10 consecutive rounds of deconvolution. Code was written for end user visualization in Shiny or batch processing in base R.

Results: Deconvolution was successful for 97.2% (1279/1315) of specimens. Agreement between the software and reviewer interpretation was 90.6% (11,927/13,150) across all deconvolutions and was consistent for 84.7% of samples in all 10 rounds of deconvolution. Discordant interpretation in 15.3% of samples was often attributed to splitting of large peaks with sharp maximums, large percentage (but small absolute) changes in calculated AUC for monoclonal proteins under 0.5 g/dL, or drifting baseline signal between channels. Mean and median percent CV for consecutive rounds of monoclonal quantitation was 5.9% and 1.16% respectively (protein range 0.02-4.5 g/dL). Deming regression of reviewer vs. software quantitation had a calculated slope of 0.728 and Pearson's r coefficient of 0.933.

Conclusion: Deconvolution and automatic quantitation of monoclonal protein peaks is possible using the Levenberg-Marquardt algorithm for immunotyping data. Future work aims to improve interpretation agreement with reviewers using more advanced ranking of matched peaks.



B-146

Discriminative Index: A Novel Indicator for Evaluating Machine Learning Algorithms in Laboratory Medicine

J. Yu[1], W. Lin[2], W. Lin[3], Y. Wang[4], Y. Tseng[5], H. Wang[3]. *[1]Chang Gung Memorial Hospital, Taoyuan City, Taiwan, [2]Syu Kang Sport Clinic, Taipei City, Taiwan, [3]Department of Laboratory Medicine, Chang Gung Memorial Hospital, Taoyuan City, Taiwan, [4]Department of Medicine, Harvard Medical School, Boston, MA, [5]Department of Computer Science, National Yang Ming Chiao Tung University, Hsinchu City, Taiwan*

Background:

In clinical scenarios, incorrect predictions by machine learning (ML) models are inevitable. One way to reduce misleading predictions is to avoid reporting predictions that fall within a predefined "gray zone". This method improves predictive performance by reporting only the less uncertain cases. However, the cost-effectiveness of applying the "gray zone" rule in an ML model is unclear without massive computation. Thus, this study aims to propose a novel metric to evaluate the effectiveness of using gray zones and validate the metric in real-world ML models.

Methods:

This study defined a statistical metric called the "discriminative index" (D-index) for evaluating the effectiveness of gray zones. To calculate the D-index, the predictive outcomes of the ML model are first transformed into two probability distributions based on the truth labels (e.g., positive or negative). The D-index is then derived from the kurtosis of these two distributions. To validate the metric, we applied the D-index to three different antibiotic susceptibility-predicting ML models (namely, convolutional neural network (CNN), random forest (RF), and XGBoost (XGB)) based on mass spectrometry data. We assessed the performance and unpredicted case numbers of each model with different gray zones and correlated the results with the proposed D-index.

Results:

The D-index values for the CNN, XGB, and RF models were 4.36, 0.38, and -1.66, respectively. When applying the "gray zone" rule to achieve 90% area under the re-

ceiver operating characteristic, the CNN, XGB and RF models retained up to 90%, 68%, and 62% of total cases, respectively. A higher D-index value indicates a more effective application of the gray zone rule.

Conclusion:

The D-index is a simple and statistically insightful metric for evaluating the cost-effectiveness of applying gray zone rule in an ML model. This metric has been validated in three mass spectrometry-based predictive models and has shown promising results. The D-index can be a useful tool for comparing and applying different ML algorithms.

B-147

Evaluation of laboratory database: is there a causal relationship between COVID-19 and type 1 diabetes

M. C. Castelo[1], M. Cruz[2], S. P. Bandeira[1], A. C. Lopes[3], L. L. Cavalcante[1]. *[1]DASA, Fortaleza, Brazil, [2]DASA, Salvador, Brazil, [3]DASA, Florianópolis, Brazil*

Background:Type 1 diabetes mellitus (T1DM) constitutes about 5% of all diagnosed cases of diabetes. The global incidence is increasing over the past few years. To date, the pathophysiology of the disease is not fully understood. with descriptions of activation of the immune system by some viral agents. SARS-CoV-2 has been suggested as a potential inducer of new-onset type 1 diabetes mellitus (T1DM). DASA has one of the largestData and Analytics areas in Diagnostic Medicine. The use of laboratory data can represent an important tool in broader knowledge of this relationship. This study aimed to evaluate the frequency of new-onset T1DM in the years 2019, 2020 and 2021. **Methods**: This study was done through an observational, retrospective big data laboratory approach with a network that extends over a large part of Brazil to analyze whether there was an increase in T1DM cases. Individuals aged 18 years or younger were evaluated from January 2019 to December 2021. The subjects were characterized with possible new-onset T1DM through the presence of glycated hemoglobin equal to or greater than 6.5% in the presence of at least one disease-related antibody. The antibodies evaluated were: anti-GAD, anti-islet, anti-insulin and anti-zinc transporter type 8. For data evaluation, the means and standard deviations and the chi-square test were used. The results of patients who underwent RT PCR COVID19 were evaluated. **Results**:A total of 431060 subjects (<18 years) were evaluated, with 229201 female (53.17%). Table 1 shows the distribution of data over the years evaluated, the number of patients with glycated levels greater than 6.5% and the number of probable new cases of DM1 from the positivity of at least 1 antibody. In the years evaluated, there was no statistical difference in the incidence of the disease between the sexes (2019 - 51.2%; p:0.92, 2020 - 50.3%; p:0.99 and 2021 - 48.9%; p: 0.67). The mean age with standard deviation in the years 2019, 2020 and 2021, respectively, was: 12.4%±4.4; 12.6±4.3 and 12.5%±4.3. The mean values of glycated hemoglobin of patients with a value ≥ 6.5% with standard deviation in the years 2019, 2020 and 2021, respectively, were: 9.3%±2.1; 9.2±2.5 and 9.3%±3.4. When comparing the incidence of the disease between the years, there was a significant increase in cases when comparing the years 2021 with 2020 and 2019 (p: 0.01 and p<0.01, respectively). Of the total number of patients in 2020, only 5 underwent RT PCR COVID 19, with no positive result, and in 2021 a total of 32, with 7 positive, corresponding to 21.8% of cases. **Conclusion**: The present study, using a large database, seems to demonstrate an increase in the incidence of DM1 among children diagnosed with diabetes. Prospective studies that evaluate the possible relationship of the disease with the SARS-CoV-2 virus may show this agent as a trigger of disease-related autoimunity.

B-148

Analytical Validation and Performance Characteristics of A Targeted Metagenomics Next-generation Sequencing Respiratory Pathogen ID/AMR Panel

A. Vest, H. Houdeshell, E. Arnold, I. Harrison, M. Hardison, C. Clark. *Aegis Sciences Corporation, Nashville, TN*

Background: The COVID-19 global pandemic underscored the importance of access to diagnostic testing, broad community surveillance, and genomic analysis of pathogen variants. The dearth of testing in the initial days and months of the pandemic highlighted the need to improve public health infrastructure. The subsequent onset of viral evolution, producing new and functionally different CoV2 strains, increased the importance for the availability and scalability of pathogen genome sequencing and data sharing pipelines through national surveillance programs. As SARS-CoV-2 moves from a pandemic to endemic state, the scientific and medical community must not forget the lessons learned and continue to reinforce the value of ongoing monitoring of circulating pathogens.Methods: Aegis Sciences performed an analytical validation of the Respiratory Pathogen ID/AMR Target Enrichment Panel (RPIP, Illumina RUO), a next-generation sequencing assay that uses target capture-based enrichment for detection, quantification, and characterization of 282 viral, bacterial, and fungal respiratory pathogens and over 2000 antimicrobial resistance (AMR) genes. A total of 1598 validation samples were sequenced via the NextSeq 550 Dx (Illumina) and demultiplexed and assembled FASTQ files were analyzed with Explify RPIP Analysis application in BaseSpace Sequencing Hub (Illumina). Samples tested were contrived specimens using 60 different pathogen isolates various concentrations and clinical comparative samples, and the study included the evaluation of concentration versus genome coverage, inclusivity, exclusivity, and orthogonal analyses. Results: RPIP is a highly sensitive, robust sequencing assay that can detect organisms at very low concentrations. Evaluation of copy number versus genome coverage revealed that 53 out of 60 organisms were detected at 1 copy/µL (PPV=86.67%), and 60 out of 60 were detected at 10 copies/µL (PPV=98.33%) and 50 copies/µL (PPV=96.67%). The average coverage across the genome improved with increasing concentration and was 59.97% at 1 copy/µL, 72.45% at 10 copies/µL, and >90% at 50 copies/µL. Inclusivity and exclusivity studies revealed an increase in false positives at low concentrations (1 copy/µL=35.11%) and a reduction of false positives at higher concentrations (10 copies/µL=20.3%) of isolates, and higher copy numbers of isolates allow for the true positives to be distinguished from contaminants and off-target organisms with very low coverage and quantification results. Orthogonal studies with upper respiratory clinical specimens tested previously by validated PCR methods revealed the following performance characteristics for respiratory pathogens, including SARS-CoV-2, influenza, parainfluenza, and rhinovirus: PPV=81.11%, NPV=99.66%, Sensitivity=98.46%, and Specificity=93.89%. Conclusions: Completion of these studies demonstrated that next-generation sequencing is a cost effective, high-throughput option for broad pathogen tracking and surveillance. Laboratories, like Aegis, must continue to leverage collaborative relationships with public and private sector entities to expand resources, foster innovation, and broaden capabilities to quickly respond to biological threats.

B-149

Next-generation Sequencing Via Targeted Metagenomics for Broad Antimicrobial Resistance Surveillance

H. Houdeshell, A. Vest, E. Arnold, X. Thacker, I. Harrison, C. Clark, M. Hardison. *Aegis Sciences Corporation, Nashville, TN*

Background: Antimicrobial-resistant infections secondary to the overuse of antibiotics are a major public health threat. The standard of care for identifying resistance is antimicrobial susceptibility testing, the phenotypic characterization of minimum inhibitory concentrations (mic) for antibiotics in the presence of bacterial growth, but these methods are labor intensive, have long turnaround times, and face challenges with non-cultivable bacteria. Molecular methods for antimicrobial resistance (AMR) ascertainment are high-throughput, robust, and sensitive and have gained interest as a genotypic complement to traditional phenotypic methods. Since the COVID-19 pandemic, next-generation sequencing has emerged as the leading technology for tracking and surveillance of infectious diseases and antimicrobial resistance, emphasizing the need for flexible sequencing panels that are easily interchangeable and capable of detecting pathogens and AMR genes. Methods: In a collaboration between Illumina, a national retail pharmacy, and Aegis Sciences Corporation, residual, self-collected nasal swab samples (n=4390) submitted for COVID-19 testing from patients across the U.S. were sequenced using the Respiratory Pathogen ID/AMR Panel (RPIP, Illumina – For Research Use Only), a target capture-based next-generation sequencing panel targeting 282 respiratory pathogens and 2097 relevant AMR markers. Sequencing data was analyzed using the Explify RPIP Analysis app (BaseSpace, Illumina) and paired with self-reported patient data submitted via an online questionnaire. Data including pathogens and anti-microbial resistance (AMR) genes detected, age, race, gender, residing state of the patient, collection month, self-reported symptoms, and health risks were collated, imported into Microsoft PowerBI software, and used to create interactive visuals for a surveillance dashboard. Results: In this cohort of sequenced specimens, 85.1% (n=3,576) had one or more viral, bacterial, or fungal pathogens present, 41% (n=1816) had one or more AMR genes present, and 38.4% (n=1,685) had one or more pathogens and AMR genes co-detected. Twenty-six different AMR genes were identified with the most prevalent being Erm 23S ribosomal RNA methyltransferase (65.5%; n=1115). Erm was identified in samples from all 50 US states and was co-detected with staphylococcus aureus (18.2%; n=216) and streptococcus pneumoniae (4.9%; n=57), among others. Following Erm in prevalence, this cohort of samples harbored genes for trimethoprim resistant dihydrofolate reductase (dfr=27.9%), tetracycline-resistant ribosomal protection protein (tet=21.0%), and macrolide phosphotransferase (MPH=14.2%). The data also revealed AMR gene-pathogen co-detections listed as CDC serious threats, such as the mecA gene responsible for methicillin resistance in staphylococcal species. The mecA gene was

co-detected in 5.3% (n=28) of staphylococcus aureus positive samples in this cohort. Finally, although not classified as a serious threat, neuraminidase inhibitor resistance, associated with less susceptibility to anti-virals, was found in 12% (n=15) of all influenza A (H3N2) infected samples (n=112). Conclusions: Completion of these studies demonstrated that next-generation sequencing using targeted metagenomics with RPIP is a cost effective, high-throughput option for large-scale surveillance of AMR genes in potential respiratory infections.

B-150

Impact of Clinical Catastrophic Events on Individual Reference Values for 20 Analytes: Results of a Six-Measurement Study.

T. F. de Almeida, I. F. Ferreira dos Santos Silva, T. Branchi Evans Salvia, D. Shoji Hayama, A. Carvalho da Silva, C. L. Pitangueira Mangueira. *Albert Einstein's Hospital, São Paulo, Brazil*

Reference values are an essential aspect of clinical laboratory testing, providing a basis for comparison of results against established norms for specific patient populations. These reference values are used to evaluate diagnostic test results, monitor disease progression, and assess treatment effectiveness. The accuracy and reliability of these values are crucial to the diagnosis and treatment of disease and illness and also important in the monitoring of disease progression and treatment effectiveness. By tracking changes in test results over time, healthcare providers can assess the effectiveness of treatments and make adjustments as needed. This information is critical in the management of chronic conditions such as diabetes and hypertension. Despite their importance, reference values can be subject to variability due to a variety of factors. These include differences in laboratory testing procedures, variations in patient populations, and differences in the methods used to establish reference ranges. To address these issues, clinical laboratories can use individual references calculated based on previous test results. Nevertheless, individual reference values can be subjected to pathophysiological alterations that can modify the values of specific analytes. The aim of this study was to investigate the impact of a clinical catastrophic event, specifically troponin levels above 1000, on the calculation of individual reference values for 21 analytes. Six measurements were taken before and after the event from a sample of 5 to 450 patients. The results showed that the mean and covariance coefficient of the majority of the analytes remained unchanged despite the presence of the clinical event. Applying a Wilcoxon test to the mean before and after the event 10 out of the 21 analytes differ significantly before and after the event. These analytes included glucose, BNP, chloride, gamma GT, hematocrit, hemoglobin, potassium, C-reactive protein and urea. The mean and covariance of the remaining 11 analytes, which included platelets, creatinine, alkaline phosphatase, magnesium, sodium, AST, ALT, TSH, fibrinogen, prothrombin time, and activated partial thromboplastin time (aPTT), were not significantly impacted by the clinical event. These results suggest that despite the presence of a clinical catastrophic event, individual reference values can still be calculated for the majority of the analytes. This is important for the accurate interpretation of clinical laboratory test results, especially in the presence of acute illnesses or injuries. However, caution should be exercised when interpreting results for the three analytes that were significantly impacted by the event. Further studies are needed to determine if similar results can be obtained for other clinical catastrophic events and analytes.

B-151

Estimation of the uric acid reference interval by the indirect method using an unsupervised machine learning tool and r programming language

D. G. Canali[1], A. C. Lopes[2], L. M. Vieira[3], G. Y. Garoli[4], C. d. Sabino[4], C. M. Dias[4], G. Zampieri[4], K. G. Muller[5]. *[1]Diagnóstico da America, São Jose, Brazil, [2]Diagnóstico da America, São José, Brazil, [3]Diagnóstico da America, Belo Horizonte, Brazil, [4]Diagnóstico da America, São Paulo, Brazil, [5]Diagnóstico da America, Joinville, Brazil*

Background: Determining the Reference Intervals (RI) of quantitative clinical laboratory tests is crucial for patient diagnosis, treatment, and monitoring. It is also a concern for laboratories seeking to meet accreditation requirements. Our study aimed to evaluate the manufacturer's RI for uric acid (3.7-9.2mg/dL) through indirect methods using a programming tool in R language in a Brazilian laboratory. **Methods:** We performed a data mining study for uric acid using the laboratory's LIS database from January to December 2021. Outpatients >18 years old, analyzed on the Siemens® platform with results within linearity were included. Exclusion criteria were total cholesterol >200mg/dL, triglycerides >150mg/dL, glycemia >126mg/dL, ferritin >300ng/mL and creatinine >1.5mg/dL at any time in the patient history and CRP >10mg/dL in the same month. We used a tool that integrates different algorithms including preliminary treating, grouping, and statistical reduction of test results. The Expectation-Maximization (EM) algorithm calculates the mean, standard deviation, and weight of the distribution for each cluster. Additionally, it integrates algorithms that determine the global mean/median, modes, antimodes, kurtosis, and skewness, to find values that best represent the reference population's distribution (Figure). **Figure.** Manufacturer's and estimated RI for Male and Female partitions. **Results:** The final database included 49.489 subjects, partitioned in 26.439 women and 23.050 men. Statistically estimated RI were 2.8-6.7 mg/dL for women and 3.8-8.6 mg/dL for men. Considering the RI proposed by the manufacturer, 23.8% of women were outside the RI provided by the manufacturer, all of them below the lower reference value. **Conclusions:** Our results confirm the importance of verification/establishment of reference intervals by clinical laboratories and suggest that estimated RI using this software is appropriate for the assisted population, demonstrating consistency with physicians' concerns. After specialist analysis, the manufacturer´s RI has been replaced by the calculated population-specific RI.

B-152

Estimation of creatinine reference interval (RI) by the indirect method using an unsupervised machine learning tool and R programming language

D. G. Canali[1], A. C. Lopes[2], L. M. Vieira[3], B. d. Santos[4], C. d. Sabino[4], C. M. Dias[4], G. Zampieri[4]. *[1]Diagnóstico da America, São Jose, Brazil, [2]Diagnóstico da America, São José, Brazil, [3]Diagnóstico da America, Belo Horizonte, Brazil, [4]Diagnóstico da America, São Paulo, Brazil*

Background: Creatinine is widely used in diagnostic medicine as a primary renal marker. Clinical laboratory measurement of serum creatinine is not standardized, and the RI varies slightly between different manufacturers. Roche brings as reference ranges 0.5-0.9mg/DL for women and 0.7-1.2mg/dL for men. After clinical manifestations of elevated female results without clinical correspondence, the laboratory proceeded with reevaluation of RI. **Methods**: We retrospectively studied the creatinine results of the laboratory database of samples run from January to October 2022 in two different technical areas in Brazil (São Paulo and Rio de Janeiro). The laboratory platform was Roche® 502/702, Jaffe method to creatinine. The LabRI tool was used with parametric, non-parametric, and robust statistical treatments to exclude outliers and algorithms in the R Language and latent abnormal values of outpatient patients. The partitions proposed were gender (male and female), age (18-60 years old and over 60 years old). The proposed RI for verification were those proposed by Rosenfeld, LG et al in REV BRAS EPIDEMIOL 2019; 22 (SUPPL2):E190002.SUPL.2 - F: 0.5-0.9 and M: 0.7-1.2. Exclusion criteria were patients with urea > 48.5mg/dL, glycemia > 200mg/dL, 24h proteinuria > 150 mg/24h, LDL > 130mg/dL, uric acid > 130mg/dL, anti-nucleus positive factor, as well as clinical data compatible with chronic renal failure or dialysis, hypertension and diabetes. **Results**: After exclusion, retrieved 225,181 individuals 52% female. Between the different sites, the results of the partitions did not undergo significant changes. For women in both age partitions, the values did not vary significantly. For men, there was no significant variation in the lower limit of IR, and we had a variation of 0.20 (18-60: 0.69-1.31 and > 60: 0.69-1.41) in the upper limit, mainly for the age partition > 60 years old, below the 95% confidence interval of the proposed references. **Conclusions**: The adoption of the Roche IR and the Brazilian article was approved for the female gender (0.5-0.9) with 118,100 individuals, and a new IR was defined for the male gender (0.7-1.2) with 107,081 adult individuals, including the elderly over 60 years old, for both genders.

B-153

Acceleration vs. time data for two pneumatic tube specimen transport routes: comparison of distributions of jerk (d(acceleration)/dt) and theta (change in angle)

C. J. Mattiello, D. F. Stickle. *Jefferson University Hospital, Philadelphia, PA*

Background: As part of a Quality Assurance investigation regarding specimen integrity, we had occasion to compare properties of pneumatic tube transport (PTT) of specimens between a collection site of origin and two different laboratory destinations. We obtained acceleration vs. time data using an accelerometer. Borrowing from public transportation safety literature, we examined these profiles in terms of distributions of jerk, which is the first derivative of acceleration (d(acceleration)/$dt \equiv g/s$), and distributions of theta, the angle between successive acceleration vectors. **Methods**: Primary data for acceleration vs. time were collected using a USB accelerometer (X16-1E, Gulf Coast Data Concepts LLC, Waveland, MS). The device

produces a CSV file for acceleration along 3 mutually orthogonal axes in three dimensions (x, y, and z) as a function of time (rate=12 Hz). For data collection, the device was wrapped in bubble wrap and placed within a PTT carrier. Data were collected for transport from the collection site to destination laboratory 1 (Route A), and from the collection site to destination laboratory 2 (Route B). Each acceleration datapoint represented a 3-dimenstional vector (having both magnitude and direction). We calculated jerk as the magnitude of the vector representing the difference in the acceleration vectors between successive data points. We calculated angular changes in direction of acceleration (theta) from the dot product of successive acceleration vectors. Variation in theta may be thought of as a measure of "rattling". **Results**: Routes A and B had practically identical transit times of approximately 300 s (5 min). Accelerations ranged in magnitude (M) from 0-8 g. Route A demonstrated a 2 min interval of essentially constant velocity, whereas Route B demonstrated nearly continuous variation in acceleration. For Route B, M>1.2 g comprised 29.0% of results compared to 13.5% of results for Route A (ratio=2.1). Jerk ranged from 0-7 g per time interval of 1/(12 Hz). For Route B, jerk>0.1 g per time interval comprised 63.0% of results compared to 28.3% of results for Route A (ratio=2.2). Theta ranged from 0-180 degrees. For Route B, theta>5 degrees comprised 59.3% of results compared to 26.6% of results for Route A (ratio=2.2). By measures of acceleration, jerk, and theta, Route B is a "rougher" ride for specimens compared to Route A. The similarity of the ratios given above (range 2.1-2.2) indicate that differences in acceleration, jerk, and theta run in parallel as variables for comparison between these two PTT routes. **Conclusions**: In transportation safety literature, jerk is a primary variable in the category of "conditions in which people get knocked down." For specimen transport, the analogy is that jerk might be a variable with respect to "conditions that may activate or disrupt cells." In our example, comparisons of distributions for acceleration, jerk and theta ran in parallel. Thus, in evaluation of effects of PTT on test results, analysis of acceleration data alone might mask identification of cumulative effects of jerk and theta as possible primary variables. Experimentally, however, it would be very difficult to establish distinction between acceleration, jerk, or theta as primary variables.

B-154

Characterizing ability of the serum potassium (K) to flag hypokalemia or hyperkalemia as observed in plasma: a simulation study

D. F. Stickle[1], K. R. Koob[1], C. R. McCudden[2]. *[1]Jefferson University Hospital, Philadelphia, PA, [2]University of Ottawa, Ottawa, ON, Canada*

Background: Plasma has long been the recommended specimen type for K measurement. However, serum usage persists, including use due to recent plasma tube shortages. In comparison to plasma K , serum K is generally right-shifted (higher). This shift varies across individual samples according to a normal distribution. Consequentially, K results outside of the population reference interval (RI) for plasma (hypokalemia or hyperkalemia) are unlikely to be strictly concordant with classification in serum according to the serum RI. Serum may thus have a lower sensitivity to detect either hypokalemia or hyperkalemia relative to plasma. We examined this premise from a theoretical standpoint by simulation.

Methods: We used longstanding and widely implemented textbook K reference intervals (Tietz, 4th Ed.) for plasma (PRI = 3.4-4.5 mmol/L) and serum (SRI = 3.5-5.1 mmol/L). The difference between plasma K and serum K is characterized by a normally distributed function where serum = plasma + 0.35 ± 0.3 mmol/L. This transformation was applied to an at-large patient data distribution from a large academic medical center to generate a theoretical serum K distribution from real world plasma patient K results. The simulated serum K data were characterized as to whether hypokalemic and hyperkalemic plasma K specimens as defined by PRI were also classified in serum as being either below or above lower and upper limits of SRI, respectively.

Results: Primary data were a plasma K patient distribution for a three month interval (n = 59,570; median = 4.1 mmol/L; hypokalemia = 6.5%; hyperkalemia = 16.7%). Simulated serum K data from the plasma transformation (n = 100,000) yielded a right-shifted distribution (median = 4.4 mmol/L) with 4.1% of results below the lower limit of SRI, and 11.4% of results above the upper limit of SRI. For samples originating as hypokalemic according to the PRI, sensitivity for detection by being flagged as below the lower limit of the SRI was 44.1% (specificity = 98.7%). For samples originating as hyperkalemic according to the PRI, sensitivity for detection by being flagged as above the upper limit of the SRI was 58.7% (specificity = 97.6%).

Conclusions: Simulation results indicate that serum K should best be thought of as an inferior substitute marker for plasma K. Depending on reference intervals employed, sensitivity of serum RI for detection either of plasma hypokalemia or hyperkalemia may be significantly less than 100%. This can result in a significant degree of misidentification of hyperkalemia or hyperkalemia as being within SRI. In our simulation, 55.9% of plasma hypokalemia results were in serum classified as being within the SRI; 41.3% of plasma hyperkalemia results were in serum classified as being within the SRI. Additionally, note that a switch from serum to plasma will likely precipitate a significant increase in flag rates for plasma K outside of the PRI compared to flag rates observed for serum K outside of the SRI. These results follow simply from the fact that serum K includes a random component not present in plasma.

B-155

Sigma Metric Equation Proposed by James O. Westgard is Wrong

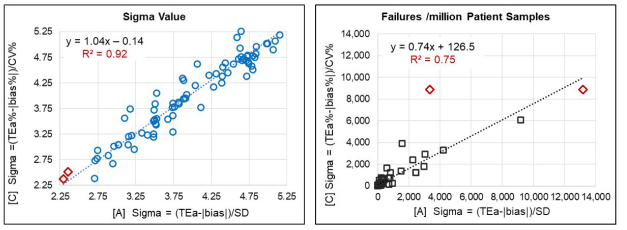
Z. C. BROOKS. *AWEsome Numbers Inc, Worthington, ON, Canada*

Background 2022 AACC Poster #132 of the same title described how the Westgard sigma metric equation of [A] SM = (TEa - Bias)/SD proposed by James O. Westgard is NOT wrong when compared to the industrial practice of calculating sigma as [B] the difference between the mean and nearest upper or lower tolerance limit divided by the SD. Formulae [A] and [C] produce the same results. The often-recommended practice of calculating sigma as [C] Sigma = (%TE -|%Bias|)/CV%, however, produces results that differ from either formulae described above whenever bias is not zero. This formula [C] using percentages is therefore wrong.

Methods Sigma values were calculated as [A] (TEa - |Bias)|/SD and as [C] (%TE -|%Bias|)/CV% on fourteen routine chemistry analytes and four cardiac markers tested on two Ortho Vitros 5600 analyzers from September to December 2022. CLIA TEa limits were selected as TEa limits for each analyte; bias was calculated as Current Mean - Peer Mean. Sigma tables were used to convert sigma to defects per million results. Eight-six of 320 data sets with sigma metrics from 2.25 to 5.25 were graphed to compare calculated sigma values and #failures/year.

Results Comparison of sigma metrics calculated from formula [A] and [C] revealed 1. An $R^2 = 1.00$ if all 320 values with sigma metrics from 0.71 to 30.8; 2. An $R^2 = 0.92$ when the range was truncated to the 86 samples with sigma from 2.25 to 5.25; and 3. An R^2 value of only 0.75 when the same sigma values are converted to the number of results failing TEa limits per one million patent samples.

86 QC Samples Comparing results from sigma formulae using [C] Percent vs. [A] Units of Measure



Conclusion: Calculations of sigma metrics based on %Bias and %CV are incorrect. This is exacerbated when sigma is converted to the number of patient failures per million results.

B-156

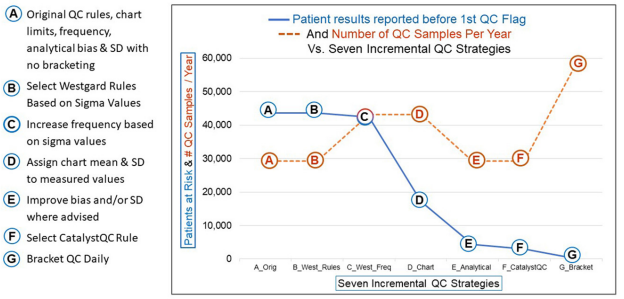
Impact of Seven Incremental Scenarios of QC Strategies

Z. C. BROOKS. *AWEsome Numbers Inc, Worthington, ON, Canada*

Background CLSI EP23A states that "At the least, the ability of the QC procedures to detect medically allowable error should be evaluated." It also defines that "The QC strategy using QC samples should include the following for each measuring system:•The type and number of QC samples tested per test event •The statistical QC limits used to evaluate the results •The frequency of QC sample test events •The frequency of periodic review •The actions taken when results exceed acceptable limits"

Methods Eighty QC sample mean and SD values were compared to Peer Means and TEa limits for 14 routine chemistry analytes and four cardiac markers tested in December 2022. The number of patient results was normalized to 200 and 60 per day for routine and cardiac; the number reported before the first QC flag after a simulated shift to a 5% error was calculated using CatalystQC software for seven incremental scenarios of quality control beginning with [A] Original QC rules (1-3s/2-2s), chart limits, frequency (once per day), analytical bias & SD; [B] Westgard Rules based on sigma values; [C] Westgard QC frequency; [D] Chart means & SDs set to measured values; [E] Improved bias and/or SD where advised by CatalystQC software; [F] Single CatalystQC rules; [G] QC bracketed at the end of each day.

Results The number of patients at risk decreased with each component of the QC process. Observed and advised number of QC samples per year was inconsistent.



Conclusion: Selecting Westgard rules and frequency alone will not reduce patient risk significantly. Assigning QC chart values to recent mean and SD and improving analytical bias and SD have the most impact on reduction of patient risk. CatalystQC single rules are more effective than Westgard rules. Bracketing QC with verified-effective QC will reduce patient risk to zero.

B-157

Metrics to express risk in terms of A. risk sources, B. potential events, C. their consequences and D. their likelihood.- as recommended by ISO 31000

Z. BROOKS. *AWEsome Numbers Inc, Worthington, ON, Canada*

Risk Metrics to express risk in terms of A. risk sources, B. potential events, C. their consequences and D. their likelihood. (as recommended by ISO 31000) compared to sigma metrics

Background:

ISO 31000 Risk Management Guidelines provides guidelines on managing risk faced by all organizations. “Risk is usually expressed in terms of A. risk sources, B. potential events, C. their consequences, and D. their likelihood.” I compared risk metrics to implement these guidelines in medical laboratories to sigma metrics to examine the most effective way to enable risk evaluation as ‘the process of comparing the estimated risk against given risk criteria to determine the acceptability of the risk’ (CLSO EP 23A.)

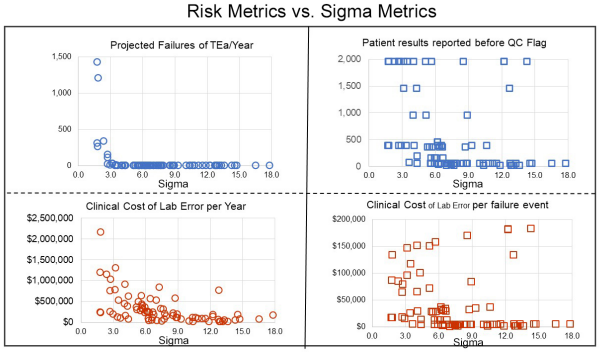
Method:

Eighty QC sample mean and SD values were compared to Peer Means and CLIA TEa limits for 14 routine chemistry analytes and four cardiac markers tested on two Ortho Vitros 5600 analyzers in December 2022. Risk evaluation was divided into Current Risk, driven by analytical accuracy and precision, and Potential Risk driven by effectiveness of the daily QC system to detect a simulated shift to a 5% error rate.

Current risk consequences were quantified as the number of failures per million opportunities and per year, and the estimated healthcare cost of lab error based on the costing process established for calcium in 2004.[i]

Potential risk consequences were quantified as the number of failures reported and healthcare cost of lab error following a simulated shift to create a 5% error rate.

Results:1.pdf



Conclusion:

There is a good relationship between sigma and projected failures per year. Sigma is NOT a good indicator of cost of error per year, or the number or cost of failures reported after a simulated failure.

Endnotes[i] National Institute of Standards and Technology. Planning Report 04.1. The impact of calibration error in medical decision making.

B-158

Application of Statistical Analysis as a Tool to Monitor the Variation of SARS-CoV-2 and Other Respiratory Viruses

G. C. Carvalho, J. M. Nurmberger, B. B. Perez, D. R. Ramadan, S. Tufik. *Associação Fundo de Incentivo a Pesquisa, São Paulo, Brazil*

Background: After the emergence of SARS-CoV-2 and its worldwide spread, only a few studies have correlated its co-circulation with other respiratory viral pathogens (RVP). The current report aims to identify the pattern of SARS-CoV-2 positivity in individuals co-infected with influenza A (FluA), influenza B (FluB), or respiratory syncytial virus (RSV) in São Paulo, Brazil. **Methods:** Study Population and Characteristics: This study was a retrospective analysis from a clinical laboratory database in São Paulo, Brazil from January 15 to March 10, 2022. Test results for respiratory viral co-infections were recorded for individuals with SARS-CoV-2, FluA, FluB, or RSV. Nasopharyngeal swab samples were evaluated for the molecular diagnosis of respiratory virus infections using a real-time multiplex-PCR panel to search for specific genes for the detection of SARS-CoV-2, FluA, FluB, and RSV. Only samples positive for one or more pathogens (including SARS-CoV-2) were included in the study. We further stratified the specimens by SARS-CoV-2 positivity, comparing those that also tested positive for an RVP in each group. Statistical Analysis: Kruskal-Wallis analysis of variance followed by Dunn’s multiple comparison tests was used. Results with $p<0.05$ were considered statistically significant. Georeferencing analysis of SARS-CoV-2 mono-infection or co-infection with another respiratory virus was performed using an online available software application. **Results:** A total of 28,932 samples were positive for at least one RVP, meeting the study criteria. Among those, a total of 93.13% were from individuals diagnosed with SARS-CoV-2 only, 2.99% positive for SARS-CoV-2 and FluA, and 0.99% positive for SARS-CoV-2 and RSV. There was no significant difference observed between genders nor individuals with mono-infection or co-infection. The analysis of age group showed a significant variation in positive cases between SARS-CoV-2 vs. SARS-CoV-2 and FluA in the 0 to 10-year-old population ($p=0.017$) and between SARS-CoV-2 vs. SARS-CoV-2 and RSV in the 0 to 5-year-old population ($p=0.018$). The highest number of positive samples for both SARS-CoV-2 and RSV was detected in the southern region of São Paulo (55.7%), compared to the north and east regions (44.3%). **Conclusion:** Differences in diagnostic patterns of COVID-19 compared to other respiratory viral infections RVP co-infections are important for a better understanding of clinical outcomes, improved patient management, and better public health planning.

B-159

Data Mining With the Bhattacharya Method as a Tool for Reference Interval Determination in Populations With Thyroid Disorders

C. D. Partel, G. C. Carvalho, M. C. Martino, D. R. Ramadan, S. Tufik. *Associação Fundo de Incentivo a Pesquisa, São Paulo, Brazil*

Background: Establishing a precise reference interval (RI) is crucial for laboratory diagnosis, population studies, and aiding physicians in patient follow-up treatment. Data mining and Bhattacharya analysis can be an efficient and cost-effective way to determine RIs for thyroid disorders based on large datasets. Bhattacharya analysis is a simple resolution method that detects distributions into Gaussian components. Unlike traditional reference interval studies using parametric or non-parametric statistics, Bhattacharya analysis does not exclude results from an “unhealthy” population before analysis. This study aimed to define the RI for thyroid-stimulating hormone (TSH) and free thyroxine (fT4) from three distinct regions in São Paulo, Brazil, using the Bhattacharya method. **Methods:** The study was a 20-month retrospective analysis of a large laboratory database in São Paulo from February/2021 to September/2022. Individuals aged 21 or older with available TSH or fT4 results were selected, including only outpatient results. Three groups from different regions (R1, R2, R3) were studied and the indirect Bhattacharya method was used to determine each region’s RI values. The midpoint, upper and lower reference limits (URL and LRL), were calculated, and a data plot was created using Microsoft Excel. One-way ANOVA was used for multiple group comparisons and results with $*p<0.01$ were considered statistically significant. **Results:** A total of 234,903 TSH and fT4 records were obtained. The three groups showed significant differences in their mean age and TSH and fT4 results ($p<0.001$). The mean age was 49.41±16.9 for R1, 46.9±9.6 for R2, and 53±16 years old for R3. The average TSH value for all three regions was 2.44 µIU/mL (SD 0.18, CV 7.2%), and the average fT4 value was 1.17 ng/dL (SD 0.06, CV 4.7%). The central

bin was selected for the best data distribution and close to the average or median for each group. The derived RIs for R1, R2, and R3 were compared for consideration of a common RI. Other criteria, such as the best bin size and at least four bins for the graph curve, were also applied. Altogether, Bhattacharya's analysis identified the following estimates from all collection points for TSH and fT4: TSH Average LRL 0.43 µIU/mL (SD 0.07, CV 16.6%)/TSH Average URL 6.45 µIU/mL (SD 0.85, CV 13.1%)/TSH Average Range 6.03 µIU/mL (SD 0.88, CV 14.6%)/fT4 Average LRL 0.82 ng/dL (SD 0.07, CV 8.1%)/fT4 Average URL 1.47 ng/dL (SD 0.07, CV 4.4%)/fT4 Average Range 0.65 ng/dL (SD 0.02, CV 3.5%). 9.7% of individuals in R3 had results outside the accepted RIs for TSH and fT4, compared to R2 as a standard population with the lowest possibility of relevant thyroid disorders. **Conclusion:** Bhattacharya's non-parametric method could be useful to determine reference values and laboratory analysis of populations with distinct characteristics. The study highlights the importance of choosing the correct control group in comparison studies, and the potential bias in unifying distinct regions to determine RIs. The low cost of data mining makes it possible for nearby laboratories to perform similar studies using the Bhattacharya method to establish RI results suitable for specific patient populations.

Mass Spectrometry and Separation Sciences

B-160

Quantification of titanium using Inductively Coupled Plasma Mass Spectrometry ICP-MS to monitor toxic levels in blood

M. O. Soares[1], L. T. Paulucci[2], F. P. Mazete[2], D. R. Ramadan[2], S. Tufik[2], E. K. Sugawara[2]. *[1]AFIP - Associação Fundo de Incentivo a Pesquisa, São Paulo, Brazil, [2]AFIP - Associação Fundo de Incentivo à Pesquisa, São Paulo, Brazil*

Background: When titanium is used as a metallic implant, it can potentially cause toxicity to humans due to wear of the implant. As the implant wears, small particles of titanium and other metals may be released into the surrounding tissue. These particles can cause an immune response, leading to adverse reactions such as allergies and implant-associated infections. Additionally, the wear of the implant can also lead to the release of metallic ions, which can have toxic effects on surrounding tissue and cells. Workers may be exposed in factories that use the metal in the manufacture of paints, varnishes, ceramics, plastics, rubber, soldering rods, floor coatings, food dyes, industrial textiles, cosmetics, glass products, pharmaceuticals, manufacturing of rubber tires and in the production of electronic components. This highlights the importance of regular monitoring and maintenance of titanium prostheses to minimize the wear and toxicity as well as monitoring commonly exposed professionals. The aim of the study was to validate a reliable method for quantification of titanium in blood.

Methods: Validation was performed using an Inductively Coupled Plasma Mass Spectrometry, 7850 ICP-MS. Analytical specificity is ensured using single particle analysis with fragmented ions that are exclusive to Ti, quantifier ion 47 Da. The procedure involves a small amount of 125 µL of blood, followed by dilution with 100 µL of internal standard at concentration of 1.0 µg/L more 2.275 µL of diluent containing 1-Butanol, Ammonium Hydroxide, EDTA and Triton X-100. After that, samples are submitted to hydrolysis to 37ºC for 40 minutes. The internal standard was purchased from Agilent at a concentration of 10.0 µg/mL. Finalizing the preparation samples are followed by aspiration into the SPS 4 model injector. Quantitation is achieved by the comparison of the responses from a given sample with the responses of calibrators with known concentrations prepared through *Multi Element Cabibration* at concentration of 10.0 µg/mL of Ti. Linearity was observed in the expected concentration range from 2.0 to 7.0 µg/L and blood samples were evaluated at six different concentrations and six times each at the same time of the study. Diluent was used as a biological matrix for the study and the standard acquired was Agilent.

Results: The linearity coefficient of determination (R) was 0.9998. The method showed 100% selectivity and no residual effect interference that was evaluated in blood matrix. To determine the average inter-assay CV%, three different concentrations were analyzed over three days and the results for each low, medium, and high concentration level were 4.27, 4.22 and 3.33%, respectively. The accuracy of the method was cheeked by analyzing samples of known concentration and expressed as a percentage. A time total analysis was 3.5 min.

Conclusion: The method was efficient for the determination of titanium in blood. The efficiency and selectivity promoted by the ICP-MS technique, combined with the reliable preparation method, can be used in the diagnosis of wear in metallic titanium prostheses and occupational exposure.

B-161

Mass Spectrometric Assay for the Early Diagnosis of Barth Syndrome

S. Byeon[1], H. Vernon[2], D. Oglesbee[1], A. Pandey[1]. *[1]Mayo Clinic, Rochester, MN, [2]Johns Hopkins University, Baltimore, MD*

Background: Barth syndrome (BTHS) is an inborn error of phospholipid metabolism caused by pathogenic variants in the *TAFAZZIN* gene locus on a chromosome X. *TAFAZZIN* encodes a phospholipid transacylase enzyme, tafazzin, that promotes cardiolipin (CL) remodeling. Deficiency of tafazzin activity in BTHS results in accumulation of monolysocardiolipin (MLCL) and decreased abundance of mature CL, making an increased ratio of MLCL to CL as a diagnostic marker for BTHS. 52:2-MLCL to 72:8-CL in dried-blood spot (DBS) has been used for provisional identification BTHS using LC-MS/MS. A development of multiplexed method that targets several species of MLCL and CL can enhance the diagnosis of BTHS. Methods: A targeted LC-MS/MS with scheduled multiple reaction monitoring (MRM) mode in negative electrospray ionization was developed using a triple quadrupole MS. DBS from patients with BTHS were obtained at Johns Hopkins University and provided by Barth Syndrome Foundation while control DBS were provided by Biochemical Genetics Laboratory at Mayo Clinic. Internal standards and chloroform:methanol (1:1, v/v) were added to DBS (1/8" diameter) and sonicated. Supernatants were collected and dried under nitrogen gas. Followed by reconstitution in methanol, samples were subjected to LC-MS/MS analysis. Results: More than 20 species of MLCL and CL species having various fatty acyl chains were detected and quantified. Remodeled CL species were decreased while MLCL and nascent CL species were found to be increased in known patients of BTHS (n=8) compared to controls (n=8). Notably, several ratios of lipids including 52:2-MLCL/72:8-CL and 54:2-MLCL/70:6-CL showed >300-fold elevation in the patients. Conclusion: Various species of MLCL and CL that can be used to diagnose BTHS can be measured from an easily accessible specimen such as DBS using this method.

B-162

Direct quantitation of five immunosuppressant drugs in dried whole blood spots by a fully automated DSM-LC-MS system

R. Gibson, J. Guo, S. Samra. *Thermo Fisher Scientific, San Jose, CA*

Background: Liquid chromatography-mass spectrometry (LC-MS) is increasingly used in clinical research to quantify immunosuppressant drugs in whole blood as it can offer higher sensitivity and selectivity than other analytical techniques. LC-MS systems may be coupled with upstream dried matrix spot modules within one high-throughput, integrated and online workflow, allowing the extraction of matrices such as whole blood from pre-collected sample cards collected in a quick and minimally invasive manner.

In this poster, such a workflow will be utilized to demonstrate the quantitation of five commonly monitored therapeutic immunosuppressants; cyclosporin A, everolimus, mycophenolic acid, sirolimus and tacrolimus.

Methods: Calibration and QC samples were prepared in whole blood. 10 µL of each calibrant was then spotted in triplicate on DBS cards. Immunosuppressants and standards were extracted using a Thermo Scientifc™ Transcend™ DSX-1 Systems via flow-through desorption with a heated clamp. TurboFlow allowed interference removal in the extracted samples prior to analytical separation. An integrated software, Aria MX, controlled each step of sample desorption and separation. Analyte quantitation was performed by a TSQ Altis™ mass spectrometer, and the data was analyzed using TraceFinder™ 5.1 General Quan software.

Results: Five commonly therapeutically monitored immunosuppressant drugs were quantified from blood spotted onto DBS cards using a fully automated workflow, resulting in a highly sensitive 5-minute method. Calibration curves were fitted using a weighting factor of 1/x with requirements of $R^2 > 0.98$, RSD/CV < 15%. The calibrants were used to determine the limits of detection (LODs) and quantitation (LOQs) shown in the below table.

Conclusion: A complete, integrated and fully automated workflow has been developed to rapidly and robustly quantify immunosuppressant drugs from dried blood spots. Quick and reproducible extraction of each target analyte and efficient chromatographic separation facilitated a 5-minute method for the quantitation of five of the most commonly therapeutically monitored immunosuppressant drugs.

Limits of detection and quantitation for five immunosuppressant drugs in dried whole blood				
	Retention Time (minutes)	LOD (ng/mL)	LLOQ (ng/mL)	R^2
Cyclosporin A	3.03	6.25	25.0	0.982
Everolimus	2.93	1.25	1.25	0.980
Mycophenolic Acid	2.71	12.5	50.0	0.985
Sirolimus	2.92	2.50	5.00	0.984
Tacrolimus	2.91	1.25	1.25	0.996

B-163

Development and Validation of a New Quantitative LC-MS/MS Method for Vitamin K1 in Plasma and Serum

Z. Wu, A. K. Ghoshal. *Quest Diagnostics Nichols Institute, Valencia, CA*

Background: Vitamin K1 (phylloquinone) is a fat-soluble cofactor; its deficiency depresses synthesis of plasma prothrombin (factor II) and other factors, causes excessive bleeding, and increases the risk of bone fractures and osteoporosis. Biochemical and metabolic studies on vitamin K1 require both highly sensitive and specific techniques. Analytical challenges to quantitation include the presence of interfering lipids, molecular instability, and extremely low physiological levels. High performance liquid chromatography (HPLC) with fluorescence and electrochemical detection (FLD-ECD) methods used for vitamin K1 involve labor-intensive, liquid-liquid extraction procedures and extremely long run times. Our goal was to develop and validate a high throughput and accurate method for measuring vitamin K1 in plasma and serum using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Methods: Plasma or serum specimens were mixed with stable isotope internal standard in an extraction solution containing acetonitrile to release vitamin K1 and precipitate proteins. After centrifugation, the supernatant was then extracted using a HybridSPE® (Supelco) plate to remove the remaining precipitated proteins and phospholipids that may contribute to ion-suppression. Certified standards were used to derive a 6-point calibration curve. Extracts (plasma or serum) and calibrators were analyzed on a SCIEX 6500+ LC-MS/MS with a Thermo TX-4 HTLC multiplexed 4-channel system for increased throughput. Chromatographic separation was achieved on a Biphenyl analytical column. Precision, accuracy, linearity, recovery, and stability were evaluated. Random specimen discards, previously submitted for vitamin K1 testing, were used for a split-sample comparison study vs the current HPLC-FLD-ECD method.

Results: Compared with the HPLC-FLD-ECD method, the LC-MS/MS method employed a simplified automated hybrid solid phase extraction procedure, used less specimen volume (100 µL vs 700 µL), increased total throughput (4 hours vs 32 hours per 96-well plate), was more sensitive (limit of quantitation [LOQ] 6.1 pg/mL vs 25.0 pg/mL), and provided greater molecular specificity for vitamin K1 through multiple reaction monitoring (MRM). The intra-assay and total inter-assay precision coefficients of variation (CVW, CVT) in plasma samples (n=100) were: (6.8%, 5.8%) at 100 pg/mL, (3.5%, 3.0%) at 800 pg/mL, and (3.0%, 3.9%) at 2,000 pg/mL. In spiked plasma and serum samples (n=10), the linearity range was 40-20,000 pg/mL and percent recovery were 98%-107%. Least-squares regression analysis comparing the results for split plasma extracts by LC-MS/MS vs HPLC-FLD-ECD (n=100) yielded correlation coefficients for vitamin K1: 0.9917, y = 0.927x + 0.827, range 40-7,000 pg/mL. Vitamin K1 was stable in plasma and in serum stored in amber plastic screw-capped vials for 8 days at 2-8°C and 30 days at -20°C.

Conclusion: We have developed and validated an LC-MS/MS method that provides rapid, sensitive, precise, and accurate evaluation of vitamin K1 in plasma and serum. Advantages over the existing HPLC-FLD-ECD method include increased throughput and improved sensitivity and specificity. Vitamin K1 results obtained using this method can be used by clinicians to guide clinical intervention to improve or normalize metabolism or prevent the potential adverse consequences associated with inadequate dietary intake.

B-165

Establishment of a Labile Bound Copper and Labile Bound Copper Ratio Reference Interval in a Healthy Adult Population

A. Bitzer, P. Day, J. Fox, M. Wermers, P. Jannetto, J. Bornhorst. *Mayo Clinic, Rochester, MN*

Background: While clinical tests are available to aid in the diagnosis of Wilson's Disease (WD), some such as total copper determination lack sensitivity and specificity, while others such as liver tissue copper assessment are costly and invasive. WD affects copper metabolism and is known to cause low serum copper concentrations. In addition, an elevated percentage of total serum copper exists as non-protein bound or labile bound copper (LBC). Previous studies have shown that measuring the ratio of labile bound copper to total serum copper may aid in the diagnosis of WD. To support the utility of a laboratory developed test using serum LBC ratios to aid in the diagnosis of WD, serum samples from healthy donors were used to establish a reference range for LBC and the ratio of LBC to total copper in adults.**Methods:** Serum samples were collected in trace metal free tubes from 214 donors (n=110 male, n=104 female) between the ages of 19-80 years old, selected against pre-determined exclusion criteria including acute hepatitis, chronic hepatitis, renal failure, biliary cirrhosis, celiac disease, and leukemia. Prior to analysis, samples went through a centrifugation-based preparation process to isolate the LBC fraction using molecular weight size exclusion filters and EDTA chelation. Samples were then analyzed via ICP-MS in kinetic energy discrimination (KED) mode to directly measure the LBC and total copper concentrations. LBC ratio values were determined from the LBC/total copper concentrations. **Results:** Preliminary reference ranges were developed for both LBC and the LBC ratio based on the 2.5th and 97.5th percentiles with 95% confidence intervals. Established sex specific reference intervals for total copper were deemed acceptable per CLSI guidelines in document EP28-A3c, with ≤10% of results from this sample set falling outside of the established intervals. No statistically significant relationship was found in the mid-95th percentile between LBC concentration or ratio with age or sex. The reference intervals for LBC concentration were calculated as 13.41 to 104.66 and 11.69 to 106.50 ng/mL for females and males respectively. The reference intervals for LBC ratio were calculated as 1.04 to 8.12 and 1.17 to 10.48 for females and males respectively. **Conclusions:** A reference range of LBC and LBC ratio has now been determined for the healthy adult population. Further testing can now be done on disease state populations to determine the sensitivity and specificity of LBC ratio in the detection, monitoring, or diagnosis of WD.

B-166

Effect of Urine Adulteration on Quantifying both Delta 8- and Delta 9-carboxy tetrahydrocannabinol using a new UPLC-tandem Mass Spectrometry Method

Y. Chai, M. Bjergum, A. Maus, R. Lahr, J. Tufariello, P. Ladwig, L. Langman, P. Jannetto. *Mayo Clinic, Rochester, MN*

Background:

The legality and use of marijuana is a controversial topic. With increased prevalence of usage, cannabis detection for legal, medical, and workforce drug testing is on the rise. LC-MS/MS quantification of delta 9-carboxy tetrahydrocannabinol (delta 9-THC-COOH) in urine is accepted as the standard to detect marijuana use. One challenge faced by testing laboratories has been identifying adulterated samples. Many products/reagents can be used to manipulate the urine to avoid positive drug testing results. In this study, we developed a dilute-and-shoot strategy to separate and quantify both the delta 8- and 9-THC-COOH in urine samples and tested the effects of adulteration using a combination of nitrite and/or acids which can be used to mask cannabinoid use.

Methods:

Five authentic delta 8- and 9-THC-COOH positive specimens, and one spiked sample were treated with strong acids (1N HCL or 10% TCA), 500 µg/mL nitrite, or a combination. After hydrolysis with sodium hydroxide, the analytes were quantified using a 6495 Mass Spectrometer coupled to a StreamSelect LC system (Agilent). LC separation was performed with a CORTECS T3 column utilizing a shallow gradient. In addition, twelve urine specimens with varying concentrations of delta 8- and 9-THC-COOH were treated with nitrite and screened using a delta 9-THC-COOH immunoassay on the Roche Cobas C501 and analyzed by LC-MS/MS.

Results:

Nitrite did not change the physical appearance or the pH of the urine samples. Strong acid treatment also did not alter the appearance but did drop the urine pH ≤2. Pretreated specimens with 1N HCL, 10% TCA, or 500 µg/mL of nitrite did not affect the delta 8- and 9-THC-COOH concentrations up to 6 days (refrigerated); however, specimens treated with strong acid combined with 500 µg/mL of nitrite for 30 minutes made both analytes undetectable. Additional experiments confirmed that 0.5 and 1 mol/L of nitrite lowered the concentration of all analytes, including internal standard, to undetectable levels in 48 hours. Twelve additional specimens were collected, 3 with delta 8-THC-COOH, 3 with delta 9-THC-COOH, and 6 with both. All 12 samples screened positive using the Roche immunoassay and were quantified using the LC-MS/MS method. After treatment with 0.5 mol/L of nitrite, 10 of the 12 samples were negative using the immunoassay, but 2 samples remained positive. However, all ni-

trite treated specimens were negative (<LLOQ) using the LC-MS/MS method and the internal standard was eliminated. Later experiments confirmed that the delta 8- and 9-THC-COOH could be eliminated with as little as 0.1 mol/L nitrite for 30 minutes.
Conclusion:

The unique dilute-and-shoot method can separate and quantify delta 8- and 9-THC-COOH. In addition, high concentrations of nitrite were shown to degrade both analytes along with the isotopically labeled internal standard. As a result, labs can distinguish true negative delta 8- and 9-THC-COOH (no analyte peaks) from nitrite adulterated specimens since the adulterated samples have no peaks for both the analytes and internal standard. A limitation of the study is that only sodium nitrite was tested. Other adulterants, such as potassium nitrite, pyridinium chlorochromate, peroxide etc. require further investigation.

B-167

Development and Validation of Liquid Chromatography-Tandem Mass Spectrometry Method for Therapeutic Drug Monitoring of Treosulfan and Busulfan

J. Ryu, S. Lee, S. Kim, W. Lee, S. Chun, W. Min. *Asan Medical Center, Seoul, Korea, Republic of*

Background: Treosulfan and busulfan are alkylating agents administered as the conditioning regimen before hematopoietic stem cell transplantation. High busulfan exposure is associated with drug toxicity and low busulfan exposure with rejection or disease recurrence. There is also an association between treosulfan exposure and early toxicity, such as skin toxicity and mucositis. As both busulfan and treosulfan have narrow therapeutic ranges, therapeutic drug monitoring (TDM) is essential. Since the clinical laboratory tests for both drugs are few, a method that can simultaneously measure both drug concentrations under the same conditions would be helpful in practice. In this study, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous measurement of treosulfan and busulfan was developed and validated.

Methods: For preparing calibrators and quality control materials, commercialized reference standards were used for treosulfan, an active metabolite of treosulfan, S,S-EBDM, and busulfan. Treosulfan-D4 and busulfan-D8 isotopes were used as internal standards (ISs). The analytes were separated from 25 µL of plasma after protein precipitation with acetonitrile containing ISs. After centrifugation, the supernatant was diluted with 3% formic acid and then injected into the liquid chromatography system followed by tandem mass spectrometry. Chromatographic separation was performed using ACQUITY I-Class plus ultraperformance liquid chromatography (UPLC) system coupled with a XEVO TQ-XS mass spectrometer operated in positive ion electrospray ionization mode. The analytes and ISs were detected in the multiple reaction monitoring (MRM) mode. The limit of detection (LoD), limit of quantification (LoQ), selectivity, linearity, accuracy, precision, carryover, matrix effect, and sample stability were validated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Retention times for treosulfan, S,S-EBDM and IS for both analytes were 0.44 min, 0.43 min, and 0.44 min, respectively. Retention times for busulfan and IS for busulfan were 1.20 min and 1.18 min, respectively. The mass-to-charge (m/z) transitions used for quantification in the MRM mode were as follows: treosulfan 296.2>87.1; S,S-EBDM 200.2>87.1; and busulfan 264.1>55.1. The total run time was 4 min. The LoDs of treosulfan, S,S-EBDM, and busulfan were 0.21, 0.23, and 0.001 µg/mL, respectively. The linear range of the calibration curves of treosulfan, S,S-EBDM, and busulfan spanned concentrations of 3.13-100, 0.63-5.00, and 0.16-5.00 µg/mL, respectively. The total precision of the developed method fulfilled the analytical criteria, except for S,S-EBDM. The LC-MS/MS method was adequately selective and accurate and showed no carry-over. It provided an acceptable matrix effect for treosulfan and busulfan; however, the matrix effect could not be excluded for S,S-EBDM. Busulfan and treosulfan were stable in plasma for 2 h at room temperature. When the samples were stored at 10°C, busulfan was stable for up to a week and treosulfan was stable for up to 3 days, and both were stable for up to a week when stored frozen at -20°C.

Conclusion: We developed the LC-MS/MS method to simultaneously measure treosulfan and busulfan. The method met the validation requirements of the CLSI guidelines and showed good performance. Therefore, this method is expected to be helpful in TDM after treosulfan or busulfan treatment in clinical laboratories.

B-169

Beta-hydroxybutyrate/acetoacetate Ratio as Indicator for Mitochondrial Diseases Utilizing a Novel LC-MS/MS Based Ketone Body Panel

R. H. Kemperman, R. D. Ganetzky, S. R. Master. *Children's Hospital of Philadelphia, Philadelphia, PA*

Background: Inherited mitochondrial diseases, such as Leigh Syndrome cause defects in the energy metabolism, resulting in abnormalities in the mitochondrial redox state, defined as the ratio between nicotinamide adenine dinucleotide (NADH) and oxidized nicotinamide adenine dinucleotide (NAD+). Accurate and reproducible measurement of the NADH/NAD+ ratio directly is challenging due to its instability. The hepatic intramitochondrial NADH/NAD+ ratio is directly proportional to the ratio between the ketone bodies: beta-hydroxybutyrate (BHB) and acetoacetate (AcAc). Therefore, measuring the ketone body ratio is a more stable approach to estimate the mitochondrial redox state. Due to AcAc stability challenges and the presence of BHB structural isomers (i.e., alpha-hydroxybutyrate (AHB), gamma-hydroxybutyrate (GHB), and beta-hydroxyisobutyrate (BHIB)), no clinically validated assay that measures BHB, AcAc, and their ratio was available as a single test. Existing assays quantify AcAc and BHB separately using spectrophotometry, enzymology, or indirectly via gas chromatography, resulting in significantly reduced accuracy and large specimen quantity requirements. Lack of integration is particularly challenging for calculating a reproducible ratio. To improve clinical care, a novel liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) based ketone body panel requiring only 10 µL of serum or plasma was developed and clinically validated.

Methods:10 µL of serum or plasma was taken and kept on ice, followed by a protein precipitation, and drying down of the acquired supernatant. Thereafter, all five analytes were separated using a 6.5 min reversed phased LC column (Acquity, Waters) and detected by MS/MS analysis (Xevo TQ-S, Waters). Data were processed and curated with Ascent V4 (Indigo). Patient samples from the mitochondrial clinic (Children's Hospital of Philadelphia) were analyzed and results were paired with known diagnoses.

Results:A new robust and sensitive ketone body panel was developed that not only accurately and reproducibly measures concentrations of BHB, AcAc, and their ratio, but also the BHB isomers AHB, GHB, and BHIB. To ensure quality, a thorough validation and stability study has been performed. All analytes reported a linear range over three orders of magnitude, i.e., 0.0025-1.5 mM (AcAc and AHB), 0.0050-1.5 mM (BHB and BHIB), and 0.0025-1.2 mM (GHB), and a dilution up to 50x is permitted. The precision was assayed over 20x days, 2x replicates, and 2x injections for each analyte at three levels determining the inter-assay (day), intra-assay (replicate), and intra-assay (injection) coefficient of variation (CV); all the %CVs were reported below 6.5%. Spike and recovery were performed to determine the accuracy, results were obtained from ten random patient samples; an average of 99.9% (AcAc), 102.7% (BHB), 95.3% (AHB), 85.7% (GHB), and 87.5% (BHIB) was measured. Additionally, at least 40 samples were cross-examined for BHB using two independent assays, a mean bias of 0.01 mM and a Pearson's R=0.996 were reported. Specificity was determined for all analytes against 16 common therapeutic drugs, bilirubin, hemoglobin, triglycerides, and high protein levels at < 15% bias. Finally, new reference ranges have been established and were used to calculate accuracy rates.

Conclusion: Improved mitochondrial disease screening and diagnostics is established utilizing the LC-MS/MS based ketone body panel.

B-170

Identifying metabolic phenotyping variability in a cellular model of Barth Syndrome

J. Kodger. *Cleveland State University, Cleveland, OH*

Introduction: Barth Syndrome is an X-linked multisystem disease caused by the mutation in the TAZ gene (TAZ, G 4.5, OMIM 300394) that encodes for acyltransferase taffazin. Taffazin is a mitochondrial protein highly expressed in heart and skeletal muscle and has a central role in cardiolipin remodeling process. TAZ is the only known single gene defect in cardiolipin remodeling, with more than a hundred TAZ mutations identified. However, no systematic correlation has been established between TAZ genotypes and clinical or biochemical phenotypes in Barth syndrome. There is a large phenotypic heterogeneity among affected individuals sometimes even within the same family. This raises a possibility of genetic and metabolic modifiers involved in TAZ clinical and metabolic phenotypes. The aim of this study is to apply LC-MS/MS untargeted and targeted metabolic analyses to explore possible correlations between two TAZ genotypes and metabolome in a cellular model of Barth Syndrome. **Methods:** Mutant TAZ 3(c.647G>T), TAZ 4(c.778-24_778-7delinsA)

fibroblast and matching by donors' age and gender control fibroblast lines (healthy#1, healthy #2) were cultured under identical conditions. Cells (n=6 for each genotype) were harvested and extracted followed Bligh and Dyer protocol. Extracted metabolites were analyzed by Agilent 6545 Q-TOF coupled with 1290 Infinity II HPLC. Chromatographic separation was achieved by using a Water Premier BEH Z-HILIC Column (2.5µM, 2.1 x150 mm) and Waters X-select HSS T3 (2.5uM, 2.1 x100 mm) columns for polar and non-polar metabolites respectively. Raw data was normalized with internal standard and total cellular protein amounts. Statistical and pathway enrichment analyses were performed with a chemometric platform Mass Profiler Professional (Agilent) and Metaboanalyst webtool. **Results:** Data was treated first as for two separate genotypes (healthy vs TAZ) followed and compared to the analysis with four separate genotypes (healthy#1, healthy #2 TAZ c.647G>T, TAZ c.778-24_778-7delinsA). Metabolites contributing to discrimination of the genotypes based on metabolic profiles were identified by using a univariate analysis ($p<0.05$) and a supervised PLSDA (VIP>1 for small polar metabolites and VIP>3 for non-polar molecules). The differentially expressed metabolites included amino acids, acylcarnitine, Krebs cycle intermediates, ATP, 3-methylglutaconic acid, fatty acids and a variety of phospholipids. Pathway enrichment analysis revealed that several metabolic pathways were involved. **Conclusions:** Our analyses reveal that genetic variations in TAZ gene cause the differences in the abundance of certain metabolites and affected pathways. This leads us to the conclusion that from the point of view of metabolic pathophysiology, TAZ should be considered beyond a single-gene defect and a more personalized approach is needed in search for potential therapies.

B-171

Comparison of Intact PTH and PTH Assays using LC-MS/MS Among Non-Dialysis Dependent Chronic Kidney Disease Patients

H. Park, M. Lee, K. Lee. *Seoul Clinical Laboratories, Yongin-si, Korea, Republic of*

Background: Parathyroid hormone (PTH) is an important regulator of calcium and phosphate homeostasis and bone remodeling. It is metabolized into PTH fragments, which are measured to a different extent by PTH assays of different generations because of differences in fragments recognized and lack of assay standardization. Therefore, we developed an LC-MS/MS method for 1-84 PTH and aimed to compare with immunoassay among patients with chronic kidney disease (CKD) stages 3 to 5 not yet on dialysis.

Methods: Blood samples were collected from 252 patients with CKD stages 3 to 5. PTH concentrations were measured simultaneously by using the second-generation - PTH intact and immunocapture in situ digestion LC-MS/MS spectrometry assays. The severity of the kidney disease was based on the eGFR calculated by using the Modification of Diet in Renal Disease (MDRD) formula.

Results: Serum 1-84 PTH concentrations were strongly correlated but significantly lower than the intact PTH concentrations ($r=0.956$, $P<0.0001$). This finding was consistent among CKD stages 3 to 5. PTH concentrations by both assays (intact and 1-84 PTH) positively correlated with BUN ($r=0.613$, $r=0.61$; $P<0.0001$, respectively), phosphorus ($r=0.423$, $r=0.427$; $P<0.0001$, respectively) and negatively correlated with blood calcium ($r=-0.355$, $r=-0.421$; $P<0.0001$, respectively), protein ($r=-0.289$, $r=-0.305$; respectively, $P<0.0001$) and the estimated glomerular filtration rate ($r=-0.468$, $r=-0.485$; $P<0.0001$, respectively).

Conclusion: Among patients with CKD stages 3 to 5 not on dialysis, the 1-84 PTH assay detected significantly lower PTH concentrations compared with intact PTH assay. This LC-MS/MS method can provide accurate and precise PTH results in patients with severe chronic renal failure. Additional studies that correlate the diagnosis and management of CKD mineral and bone disorders are needed to determine whether 1-84 PTH assay results are better surrogate markers in these early stages of CKD.

B-172

Development and validation of an LC-MS/MS assay for quantification of Glecapravir/Pibrentasvir in human plasma

K. Garza, A. Pandey, M. Marzinke. *Johns Hopkins University School of Medicine, Baltimore, MD*

Background: The global prevalence of chronic hepatitis C virus (HCV) infection is 58 million. Untreated chronic HCV infections can lead to serious health complications, including liver cirrhosis, damage, and disease. Direct acting antiviral (DAA) therapies for HCV infections are effective in the treatment and management of chronic infections. Glecapravir (GLE) and pibrentasvir (PIB) are DAAs that may be delivered alone or as a fixed-dose oral formulation to treat adolescent and adult patients with chronic HCV infections (genotypes 1-6) with or without cirrhosis. Here, we describe a liquid chromatographic-tandem mass spectrometric (LC-MS/MS) assay for the multiplexed quantification of GLE and PIB in human plasma, with future applications in clinical trials to evaluate the pharmacokinetics of intramuscularly delivered GLE/PIB.

Methods: Calibrators were prepared in human K_2EDTA plasma at final concentrations ranging from 0.25-2,000 ng/mL GLE and 0.25-1,000 ng/mL PIB. Samples at the lower limit of quantification (LLOQ), low, mid, high, and dilution quality control (QC) levels were prepared at 0.25, 0.75, 800, 1800, and 20,000 ng/mL for GLE and 0.25, 0.75, 400, 800 ng/mL, and 10,000 ng/mL for PIB, respectively.Plasma samples containing GLE and PIB were combined with isotopically labeled internal standards and subjected to protein precipitation. Samples were evaporated to dryness, reconstituted in 50:50 mobile phase A (MPA, 0.1% formic acid in water): mobile phase B (MPB, 0.1% formic acid in 80:20 acetonitrile:water) and subjected to LC-MS/MS analysis.Chromatographic separation and analyte quantification occurred on an API 6500+ (Sciex, Foster City, CA) interfaced with an LC-30 (Shimadzu, Kyoto, Japan) operated in positive ionization and selective reaction monitoring modes. Chromatographic separation occurred using a Zorbax Eclipse C18 column under a gradient elution from MPA to MPB. Transitions monitored for analytes of interest were as follows: GLE (839.4 à 684.3 m/z), PIB (557.5 à 871.4 m/z), and their internal standards (GLE -IS 843.5 à 684.3 m/z; PIB-IS 561.5 à 629.3 m/z).The LC-MS/MS assay was validated in accordance with Food and Drug Administration Bioanalytical Method Validation Guidance for Industry recommendations.

Results: Inter-assay precision and accuracy ranged from 4.34% to 15.6% and -4.90% to 10.6% for GLE and 3.20% to 9.80% and -7.48% to -1.46% for PIB, respectively. GLE and PIB in plasma are stable following six freeze thaw cycles at <-70°C, with a percent difference (%DIF) ≤±10.8% for GLE and ≤±12.4 for PIB. Additionally, GLE and PIB are stable at room temperature for up to 67 hours and demonstrated re-injection stability at 4°C for up to 101 hours (GLE: %DIF ≤±4.1%; PIB %DIF: ≤±7.8%). Matrix effects were assessed quantitatively; negligible percent matrix effects were observed for PIB (106%) and PIB-IS (111%) across the measuring range of the assay. Significant ion suppression was observed for GLE (59.9%) and GLE-IS (63.9%); however, relative matrix effects were <5% between drug and internal standard and deemed acceptable. Other assay validation assessments in alternative matrices were also deemed acceptable.

Conclusion: An LC-MS/MS method for GLE and PIB quantification in plasma has been developed and validated and may be used in downstream applications to characterize DAA pharmacology for HCV treatment.

B-174

Simple and Accurate Quantification of Four Direct Oral Anticoagulants in Plasma Using UPLC-MS/MS

C. Lin, Y. Huang, C. Cheng, Y. Yeh, S. Chang. *Chang Gung Memorial Hospital, Taoyuan, Taiwan*

Background: Direct oral anticoagulants (DOACs) constitute first-line therapy used for many thromboembolic indications, such as prevention and treatment of venous thromboembolism and stroke prevention in atrial fibrillation. This class of medication consists of the direct thrombin inhibitor (dabigatran) or direct factor Xa inhibitors (apixaban, rivaroxaban, and edoxaban). DOACs quantification may be useful in the case of critical clinical situations, including drug accumulation in long-term treatment, overdosage, thrombotic or bleeding events. This study aimed to develop and validate an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for the quantification of four DOACs: dabigatran, apixaban, rivaroxaban, and edoxaban in plasma. **Methods:** The calibration curve samples were prepared by spiking drug free plasma with DOACs. The stable isotope labeled DOACs was used as an internal standard. After addition of the internal standard and protein precipitation, the supernatant was 10-fold diluted and injected into a chromatography system consisting of a UPLC BEH C18 (2.1 × 50 mm; particle size 1.7 µm) analytical column with gradient made of mobile phase (5 mM ammonium formate pH3.5 in water and 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 analytical range was linear with a correlation coefficient of over 0.99 in the range of 0-500 ng/mL for each analyte. Intra- and inter-assay imprecision were less than 3.1 % (n = 20) and 6.0 % (n = 20), respectively. The accuracy was evaluated by spike recovery and the mean recoveries were between 97.2% and 102.1% for all analytes. This assay showed no ion suppression or enhancement and no carryover. The chromatography run time was 4.5 min. The assay was applied to quantitate DOACs in plasma samples from 93 patients. Our data showed that the DOACs concentrations vary markedly individual patients after equal dose, this therapeutic drug monitoring

of DOACs is essential to optimized drug efficacy. **Conclusion**: An accurate, and cost-effective UPLC-MS/MS method for the quantification of DOACs was successfully applied for therapeutic drug monitoring in patients.

B-175

Phytochemicals in *K. pinnata* and the Antioxidant Activity of Combined *K. pinnata* and Metformin Preparations on Normal and Diabetic Skeletal Muscle Cells

P. Ramon, F. Omoruyi, J. Sparks. *Texas A&M University-Corpus Christi, Corpus Christi, TX*

ABSTRACT

Background: Diabetes Mellitus is associated with oxidative stress, which may play a central role in developing diabetic complications. With the rise in healthcare costs, more people globally are choosing to complement their pharmacological regimen with dietary supplements from natural products. In this study, the compound composition of *K. pinnata* and the effects of combined preparations of *K. pinnata* and metformin on antioxidant activity in human skeletal muscle myoblasts (HSMM) and human diabetic skeletal muscle myoblasts (DHSMM) were investigated. **Methods:** *K. pinnata* leaves were crushed and extracted with hot water, then gravity filtered and freeze-dried. A 1 mL aliquot of the *Kalanchoe pinnata* extract solution was utilized to determine the chemical composition of the herbal extract. Liquid chromatography-mass spectrometry (LC-MS) was run in negative ion mode, with the *K. pinnata* extract analyzed in triplicate. A computer program further analyzed the LC-MS data to determine the chemical components in *K. pinnata aqueous extract.* A database algorithm within Compound Discoverer was used to identify the compounds in *K. pinnata* aqueous extract. For subsequent cell culture experiments, the *K. pinnata* leaf extract was weighed and combined with aqueous metformin solutions to prepare various combinatorial treatments. Human skeletal muscle myoblast cells and diabetic human skeletal muscle myoblast cells (Lonza Inc, Walkersville, MD) were cultured and incubated at 37 °C with 5% CO2. A CCK-8 cytotoxicity assay was performed to determine cellular viability, while trypan blue was used to assess the cellular proliferation of in-vitro cells. The effects of combinatorial preparations of *K. pinnata* and metformin on antioxidant indices were determined in HSMM, DHSMM, and HSMM H_2O_2 stress-induced cells. **Results:** Liquid chromatography-mass spectrometry (LC-MS) showed that *K. pinnata* contains biologically active flavanols. The main compounds identified in locally grown *K. pinnata* were quercetin, kaempferol, apigenin, epigallocatechin gallate (EGCG), and avicularin. Antioxidant results indicated that a combinatorial preparation of *K. pinnata* with metformin might modulate antioxidant responses by increasing the enzymatic activity of superoxide dismutase and increasing levels of reduced glutathione. A combination of 50 µM and 150 µg/mL of metformin and *K. pinnata* resulted in a significant increase in reduced glutathione levels in non-diabetic and diabetic human skeletal muscle myoblasts and H_2O_2 stress-induced human skeletal muscle myoblasts. Additionally, a *K. pinnata* treatment (400 µg/mL) alone significantly increased catalase (CAT) activity for non-diabetic and diabetic human skeletal muscle myoblasts and an H_2O_2 stress-induced human skeletal muscle myoblast cell line while significantly lowering malondialdehyde (MDA) levels. The antioxidant activity of the treatment options was more effective at promoting cell viability after 24 hours of treatment versus 72 hours. **Conclusion:** These treatment options show promise, especially after 24 hours for treating oxidative stress-mediated pathophysiological complications associated with type II diabetes.

B-177

Quantitative measurement of vitamin B_1 in whole blood using a multiplex ESI-LC-MS/MS system

P. P. Vachali[1], E. Kish-Trier[2], R. N. Law[1], E. L. Frank[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; NanoSpot.ai, Salt Lake City, UT, [3]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; University of Utah Health, Salt Lake City, UT*

Background: Vitamin B_1 is a water-soluble vitamin required for human health. Thiamine diphosphate (TDP), the active form of vitamin B_1, plays an essential role in biochemical pathways required for energy metabolism. TDP is analyzed routinely in the laboratory to assess the nutritional status of post-bariatric surgery patients and other individuals who are at risk of vitamin deficiency. In recent years, demand for vitamin B_1 testing has increased dramatically, prompting development of a high throughput assay. Here, we present a method using protein precipitation and multiplex LC-ESI-MS/MS analysis at low pH with reversed-phase parameters that optimize chromatographic separation. The new method was validated for linearity, sensitivity, accuracy, imprecision, specificity, reproducibility, carryover, and stability. **Method**: Whole blood samples were deproteinized using trichloroacetic acid (TCA) after the addition of stable-isotope-labeled TDP (d_3-TDP) as an internal standard (IS). TDP was separated by reversed-phase chromatography on Waters XBridge™ Premier BEH C_{18} columns (2.1 x 100 mm, 2.5 µm) and analyzed on an Agilent StreamSelect™ LC-ESI-MS/MS platform. The StreamSelect™ system consists of four parallel Agilent Infinity Lab LC Series II instruments coupled to a single triple quadrupole mass spectrometer (6470B). TDP was found to interact with metal surfaces in the LC system, so the flow path was passivated using a deactivator solution which improved overall sensitivity and peak quality. TDP eluted in 3.15 ± 0.08 minutes; run times per column for a single stream and four streams were 9 minutes and 2.25 minutes, respectively. TDP was analyzed using positive electrospray ionization and multiple reaction monitoring mode with mass transitions of *m/z* 425.05 > 121.1 (quantifier) and *m/z* 425.05 > 304.00 (qualifier). **Results:** The method was linear from 20-1000 nmol/L with an R^2 >0.99. Replicate analysis of low calibrators to evaluate assay sensitivity demonstrated accuracy within 10% and imprecision of ≤5%. Spike-recovery experiments showed an accuracy of ±15%. TDP measured in purchased, matrix-matched quality control (QC) and expired proficiency testing materials met the specified range for all samples. The intra- and inter-day assay imprecision was ≤3%. The cross-analyte interference study showed the analyte response in the IS-only blank was less than 10% of the LLOQ area. Also, the IS response at the ULOQ was ≤5% of the analyte area. Repeated injections of calibrators and QC materials across the four LC streams showed excellent parity (<2% imprecision). Re-injection reproducibility of extracted samples at 24 and 48 hours was within ±2% of time zero concentration. Batch size tests indicated a high degree of robustness required to support the anticipated high testing volumes. **Conclusions:** An accurate, specific, and high-throughput LC-ESI-MS/MS method was developed and validated to measure TDP in whole blood. Simple, quick sample preparation was employed for adaption to a high-throughput platform. StreamSelect™ using four parallel LCs reduced injection times to 2.25 minutes per sample and significantly minimized mass spectrometer idle time. The assay performance evaluations met our laboratory-developed test acceptance criteria for use in routine clinical laboratory analysis.

B-178

Clinical routine ultrasensitive LC-MS/MS method development for analysis of 17β-Estradiol in human serum.

P. Y. TOKUYAMA, V. C. ROCHA, G. ZAMPIERI, P. S. OSORIO, A. C. LOPES. *DASA, SÃO PAULO, Brazil*

Background: The increasing clinical demand for the measurement of circulating 17β-Estradiol at very low concentrations consolidates the need of highly sensitive and specific analytical methodologies. These assays are suitable in situations such as antiestrogen therapy monitoring (use of aromatase inhibitor in breast cancer treatment); diagnosis of suspect inherited sex-steroid metabolic disorders; diagnosis of precocious and delayed puberty in female; hormone replacement therapy in postmenopausal women; and estrogen deficiency in men. Time-consuming methods and lack of sensitivity are common in the quantification of 17β-Estradiol in serum by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The strategy of chemical derivatization is common for analysis of estrogens because it enhances specificity and sensitivity, but this approach becomes laborious in the sample preparation process. For the purpose of solving these analytical challenges, the aim of this work was developing a reliable, sensitive and low-cost LC-MS/MS method without derivatization for clinical routine analysis. **Methods:** Isotope-labeled internal standard 17β-Estradiol-D5 were added to calibrators, quality controls (QC) and serum samples before a liquid-liquid extraction with the organic solvent Diethyl Ether. After reconstitution, the extracts were injected into a Biphenyl column 50x2.1mm, 2,6µm, chromatographic separated by solvent gradient (Ammonium Fluoride in Water – Acetonitrile) and detected in positive electrospray ionization mode. The analytical method was validated using reference material grade standards and spiked QCs in charcoal stripped pool patient serum at 4 levels by the assessment of linearity, limit of quantification (LoQ), precision inter and intra-assay, accuracy, carryover, method comparison, cross-reactivity and stability (freeze-thawing cycles, bench top, postoperative and stock solution). **Results:** The method has been validated with the analytical measurement range of 2 to 500 pg/mL. It was successfully optimized to achieve low levels of 17β-Estradiol as proposed with limit of quantitation of 2 pg/mL. Within-run and between-run precision (coefficient of variation) study yielded 1.3 – 7.2% and accuracy within 95.7 – 112.1%. No carryover, matrix effect and significant cross-reactivity was observed. Estradiol was found to be stable in serum samples under 3 freeze/thawing cycles, 4 hours at ambient temperature and 72 hours after extraction procedure in

auto-injector. The stability of stock solutions was tested in -20ºC freezer and remained stable for 1 month. Method comparison between two reference laboratories (LC-MS/MS method) was performed and the results

showed acceptable data for regression statistics (slope (a) =0.9184; intercept (b)=-0.1576), t-test statistics (bias=8,5%) and difference plot. **Conclusion:** An ultrasensitive and derivatization-free LC-MS/MS method was developed and it may be beneficial for patients with endocrine disorders associated with estradiol levels.

B-179

Development of a Reflex Mass Spectrometric Workflow for Confirmation of IgE and IgD Specificity Paraproteins Utilising the EXENT® Platform

N. Wright[1], W. Bermingham[2], A. Rollins[1], O. Berlanga[1], S. Pasha[1], S. North[1], G. Wallis[1], S. Harding[1]. *[1]The Binding Site, Birmingham, United Kingdom, [2]Immunology, Heartlands Hospital, Birmingham, United Kingdom*

Background: The EXENT® (GAM) assay is a MALDI-TOF (Matrix Assisted Laser Desorption Ionisation Time of Flight) mass spectrometry-based solution which utilises IgG, IgA, IgM, total kappa and total lambda specific paramagnetic beads, which could be used in the investigation of monoclonal proteins in multiple myeloma and other plasma cell disorders (in development by The Binding Site (TBS), part of Thermo Fisher Scientific). Rare cases of IgD and IgE myelomas will be partially typed with the current iteration of the EXENT (GAM) assay. We present here a workflow to confirm the heavy chain specificity of such paraproteins as IgD or IgE using reflex LC-MS analysis of EXENT eluates.

Methods: Samples known to contain IgD (2) or IgE (3) paraproteins were provided from either routine workload at the immunology laboratory at Birmingham Heartlands Hospital, UK or an internal library of historical myeloma samples. Capillary zone electrophoresis (CZE) and immunofixation (IFE) were performed on Capillarys2™ and Hydrasys2 Scan™ platforms, respectively (Sebia, France). Serum free light chain (sFLC) measurement was performed using Freelite® reagents (The Binding Site, UK) on a Cobas c501 turbidimeter (Roche, Switzerland). Samples were prepared via EXENT (GAM) assay, by separately incubating diluted serum with antisera specific for IgG, IgA, IgM, total kappa and total lambda conjugated to paramagnetic beads. After washes, purified immunoglobulins were treated to reduce and elute the light chain component. Samples were mixed with matrix, spotted onto a MALDI target plate and acquired by MALDI-TOF MS. Once light-chain specificity of the suspected IgD or IgE paraprotein was determined, repeat immuno-precipitation with the same light-chain bead was carried out. Eluted samples were neutralised, transferred to fresh tubes, reduced, alkylated and digested with trypsin for 3 hours. After acidification digested samples were analysed by C18 reverse phase UPLC on a BioAccord LC-MS system (Waters, UK). Data was then screened against a database of known immunoglobulin heavy chain peptides to assess the presence of heavy chain peptides specific for each immunoglobulin isotype.

Results: In each sample tested, the EXENT solution identified at least one monoclonal light chain that was only seen in the total kappa or total lambda immunoprecipitations with no corresponding monoclonal peak(s) at the same mass(es) in the IgG, IgA or IgM specificities. This finding would be consistent with the presence of clonal plasma cells producing either monoclonal free light chains only, or IgD and IgE paraproteins. Subsequent LC-MS and LC-MS^e (tandem MS) analysis of reduced, alkylated and tryptically digested eluates corresponding to these suspected IgD and IgE samples produced high quality peptide sequencing data. The data identified multiple IgD or IgE specific peptides in each sample and showed between 40-66% sequence coverage for IgD or IgE heavy-chain constant regions. Thus, confirming the presence of IgD or IgE in these samples which had been partially typed by the EXENT solution.

Conclusion: This data demonstrates that the EXENT (GAM) assay could potentially aid in detection of IgD and IgE paraproteins when combined with LC-MS proteomic techniques, as it allows efficient and facile confirmation of these rare heavy chain isotypes.

B-180

The EXENT® Solution Provides Evidence for High Prevalence of Multiple M-proteins in Monoclonal Gammopathies

S. Allen, J. Birtwistle, O. Berlanga, G. Malin, G. Lakos. *The Binding Site, Birmingham, United Kingdom*

Background: Monoclonal gammopathies (MG) are characterized by excessive production of monoclonal immunoglobulin (M protein), typically consisting of a heavy chain (HC) and a light chain (LC), or a LC only. In some patients, however, more than one restricted band may be present on serum protein electrophoresis. These additional M proteins may be present at initial diagnosis or emerge later following observation or treatment, especially autologous stem cell transplantation. Immunofixation electrophoresis may not be able to differentiate between two bands of the same isotype or other sources of an additional M protein, such as polymerization, glycosylation or therapeutic monoclonal antibody. Mass spectrometry (MS) has recently emerged as an analytically sensitive technology for the detection of M proteins. The EXENT® solution (in development by The Binding Site, part of Thermo Fisher Scientific) uses immuno-purification of immunoglobulins, followed by matrix-assisted laser desorption ionization time of flight (MALDI-ToF) MS analysis. The automated, algorithm-driven analysis of the resulting mass spectra determines the isotype, the molecular mass and the quantity of M proteins. The molecular mass of the M protein serves as a "fingerprint" and allows for easy differentiation between multiple M proteins. The goal of this cross-sectional study was to assess the prevalence of biclonal and multiclonal M proteins in monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), multiple myeloma (MM), Waldenstrom macroglobulinemia (WM) and AL amyloidosis with mass spectrometry.

Methods: Diagnostic (baseline) samples from 271 patients (35 MGUS, 66 SMM, 125 MM, 31 WM and 14 AL amyloidosis) were analyzed with EXENT® using the EXENT® Immunoglobulin Isotypes reagents. A second M protein was considered distinct from the primary M component if it had a different HC or LC component, or, if it had the same isotype, but had a different molecular mass. Samples producing an additional high-mass peak attributed to LC glycosylation have not been considered biclonal.

Results: The EXENT® solution has identified a second M protein in 201 (74%) samples, although the second M protein was below the lower limit of the measuring interval in 107 (39%) cases, and only 36 (13.5%) was ≥ 0.2 g/L: 5 out of 35 in MGUS, 10 out of 66 in SMM, 13 out of 125 in MM, 8 out of 31 in WM and none in AL amyloidosis. Among these, the second M protein had the same isotype in 17 cases and contained a different HC or LC or both in 19 cases. IgG was the most prevalent; the distribution of kappa and lambda LC was 11:13. More than two M proteins were identified in 120 (44%) patients, in 12 (4.5%) cases with a concentration of ≥ 0.2 g/L.

Conclusion: The prevalence of two or more M proteins was higher than the previously reported 3-6%. The higher prevalence is likely the result of the ability of the system to detect small monoclonal peaks that have previously gone unrecognized by traditional methods, and to distinguish between M proteins of the same isotype based on molecular mass.

B-181

Multi-centre Analytical Performance Evaluation of the EXENT^{â} Solution for Identification and Quantitation of Monoclonal Immunoglobulins

R. Stanton[1], D. McEntee[1], D. Matters[1], S. Ijaz[1], A. Khalid[1], D. Barnidge[2], L. Ouverson[2], D. Sakrikar[2], J. Ashby[1], N. Marrott[1], I. M. Ghobrial[3], H. El-Khoury[3], L. Bertamini[3], G. Fleming[3], E. Horowitz[3], A. S. Neish[4], Z. M. Solis[4], K. B. McLendon[4], S. North[1]. *[1]The Binding Site, Birmingham, United Kingdom, [2]The Binding Site, Rochester, MN, [3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, [4]Emory University School of Medicine, Atlanta, GA*

Background: Current routine methodologies for monoclonal immunoglobulin measurements may not be sufficiently sensitive to reflect the depth of response seen in patients since the introduction of novel therapies; mass spectrometry may offer a valuable, sensitive alternative approach. Here we describe the preliminary analytical performance characteristics of the EXENT® solution (in development by The Binding Site, part of Thermo Fisher scientific) that combines specific immunoprecipitation steps and mass spectrometry for the identification and quantification of IgG, IgA and IgM intact monoclonal immunoglobulins. Each intact monoclonal immunoglobulin clone can be tracked using its unique m/z value.

Methods: The Lower Limit of Measuring Interval (LLMI) was established for each immunoglobulin type following EP17-A2:2012. Linearity studies were performed according to CLSI EP06-A2:2020 using high and low pools of IgG, IgA, and IgM monoclonal samples and with additional linearity testing below 1 g/L for each specificity to more effectively demonstrate low-end linearity. Within run, between run, between analyzer, between lot and total precision for M protein concentrations and for molecular mass (m/z) of the monoclonal peaks were assessed according to CLSI EP5-A3-2014. Interference was tested following ED3:2018 using 20 potential interferents against 7 samples including high and low IgG, IgA and IgM monoclonal samples.

Results: The initial indication of the EXENT solution performance, in development, are set out below. Results suggest an analytical sensitivity of the assays around 15 mg/L at the LLMI for all specificities. Linearity over a range of 0.014-88.9 g/L for IgG, 0.011 - 68.4 g/L for IgA, and 0.11-74.2 g/L for IgM. Coefficients of variation (CVs) for M protein concentrations in precision studies were <15% for all specificities and samples. Mass/charge values were within ±1.1 to ±1.5 m/z in total precision studies, and within ±2.7 to ±3.9 m/z in between lot precision studies, respectively, for M proteins with an m/z value ranging from 11,360.6 to 11,698.5 m/z. No significant interference effects were observed when testing the 20 interferents including intralipid (20 g/L), triglyceride (15 g/L), bilirubin (400 mg/L), rheumatoid factor (200 IU/mL) and haemoglobin (10 g/L).

Conclusion: The new EXENT solution demonstrates the potential for a wide measuring interval and the ability to detect M proteins with very low concentration. Also, it could provide stable and reproducible performance for the detection and typing of monoclonal immunoglobulins.

B-182

Advantage of Core-Shell Particle Morphology Over Fully Porous HPLC Column for the Analysis of Vitamin K Compounds by LC-MS/MS

S. W. Huq, Z. Aqeel. *Phenomenex, Torrance, CA*

Background: Vitamin K's are a group of structurally similar, fat-soluble compounds. There are two natural forms K1 (Phylloquinone) and K2 (Menaquinone), where the later has short and longer chain homologues called Vitamin K2-MK-4 and Vitamin K2-MK-7 respectively. Due to the extremely hydrophobic nature and poor ionization characteristics of these compounds, LC-MS/MS analysis at low concentrations becomes challenging. In this communication we make parallel comparisons of three HPLC columns packed with fully porous and core-shell particles to showcase the superiority of core-shell columns for sensitive detection of vitamin K analytes by LC-MS/MS. Method: We compared fully porous (Gemini and Luna, Phenomenex, Torrance, CA) and core-shell (Kinetex EVO, Phenomenex) C18 columns for this study. All columns evaluated were 50x2.1 mm with a 5µm particle size to maintain low back pressure for the viscous organic modifier utilized. Deionized water and a combination of isopropanol and acetonitrile (1:1; v/v) were used as mobile phase A and B respectively for chromatographic separation. An Agilent 1290 Infinity series LC system was employed with a SCIEX 6500 QTRAP mass spectrometer under APCI for MS detection. Analyst software 1.7.2 used for data analysis. Results:The LC-MS/MS method had a 4-minute runtime for chromatographic separation of the analytes including 1.5 minutes of equilibration time. The comparison data revealed the Gemini C18 column shows some higher selectivity and resolution values, whereas both EVO and Luna C18 columns were somewhat comparable. However, the core-shell EVO C18 column displayed much higher efficiency with narrower and compressed peak widths, which dramatically increased the peak height for all 3 analytes exhibiting the highest signal/noise ratio. Conclusion:The core-shell Kinetex 5µm, EVO C18 column with its unique organo-silica grafting shows much higher efficiency and sensitive detection capability for vitamin K analysis by LC-MS/MS compared to that of conventional fully porous silica particles.

B-183

The Impact of 3-EPI-25-Hydroxyvitamin D on 25-Hydroxyvitamin D Measurement by Liquid Chromatography/Mass Spectrometry

K. A. Turner, T. W. Radecki, E. L. Boughner, K. D. Bolhuis. *Corewell Health, Grand Rapids, MI*

Background: 25-hydroxyvitamin D is the recommended target to evaluate vitamin D status. The accurate measurement of 25-hydroxyvitamin D by liquid chromatography/mass spectrometry (LC/MS/MS) is impacted by the presence of the biologically inactive epimer of 25-hydroxyvitamin D2 and D3**,** 3-EPI-25-hydroxyvitamin D2 and D3. The epimers are isomers and share the same mass and fragment transitions as 25-hydroxyvitamin D2 and D3. For an accurate measurement the epimer should be chromatographically separated from 25-hydroxyvitamin D. Herein we assess the significance of 3-EPI-25-hydroxyvitamin D in our patient population.Methods: A LC/MS/MS method was developed to chromatographically separate 25-hydroxyvitamin D2 and D3 from 3-EPI-25-hydroxyvitamin D2 and D3 on a Waters Xevo TQ-S Micro with an Acquity UPLC System using a 2.6 µm Pentafluorophenyl (PFP) column (Phenomenex). There were 231 residual serum samples analyzed.Results: The cohort tested consisted of 16 infant samples (<1 years), 62 pediatric samples (1-17 years), adult samples (≥18 years), and 20 pregnant samples. 3-EPI-25-hydroxyvitamin D2 was not detected in any sample analyzed (<1 ng/mL). The prevalence and concentration of 3-EPI-25-hydroxyvitamin D3 was variable (Figure 1). Conclusion: The specificity of 25-hydroxyvitamin D measurement by LC/MS/MS is not affected by 3-EPI-25-hydroxyvitamin D2 as it is not present in our patient population. However, 3-EPI-25-hydroxyvitamin D3 is a significant interference. The presence of 3-EPI-25-hydroxyvitamin D3 is variable with the highest prevalence and concentration in infants. The prevalence and concentration decrease with age and is minimal in pregnancy. For infant and pediatric samples, an assay that chromatographically separates 3-EPI-25-hydroxyvitamin D3 from 25-hydroxyvitamin D3 is needed. For adult samples, it may be appropriate not to separate 25-hydroxyvitamin D3 from the epimer. For this patient population a balance between accuracy and throughput must be established.

	EPI-D3	EPI-D3 (ng/mL)			EPI-D3/D3 (%)		
	Positivity (%)	median	min	max	median	min	max
All samples	32	2	1	23	3.4	1.3	72.0
<1 years	56	2	1	18	10.5	2.2	72.0
1-17 years	21	3	1	23	6.5	1.9	44.2
≥18 years	33	2	1	9	2.5	1.3	16.1
Pregnant	10	1.5	1	2	4.1	3.1	5.1

B-184

Development and validation of a high-performance liquid chromatography-mass spectrometry method for analyzing phenolic acids metabolites in feces

B. Odat, N. Abdelkader. *Cleveland State University, Cleveland, OH*

Background Phenolic acids are beneficial metabolites produced by Co-metabolism between a human host and the gastrointestinal microbiota. They play a part in signaling pathways involved in intestinal mucosa homeostasis. The synergic relationship between the microbiota and the nutrient in the gut is associated with the content of phenolic compounds found in the gut system. Recent studies have proven that phenolic acids are linked to weight loss, but such an association needs to be better understood.This research aims to develop and validate a Liquid Chromatography tandem Mass spectrometry (HPLC-MS/MS) method for quantifying phenolic acids in human fecal samples according to the food and Drug Administration (FDA) guidelines. Moreover, the research focuses on the synergic relationship between the gut microbiota and the prebiotics, and their ability to enhance the production of phenolic acids, as an increase in the phenolic acid level in the gut may reduce obesity. **Methods** high-pressure liquid chromatography is used to separate the analytes (ferulic acid, gallic acid, and caffeic acid) based on polarity, and AB SCIEX QTRAP triple quadrupole Tendon mass spectrometer is used to conduct mass spectroscopy using electrospray ionization. Solid phase extraction method was used to extract and purify the sample with C18 SPE cartridges before analysis. Quantitative analysis was performed by Analyst software. The developed LC-MS/MS method was applied to fecal samples collected from healthy populations. For testing the influence of prebiotics on the production of phenolic acids. Samples of gut microbiota (lactobacillus acidophilus) mixed with different prebiotics were monitored to measure the outcomes of phenolic acids. Samples were compared with other prebiotics such as fiber, cellulose, and inulin. Target analytes meeting significance of ($p < 0.05$) will be verified by the development of a targeted LC-MS/MS (MRM) assay. Once compounds of significance have been verified, the method will be evaluated based on the guideline from 2018, Bioanalytical method validation guidance for industry; several parameters must be considered when validating a method. These parameters include selectivity, accuracy, precision & recovery, linearity, sensitivity (LOD and LOQ), stability, and matrix effect. Validation will be applied to the stool samples from an average healthy population. **Results** Phenolic acids were extracted and quantified using a simplified HPLC-MS/MS, with acidified methanol. A total of five phenolic acids with different concentrations were identified in different fecal samples. The method was used to determine the association between gut microbes and nutrient sources and their influence on the production of phenolic acids when mixed. Initial preliminary data indicate that adding prebiotics to a probiotic media increases the peak intensity suggesting an expansion of the quantity of phenolic acid in the mixture. In addition, changing the amount or the type of prebiotics affects the outcomes. Fiber shows the most significant outcomes when compared to other prebiotics. **Conclusion** Based on this research's findings, certain types of Prebiotics may enhance the generation of phenolic acid quantity through co-metabolism between a human host and Probiotics in the gut. Fiber showed the most significant outcomes of phenolic acid formation compared to inulin and cellulose.

B-185

High Throughput ID-LCMSMS Method for Simultaneous Quantitation of Monosaccharides in Human Serum

C. Y. Tse[1], L. Zhang[1], K. Dahya[1], F. Pokuah[1], O. Sugahara[1], V. Delatour[2], U. Danilenko[1], H. W. Vesper[1]. *[1]Centers for Disease Control and Prevention, Atlanta, GA, [2]Laboratoire national de métrologie et d'essais (LNE), Paris, France*

Background: Blood glucose is one of the most common clinical tests used for diagnosis and treatment of diabetes, with clinical analyzers being the typical instrumentation used in the clinical laboratory. While glucose is an important monosaccharide for management of diabetes, dietary fructose has shown associations with non-alcoholic fatty liver disease (NAFLD). However, only limited data on serum fructose in the general population are available. Clinical analyzers that can measure glucose and other monosaccharides simultaneously from human serum are unavailable. A high throughput ID-LC/MS/MS method is needed to allow simultaneous quantitation of monosaccharides from human serum to help better understanding the associations of monosaccharides with different diseases. **Methods:** Calibrators, serum samples, and internal standards were transferred and subsequently processed on an automated liquid handler platform. Acetonitrile was added to precipitate monosaccharides from serum matrix and the precipitate was removed through filtration with 96-well protein removal plate. Clean extracts were injected on a LC-MS/MS system using a LC gradient consisting of acetonitrile and water. Quantitation was performed using stable-isotope labeled standards. Certified reference material NIST glucose SRM 917c and commercially available fructose, mannose and galactose were used for calibration. The analytical performance was assessed using NIST glucose SRM 965b serum materials and commercial serum material with monosaccharides spiked at different levels. **Results:** The method demonstrated high level of specificity for glucose, fructose, mannose, and galactose with all monosaccharides being chromatographically baseline-resolved within 9 minutes. No interferences were detected when monitoring the QI/CI ratio in commercial serum materials. The method is highly linear (all R^2 = 0.999) between 10 – 380 mg/dL and 0.04 – 12 mg/dL for glucose and other monosaccharides, respectively. The limit of detection was 0.19 mg/dL for glucose and 0.02 to 0.03 mg/dL for galactose, mannose, and fructose. For glucose, the imprecision and bias, determined with NIST SRM 965b measured over 5 days in duplicate, <4%CV and -1.44%, respectively. Inter-day imprecision, determined with samples at concentrations between 0.05 mg/dL to 9 mg/dL, was between 4 and 12%CV for all monosaccharides. The method demonstrated good agreement when compared with the CDC glucose GC-MS RMP with an intercept and slope of -1.7 (CI -4.05 – 0.38) and 1.03 (CI 1.01 – 1.06), respectively. The Pearson's coefficient is 0.999. This method allows for measurement of 85 samples in one batch in a semi-automated manner. **Conclusion:** The described high throughput LC/MS/MS method for simultaneous quantitation of four monosaccharides is sufficiently sensitive, selective, accurate and precise for measuring monosaccharides in epidemiological studies. Its suitability as comparator method for method comparison studies with regular clinical analyzers and point-of-care devices are currently ongoing.

B-186

Investigating LC Column Selectivity for 19 Steroids in a Clinical Research Setting

M. Freige, S. J. Marin. *Phenomenex, Torrance, CA*

Background: Steroids are a class of structurally related endogenous compounds. Several have the same chemical formula (and hence the same m/z) and must be chromatographically separated for accurate identification and quantitation by LC-MS/MS. Here, six bonded silica solid core or "core-shell" LC columns (see table 1) were evaluated to determine selectivity and optimum separation conditions for a 19 analyte steroid panel for clinical research. The optimum column for the separation of estrone and estradiol was also established.

Methods: Analytical reference standards (Cerilliant®, Round Rock, TX) were used to prepare diluted samples for injection. Six different core-shell 2.6µm, 50x2.1 mm LC columns (Kinetex™, Phenomenex, Torrance, CA) were evaluated (see table 1). Detection employed a 7500 triple quadrupole LC-MS/MS equipped with an ESI source used in positive and negative mode (SCIEX®, Framingham, MA) coupled to an Agilent® 1290 liquid chromatograph (Agilent Technologies, Santa Clara, CA). Table 1. Columns evaluated for a panel of 19 steroids

Column	Phase Chemistry	Property
C18	C18	Traditional bonded silica C18
EVO C18	modified C18	1-12 pH stable bonded C18
Polar C18	C18 modified with polar groups	100% aqueous stable, improved polar analyte retention
XB-C18	C18 modified with di-butyl side chains	Inactivated silica surface, altered selectivity
Phenyl-hexyl	Silica modified with phenyl-hexyl groups	Improved aromatic selectivity
Biphenyl	Silica modified with biphenyl groups	Improved aromatic and polar analyte selectivity

Results: The traditional C18 column worked best for the complete steroid clinical research panel. Estrogens had the best separation on the biphenyl phase, which demonstrated the highest level of selectivity for estrone and estradiol. The phenyl-hexyl column did not sufficiently separate all steroid isomers in the clinical research panel, however it was sufficient to separate estrone and estradiol.

Conclusion: The proposed HPLC conditions and the core-shell 2.6 µm 50x2.1 mm C18 column provided the best separation for 19 steroids with a fast 8 minute run time. For research focused on estrogens, the core-shell biphenyl column yielded the best separation.

B-187

Evaluation of clinical utility of LC-MS/MS method for thyroglobulin measurement in comparison with immunoradiometric assay and chemiluminescence microparticle immunoassay

E. Yoon[1], S. Kim[1], H. Oh[1], H. Park[2], S. Kim[2], S. Lee[1]. *[1]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [2]Division of Endocrinology and Metabolism, Department of Medicine, Thyroid Center, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of*

Background: Thyroglobulin (Tg) is the follow-up index of choice for patients with differentiated thyroid cancer (DTC), especially who were undergone total thyroidectomy. However, due to the commonly used immunoassay's inherent susceptibility to interference combined with the high prevalence of thyroglobulin antibody (TgAb) in DTC patients, the need for more reliable measurement for Tg independent from TgAb has been brought up. In this study, we developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for Tg measurement and compared its clinical usefulness and analytical performance with immunoradiometric assay (IRMA) and chemiluminescence microparticle immunoassay (CMIA).

Methods: We developed trypsin digestion-peptide immunoaffinity LC-MS/MS methods for Tg measurement. Analysis was performed using an LC-MS/MS system consisting of Agilent 1290 LC (Agilent Technologies, Santa Clara, CA) and Qtrap 5500+ MS/MS system(ABSciex, Framingham, MA). Tryptic Tg specific peptide (FSPDDSAGASALLR) and cleavable internal standard peptide (LSFYWTTTGDPF FDSHSDSLL*RSGPYMPQ, L*: 13C6, 15N) were quantitated against 5-point linear calibration curve with Access Thyroglobulin Calibrators (Beckman Coulter, Pasadena, CA). Three levels of serum control were prepared (3.95, 12.5, and 39.4 ng/mL). Lower limit of quantitation (LLoQ), linearity, precision, and carryover were validated for the assay. For method comparison, serum from patients with DTC or Hashimoto thyroiditis was collected. Tg was also measured with IRMA using BRAHMS TG Plus (Thermo Fisher Scientific, Waltham, MA), and CMIA using Architect Thyroglobulin (Abbott laboratories, Illinois, CA). TgAb was measured with IRMA using BRAMS Anti-Tg (Thermo Fisher Scientific) and the cut-off for TgAb was 60 IU/mL. Also, interference of TgAb was evaluated by comparing the Tg recovery of five sets of TgAb negative samples spiked with diluents and high TgAb samples.

Results: Within-run and between-run imprecisions were CV 7.8-13.0% and CV 8.9-12.7%, respectively. LLoQ was 0.5 ng/mL. The assay was linear through 0.5-650 ng/mL. No significant carryover was observed within the analytical measurement range. Out of the total 193 samples, the number of samples above LLoQ in both LC-MS/MS, and IRMA or CMIA were 73 samples (45 TgAb-positive and 28 TgAb-negative samples) and 69 samples (42 TgAb-positive and 27 TgAb-negative samples), respectively. The result well correlated between LC-MS/MS and IRMA (R2=0.99), and LC-MS/MS and CMIA (R2=0.98) in overall samples. When analyzed according to TgAb status, LC-MS/MS and IRMA (R2=0.84), and LC-MS/MS and CMIA (R2=0.93) correlated rather poorly in TgAb-positive samples, while TgAb-negative samples were consistent (R2=0.99 for IRMA, R2=0.97 for CMIA). We found one case with systemic metastatic thyroid cancer that showed significantly high Tg levels in

IRMA and LC-MS/MS (above the upper limit of quantitation), whereas below LLoQ in CMIA. The interference of TgAb was negligible in LC-MS/MS, while significant interference was observed in CMIA, with a mean recovery rate of 57.6% (4.3-82.5%). In one sample, when measured with CMIA, thyroglobulin level was 3.29 ng/mL when mixed with diluent, but below LLoQ (0.14 ng/mL) when spiked with TgAb-positive samples (TgAb 1700 IU/mL).

Conclusion: The LC-MS/MS method for Tg measurement was less prone to antibody interference than immunoassays. Measurement of Tg using LC-MS/MS would be useful for reliable diagnosis and proper management in TgAb-positive patients.

B-189

Evaluate the suitability of urinary reference intervals for organophosphate flame retardants in healthy adult population from Southern Taiwan

C. M. Chen[1], H. L. You[2], W. T. Huang[2]. *[1]Kaohsiung Chang Gung Memorial Hospital, Kaohsiung City, Taiwan, [2]Chang Gung Medical Foundation Kaohsiung Branch, Kaohsiung City, Taiwan*

Background: Organophosphorus flame retardants (OPFRs) are chemical additives used to prevent fires and are widely used in furniture, electronics, plastics, and building materials. Its chemical structure is similar to human hormones, which are often misidentified and used in the human body, thereby interfering with the operation of normal physiological functions and causing endocrine system disorders, so it is also known as environmental hormones. Recently, it has been confirmed that exposure to OPFRs can cause health hazards such as neurotoxicity, endocrine disruption, hepatotoxicity, and reproductive dysfunction. In view of the increasing concern about the exposure of human OPFRs, but there is no laboratory in Taiwan to detect the content of human OPFRs. This study aims to develop a biomonitoring method for OPFRs, which is used to provide reliable information on the exposure status of individual OPFRs, and to establish a reference interval for OPFRs in the urine of different diseases and reference groups and confirm the potential of OPFRs as biomarkers in environmental medicine. **Methods:** The Waters ACQUITY UPLC I-Class and Xevo TQ-XS IVD systems were developed to detect 10 OPFRs in urine: Tri-(n-butyl) phosphate (TNBP), Tris-(2-butoxyethyl) phosphate (TBEP), Tris-(1,3- dichloro-2-propyl) phosphate (TDCPP), Triphenyl phosphate (TPHP), Tris-(2-chloroethyl) phosphate (TCEP), Di-n-butylphosphate (DNBP), Bis-(butoxyethyl)-Phosphate (DBEP), Bis-(1,3-dichloro-2-propyl) phosphate (BDCPP), Diphenyl phosphate (DPHP), Bis (2-chloroethyl) phosphate (BCEP), and analytical methods. According to the CLSI C62A specifications, the methodology verification and confirmation passed the evaluation. A total of 538 patients (78 infertility, 167 hemodialysis, 96 esophageal cancer, 99 lung cancer and 98 endometrial cancer) and 175 reference groups were tested for urine samples. The ANOVA analysis was used to compare the differences in the content of 10 OPFRs compounds in the urine of subjects of different ethnic groups. ROC curve analysis was used to determine the best decision value of whether the disease was present, and Chi-square verification was used to conduct disease risk assessment and verify the feasibility of the set reference value. **Results:** The BCEP (6.23±2.19 ng/mL) of most patients with hemodialysis, lung cancer and esophageal cancer was significantly higher than the reference group (0.89±0.38 ng/mL). Patients with gynecological diseases had a higher BDCPP (4.29±1.97 ng/mL) than the reference population (0.76±0.32 ng/mL), which was consistent with the conclusions of previous animal experiments. Since it is not easy to explain the direct correlation between OPFRs and the disease. We decided to sum the detection values of 10 OPFRs. We found that their sum was greater than 2.05 ng/mL. The risk of infertility was 37.8 (95% CI: 11.07~129.14), the risk value of esophageal cancer was 83.3 (95% CI: 25.25~274.85) and the risk value of severe chronic kidney disease was 56.7 (95% CI: 17.49~183.97). **Conclusion:** Due to the different types of OPFRs used in different countries, it is impossible to directly apply the reference values of other countries to their own people. So we develop the OPFRs detect method and verify the reference values through a series of analysis and discussion. Which will help us further understand the association between these environmental hormones and diseases.

B-190

Development of an LC-MS/MS Method for Analysis of 7α-hydroxy-4-cholesten-3-one and Investigation Into its Utility as a Biomarker for Bile Acid Diarrhoea in the United Kingdom

L. Starbrook[1], A. Kumar[2], C. Ford[1], M. Brookes[2], R. Gama[1]. *[1]Black Country Pathology Services, Wolverhampton, United Kingdom, [2]New Cross Hospital, Wolverhampton, United Kingdom*

Background: Bile Acid Diarrhoea (BAD) is a common gastrointestinal condition estimated to affect 1% of the population. It is, however, significantly under-diagnosed. This is partly due to lack of diagnostic methods. In the United Kingdom (UK) the 75-SeHCAT retention scan is considered the gold-standard diagnostic method and is the only routinely available diagnostic test for BAD. SeHCAT retention of <15% is considered consistent with BAD. The SeHCAT test is, however, expensive, time consuming, and exposes the patient to a dose of radiation. 7-alpha-hydroxy-4-cholesten-3-one (7αC4) is a precursor in the bile acid synthesis pathway and has demonstrated utility as a marker of BAD. There is limited data comparing 7αC4, which is measured in the United States, to the SeHCAT test, as SeHCAT is not licensed in the United States. 7αC4 can be measured by LC-MS/MS, thus can provide a more convenient, low cost and high throughput option for diagnosis of BAD.

Aims: 1) To develop and validate an LC-MS/MS method for analysis of 7αC4. 2) To analyse serum samples taken from patients referred for SeHCAT scans. 3) To determine the utility of 7αC4 compared to SeHCAT for diagnosis of BAD.

Methods: The method was adapted from Donato *et al*. 750ul of ice-cold acetonitrile containing 50nmol/L D7-7αC4 as internal standard was added to 250ul serum. Samples were vortexed for 30 seconds, centrifuged at 3000g for 10 minutes, and supernatant transferred into a vial for LC-MS/MS analysis. The LC-MS/MS system utilised a Gemini 5um NX-C18 column coupled to a Sciex 6500 TripleQuad mass spectrometer. Ethical approval was obtained to collect serum on patients undergoing SeHCAT retention scans for investigation of BAD. 40 patients who attended for SeHCAT scans had serum collected and stored at -80 degrees Celsius until analysis for 7αC4. Sensitivity and specificity of 7αC4 was calculated and ROC curve analysis performed.

Results: The 7αC4 method demonstrated an LoQ of 1.5 nmol/L, linearity up to 1095 nmol/L, and intra-assay %CV of 3.0 - 7.7%. Using SeHCAT retention of <15% as positive for BAD, ROC curve analysis demonstrated an AUC of 0.765. 7aC4 at a concentration of 175 nmol/L provided a sensitivity of 30% and a specificity of 92%. 7αC4 at a concentration of 50 nmol/L provided a sensitivity of 93% and a specificity of 46%.

Conclusion: The 7αC4 method demonstrated good analytical performance and was suitable for use in a routine laboratory. 7αC4 results of <50 nmol/L suggest BAD is unlikely, and results of >175 nmol/L make a diagnosis of BAD likely. Results in between these two values may be considered indeterminate and referral for SeHCAT scanning may be considered appropriate. The 7αC4 assay provides an opportunity to stratify patients for SeHCAT testing in the UK, improving diagnosis and management of BAD in patients with chronic diarrhoea and streamlining the patient pathway.

B-191

Development of a Simple, Robust, and Accurate LC-MS/MS Method for the Quantification of 17 Therapeutic Drugs: Initial Validation Results

D. A. Payto, X. Tang, R. Giles, R. Wei, J. M. Colón-Franco. *Cleveland Clinic, Cleveland, OH*

Background: Therapeutic Drug Monitoring (TDM) of Zonisamide (ZON), Felbamate (FELB), Lamotrigine (LMTR), 10-Hydroxycarbamazipine (OXCARB), Carbamazepine Epoxide (CARBEP), and Pentobarbital (PENTO) is essential in developing the regimen to produce the best clinical outcomes while monitoring for possible toxicity. Historically, both HPLC-UV and LC-MS/MS have been used for the quantification of these types of drugs. HPLC-UV is often subject to interferences and extended run times. Because of this, there has been a shift to LC-MS/MS methods which are often simple, fast, robust, and suffer from less interferences. Our objective was to develop a simple, robust, and accurate LC-MS/MS method to replace the HPLC-UV methodology. **Method:** Serum (50 µL) and IS solution (100 µL; Zonisamide-13C6, Lamotrigine-13C,15N4, (±)-10,11-Dihydro-10-hychoxycarbamazepine-13C6, Carbamazepine-10,11-epoxide-13C6, Felbamate-D4, Pentobarbital-D5 in methanol with 0.5% acetic acid) were vortex mixed and centrifuged. Supernatant (50 µL) was mixed with 950 µL of water and vortexed mixed. Extract was injected on a Thermo Accucore C18 column. A quantifier and qualifier transition was monitored for all analytes. Multiple Reaction Monitoring (MRM) was performed in positive mode for all analytes except for PENTO (negative). Total chromatographic time for the 17 analytes is 7.31

minutes. **Results:** No ion suppression, differential matrix effect, or interferences were observed. Analytical Measurement Range (AMR) data for the initial 6 analytes is presented in table 1. The coefficient of variation (%CV) was <2.8% for ZON, <4.3% for FELB, <7.3% for LMTR, < 3.8% for OXCARB, <3.0% for CARBEP, and <3.5% for PENTO. Method comparison with a previously validated method were acceptable (Table 1). **Conclusion:** This simple, robust, and accurate LC-MS/MS method has been validated for the initial 6 anlytes and has been determined to be acceptable for clinical use. Validation of the remaining 11 analytes is ongoing (Pregabalin, Gabapentin, Levetiracetam, Ethosuximide, Lacosamide, Rufinamide, Primidone, Lamotrigine, Topiramate, Brivaracetam, and Perampanel)

Table 1: Method Validation Data

	ZON	FELB	LMTR	OXCARB	CARBEP	PENTO
Retention Time (min.)	1.41	2.10	2.26	2.69	2.72	3.46
AMR (µg/mL)	2.5-100.0	2.0-150.0	0.5-30.0	0.5-100.0	0.5-10.0	2.0-100.0
AMR Recovery (%)	100-118	91-108	97-112	82-102	108-116	94-111
Correlation Coefficient (R)	0.9969	0.9931	0.9918	0.9957	0.9955	0.9655
Bias (%)	-3.4	-5.3	1.1	-5.5	0.2	-8.9

B-192

An LC-MS/MS Method using the Xevo TQ Absolute Mass Spectrometer for the Combined Analysis of Aldosterone and Plasma Renin Activity in Plasma for Clinical Research

R. Wardle, S. Balloch, G. Hammond, D. Foley, L. Calton. *Waters Corporation, Wilmslow, United Kingdom*

Background: Primary aldosteronism (PA) is a common cause of hypertension, whereby uncontrollable amounts of aldosterone are produced by a benign tumour or hyperplasia of the adrenal glands. Excess aldosterone results in significant sodium reabsorption in the kidneys, increasing water retention and blood volume, thereby causing hypertension. The renin-angiotensin-aldosterone system (RAAS) regulates the production of aldosterone and in this system, renin and aldosterone should move in synchronicity with each other throughout the day. Therefore, these two components are used to assess the status of the RAAS, particularly in the evaluation of new therapies in clinical research studies. Historically, the assessments of aldosterone and plasma renin activity (PRA) have been performed using separate methods using immunoassay or more recently liquid chromatography - tandem mass spectrometry (LC-MS/MS) platforms. One of the benefits of using LC-MS/MS for clinical research is the ability to measure multiple analytes across the proteome and metabolome using the same system and even in the same analysis to provide more information in less time and save costs. Here we evaluate a single LC-MS/MS method for the combined measurement of plasma aldosterone and renin activity for clinical research purposes. **Methods:** Aldosterone certified reference material (Merck, UK) and Angiotensin I (Cambridge BioScience, UK) were used to create calibrators in 2% Bovine Serum Albumin (BSA) in Phosphate Buffered Saline (PBS). In-house QC material prepared in both 2% BSA in PBS and K_2EDTA plasma (BioIVT, UK), were used to evaluate method precision. Plasma samples were analyzed using the newly developed method and the quantified results were compared to separate independent LC-MS/MS methods for aldosterone and plasma renin activity. Plasma samples were treated with generation buffer (Sodium acetate, EDTA, acetic acid, SBTI and PMSF) and mixed for three hours at 37°C. Samples were precipitated, diluted and centrifuged prior to SPE. Sample supernatant was transferred to a Waters Oasis™ µElution 96 Well Plate, followed by a wash and elution. Using an ACQUITY™ UPLC™ I-Class System, samples were injected onto a Waters XBridge™ C_8, 2.5µm, 2.1 x 50 mm Column using a water/methanol/ammonium fluoride gradient elution profile and quantified with a Waters Xevo™ TQ Absolute Mass Spectrometer. **Results:** The method demonstrated no significant carryover or matrix effects and was shown to be linear from 10 - 2500 pg/mL for aldosterone and 0.1 - 25 ng/mL/hr for PRA. Analytical sensitivity investigations indicate the analytical sensitivity of this method would allow precise quantification (<20%) at 10 pg/mL and 0.1 ng/mL/hr, for aldosterone and PRA, respectively. Coefficients of variation (CV) for total precision and repeatability on 5 analytical runs for low, mid and high QCs were all <10% (n = 25) for aldosterone and PRA. Comparison with samples previously analyzed by an independent LC-MS/MS method demonstrated good agreement for aldosterone and PRA. **Conclusions:** We have successfully quantified aldosterone and renin activity in plasma in a single method using an SPE protocol with LC-MS/MS analysis, for clinical research purposes. The method demonstrates excellent linearity and precision, with minimal matrix effects. For Research Use Only. Not for Use in Diagnostic Procedures.

B-194

Development and validation of rapid LC-MS/MS method for simultaneous determination of 8-iso-$PGF_{2\alpha}$ and 11-$DHTXB_2$ in human urine.

N. Abdelkader, B. Odat. *Cleveland State University, Cleveland, OH*

BACKGROUND Cardiovascular disease (CVD) is one of the leading causes of death globally, the prevalence ofCVD is expected to increase from 36.9% to 40.5% between 2010 and 2030 in the United States.Atherosclerosis (AS) is thought to be the primary cause of cardiovascular diseases (CVDs),however there are several others. About half of Americans between ages 45 and 84 haveatherosclerosis and don't know it. Eicosanoids (8-iso-PGF2α and 11-Dehydrothromboxane B2) arethe most common biomarkers for predicting atherosclerosis. The objective of this study was todevelop a rapid LC-MS/MS method for simultaneous determination of 8-iso-PGF2α and 11-DehydrothromboxaneB2 (11-DHTXB2) in human urine. **METHODS** One milliliter of human urine spiked with 0.5 µl of each internal standard (1 µg/ml) was mixedwith 1 ml of heptane,1ml of ethyl acetate, and 1 ml of Acetonitrile in a glass tube followed by theaddition of 1 ml of pure water. The mixture was vortexed for 1 minute and then centrifuged (4000rpm) for f 5 minutes. The middle organic layer was transferred to another tube and dried undernitrogen and resuspended with 50µL of the initial mobile phase composition (70:30 v/v H2O:ACN) for the analysis. One milliliter of human urine spiked with 0.5 µl of each internal standard(1 µg/ml) was loaded to a conditioned SPE cartridge, then washed with 1ml of 10% ACN afterthat analytes eluted with 1 ml of pure ACN at the end eluent is collected, dried and reconstitutedin 50 µl of (70:30 v/v H2O: ACN). LC-MS/MS was performed using AB Sciex 5500 QTRAPtriple quadrupole tandem mass spectrometer coupled to Shimadzu HPLC with 10 minutesgradients for both samples. **RESULTS** The method was optimized by using standard solutions of 8-iso-PGF2α and 11-dehydrothromboxane B2 and showed separation at 5.27 and 5.45 minutes respectively. The methodapplied on pooled urine sample from healthy individuals and showed No bias in retention time.Two purification methods were used to extract the two analytes from pooled urine samples , threephase liquid extraction (3PLE) and solid phase extraction (SPE). 3PLE showed a better resultsSPE method in eicosanoids extraction which results in higher intensity signals by 2-fold for 8-iso-PGF2α and 5-fold for 11-DHTXB2.The method was shown to be linear from 0.1 to 5 ng/ml forboth analytes ($R^2 \geq 0.98$). **CONCLUSION** The three-phase liquid extraction method extracted the two analytes better than the solid phaseextraction method, which is the most used purification method for eicosanoids. Furthermore, arapid and simple LC-MS/MS method for simultaneously measuring urinary 8-iso-PGF2α and 11-DHTXB2 was successfully developed.

B-196

Lc/ms-ms quantification of myo-inositol: a novel biomarker for kidney disease

C. Omosule, C. Blair, E. Herries, C. Farnsworth, D. Dietzen, J. Gaut. *Washington University in St. Louis, St. Louis, MO*

Currently accepted clinical standards for assessing kidney function include the measurement of creatinine for calculating estimated glomerular filtration rates (eGFR) and albumin to creatinine ratios. However, creatinine, a product of muscle creatine metabolism, is an imperfect proxy for renal function because it is subject to non-renal interferences. There is an unmet need for extremely sensitive and precise biomarkers for evaluating renal function in patients given that acute kidney injury (AKI) and chronic kidney disease (CKD) are linked to high mortality, morbidity, and contribute considerably to health care expenditures.Myo-inositol oxygenase (MIOX) is a novel biomarker limitedly expressed in the proximal renal tubules. MIOX concentrations are elevated in mice with renal injury and in critically ill patients with acute kidney injury (AKI), and increases are observed 54 hours prior to changes in serum creatinine are observed. Importantly, MIOX facilitates the conversion of myo-inositol to glucuronic acid. We developed a robust LC-MS/MS method to quantify myo-inositol in human plasma and serum, hypothesizing that following renal injury (acute and chronic), plasma concentrations of myo-inositol will increase, and will correlate with increases in serum creatinine concentrations.In patients with stable renal function, the central 95% distribution of plasma myo-inositol concentrations was 16.61 - 44.2 uM (Median 27.7uM, 95% CI: 26.7-28-7). No sex-based differences in plasma myo-inositol were observed. We also observed excellent correlation between plasma myo-inositol and serum creatinine concentrations (r=0.84, 95% CI: 80-88), as well as excellent

sensitivity (99%) and specificity (100%) at a myo-inositol concentration of 50.22uM for differentiating patients with stable kidney function from those with CKD. Patients with CKD exhibited higher plasma myo-inositol concentrations (p value <0.0001) with a central 95% percentile of 51.2-309.1 uM (Median 104.1 uM, 95% CI: 92.3-113.6).These results imply that ***myo-inositol concentrations increase in accord with renal tubular injury and MIOX buildup in patients with kidney dysfunction, making it a potential biomarker for renal impairment***.

B-197

Separation of bile acid muricholic acid enantiomers by C18 reversed-phase HPLC as a function of component polarity values of the mobile phase

M. Khair, D. J. Anderson. *Cleveland State University, Cleveland, OH*

Objective: Optimize the reversed phase HPLC technique for the separation of alpha-muricholic acid and beta-muricholic bile acid enantiomers, by investigating the effects of the mobile phase's three polarity components [hydrogen bond donor acidity (α), hydrogen bond donor basicity (β) and dipolarity/polarizability (π)] on the separation of the enantiomers. **Background:** The importance of drug enantiomers in clinical pharmacology is well recognized, where often only one of the enantiomer pair effectively binds to the target protein site to exert its therapeutic effect. Same for some endogenous compounds, only one of the enantiomer pair molecules binds to an enzyme or receptor to exert its physiological action, while the other molecule is inactive. It is essential for the analytical method to separately determine the two compounds of the pair. Usual HPLC techniques for separating enantiomers employ specialized chiral columns. In this work, steroid isomers are separated on a C18 column, with the separation controlled by mobile phase (MP) component polarity adjusted with different proportions of binary organic modifiers added to the MP. **Methods:** The bile acid enantiomers alpha-muricholic acid and beta-muricholic acid were separated on a C18 column (150 mm x 2.1 mm, 3.5µm, 40 °C) using two different organic modifiers in the MP at a flow rate of 0.2 mL/min, with ESI-LC/MS detection. The isocratic MP consisted of 0.1% formic acid in 100% water for MP A and 0.1% formic acid and five or more different proportions (separate runs) of the two organic modifiers for MP B to adjust MP polarity. MP component polarities was determined from: 1) the literature values for the total polarity (P) for each of the pure solvents in the MP; 2) published values of the fraction of the total polarity for the polarity components ($\alpha+\beta+\pi=1$) for each solvent; and 3) the fraction proportion of each solvent in the MP. The experimentally determined selectivity factor is then plotted versus the polarity component value of the MP. **Results:** When the selectivity factor versus the values of the MP polarity components was plotted for six different organic modifier pair combinations on the same plot, the summed dipolarity/polarizability (and basicity polarity (β) components of the MP showed reasonable correlation (R^2=0.72), displaying mostly a linear superimposed trend for all the organic modifier pairs, while the acidity component (α, R^2=0.13) and total polarity (P) (R^2= 0.002) of the MP did not. Thus, the π+β summed MP polarities is a significant determiner of selectivity, irrespective of the organic modifier pair used. The bile acid enantiomers were baseline separated, with a selectivity factor of 1.3 being the highest value obtained. **Conclusion:** For the laboratory determination of steroid enantiomers, our results suggest that calculated component polarity values of the MP can be used to direct laboratorians for achieving desired separation of steroid enantiomers (as opposed to a trial-and-error approach for achieving the separation). This study shows that the separation of these bile acid enantiomers significantly depends on the summed π and β polarities of the MP, with poor correlation for total polarity (P) and α.

B-198

Web-based Application of an Automated Quality Assurance Monitoring System for a Pain Management Panel by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

M. Jarrells, S. Armstrong, C. Hickey, C. Bumgardner, M. Yu. *University of Kentucky, Lexington, KY*

Background: With its advantages in analytical sensitivity and specificity, quantitative drug analysis by LC-MS/MS platform allows the clinical laboratories to provide results that would meet various clinical needs, especially for those to assess patient compliance on pain medications. However, the complexity of the technology and data it generated, poses challenges and burdens to the quality assurance process. In our toxicology laboratory at the University of Kentucky, we offer a pain management panel to physicians for monitoring patients compliance purpose. This is a LC-MS/MS based assay that can simultaneously qualitative up to 52 drugs and their metabolites. Quality monitor of data from this multicomponent drug assays is laborious and inefficient. Our objective here is to develop and implement an effective automated quality assurance mechanism, and eventually improve the laboratory efficiency, reduce lab errors and enhance quality of patient care.

Methods: Using the Sciex Multiquant software, the daily processed txt file is generated and uploaded to shared drive to be analyzed using a Java based program. The processing of a file is split into three subparts: (1) saving original data, (2) calculating/verifying the quality assurance metrics with the predefined limits, and (3) producing a report. The original data uses to the database to retrieve specific information without having to reload the file multiple times. When completed, all the data included in the initial report are read along with limit variables. The metrics for quality assurance with predefined criteria includes: System suitability test sample (Retention time, Peak area), Internal standard (IS) peak area, Blank sample peak area, Calibrator accuracy and calibration curve accuracy, Quality control, Ion ratio, Relative retention time to IS. Once these calculations are finished, a report is generated in a format that allows it to be easily read on the website

Results: We are able to generated a daily QA report that is web-based application. Discrepancies within any of the statistics are color coded and given a button allows a user to "check" the measurement and provide a reason why this measurement was out-of-scope. All users are allowed to provide reasoning for an out-of-scope measurement and each of these users' comments is tracked to provide an audit trail. Once all measurements have been reviewed, a report is ready form review. The process can only be completed by a privileged user. Once the process has been completed, the report cannot be modified. With this application, we are not only able to monitor the quality of the run on the day-to-day basis, the QC and instrument behavior can also be evaluated and reviewed longitudinally.

Conclusion: An effective and efficient web-based quality assurance monitoring system was successfully developed and implemented for this multicomponent drug analysis by LC/MS-MS. The application of this program will facilitate the mass spec platform use in clinical laboratories.

B-199

Performance of the MasSpec Pen for intraoperative margin evaluation during breast conserving surgeries

K. E. Liebenberg[1], R. J. DeHoog[1], A. E. Montgomery[1], M. F. Keating[2], A. De Melo Porcari[1], E. Bonefas[1], C. Nagi[1], A. N. Barbieri[1], A. Thompson[1], A. Al-Fartosi[1], J. W. Suliburk[1], S. A. Carter[1], L. S. Eberlin[1]. *[1]Baylor College of Medicine, Houston, TX, [2]University of Texas at Austin, Austin, TX*

Background Breast cancer is the second leading cause of cancer deaths among females worldwide. Surgical resection is the most common treatment option for women diagnosed with breast cancers. Many women with in situ disease or early invasive breast cancer are candidates for breast conserving surgery (BCS), which involves removing the lesion of interest and preserving the rest of the breast. One of the greatest challenges a breast cancer surgeon faces is to achieve negative margins while optimizing aesthetic outcomes. Negative margins are highly desirable as they offer the greatest potential for prolonged disease-free survival. Accurate intraoperative surgical margin evaluation is therefore critical during BSC. Molecular technologies offer the opportunity to incorporate cancer-specific biomarkers into clinical decision-making for improved cancer detection. Our team has reported the development of an innovative handheld mass spectrometry (MS) probe, the MasSpec Pen (MSPen), for rapid and gentle molecular analysis of human tissues, including breast cancer tissues. The MSPen uses water to gently extract molecules from tissues upon contact, which are then analyzed by MS and statistical classifiers to provide a predictive classification within seconds. Here, we evaluate the performance of the MSPen for intraoperative detection of breast cancer. **Methods** The MSPen coupled to an Orbitrap Exploris 120 (Thermo Scientific) is being used to analyze both normal breast and invasive lobular carcinoma (ILC) banked tissues. Tissue samples are first thawed prior to analysis. After analysis, the region analyzed is demarcated, and the samples snap-frozen, sectioned, H&E stained, following evaluation by a pathologist. We will then apply the least absolute shrinkage and selector operator (lasso) method to build classification models for distinguishing normal breast and ILC using the histologically validated mass spectra. The MSPen system is also implemented in the operating room for testing within surgical workflow. Analyses will be carried out during BCS surgeries for patients with ILC. The ILC classifier generated from banked tissues will be used to predict on data obtained from the intraoperative analyses and validated against final histopathological reports. **Results** To date, we have analyzed 213 banked human breast tissues using the MSPen, including healthy breast (n=79), invasive ductal carcinoma (IDC) (n=64), healthy lymph nodes (n=26), and lymph nodes with metastatic IDC (n=44). The rich mass spectral data obtained from breast tissues showed high relative abundance of various metabolites and lipid species that have been previously described as potentially diagnostic of breast cancer. Using this data, we built classi-

fiers that discriminate cancer and normal breast tissues with high accuracy (overall 94%) for primary breast IDC. In a pilot clinical study, we demonstrated feasibility for ex vivo and in vivo tissue analysis using the MSPen platform during 29 breast surgeries. We are currently expanding our study to detect ILC using banked tissues and in patient tissues intraoperatively. **Conclusion** Our study in breast cancer provides evidence that the MSPen enables molecular-based diagnosis of IDC tissues using excised specimens and in in vivo and ex vivo analysis performed by surgeons intraoperatively. Current effort including ILC is expected to show similar capabilities for patients with ILC.

B-200

Preoperative Classification of Thyroid Nodules by Desorption Electrospray Ionization Mass Spectrometry Imaging of Fine Needle Aspiration Biopsies

R. J. DeHoog, A. Al-Fartosi, N. Zarrin-Khameh, R. Huang, L. S. Eberlin, J. Suliburk. *Baylor College of Medicine, Houston, TX*

Background Preoperative diagnosis of thyroid lesions by fine-needle aspiration (FNA) biopsy cytology can be challenging, and in up to 20% of cases is unachievable. Patients with an indeterminate preoperative diagnosis are often recommended for diagnostic surgery, with the majority receiving a benign diagnosis, rendering the surgery unnecessary. Consequently, new technologies that can provide accurate preoperative diagnosis of thyroid lesions are needed. Here, we have employed DESI-MS imaging along with statistical modeling to determine molecular signatures of benign and malignant thyroid lesions using banked thyroid tissue samples with known histopathology diagnosis. We then applied this methodology for analysis and classification of preoperatively collected FNA smears.

Methods A total of 199 fresh-frozen thyroid tissues including 50 normal, 55 follicular adenoma (FTA), 58 follicular carcinoma (FTC), and 36 papillary carcinoma (PTC) were sectioned at a thickness of 10um and stored at -80C until analysis. FNA biopsies were prospectively collected at Baylor College of Medicine. DESI-MS imaging was performed in the negative ion mode using a Waters Xevo G2-XS mass spectrometer fitted with a DESI-XS source. Samples were H&E stained and pathologically evaluated after analysis. Molecular profiles from tissue regions of clear histology were used to build classification models. For the FNA smears, mass spectra corresponding to clusters of thyroid cells were extracted for statistical prediction, and the predictive performance of the models on FNA smears was assessed in correlation with pathology.

Results DESI-MS analysis of thyroid tissue sections generated 151,317 individual mass spectra, comprised of hundreds of lipid and metabolite features, that were used to build and validate two classification models: PTC vs. benign thyroid, comprised of normal thyroid and FTA, and FTC vs. benign thyroid. For each of these models, the data was randomly split with two-thirds used as a training set to generate the model and one-third used as an independent validation set to assess the performance of the model. For the PTC vs. benign thyroid model, a prediction accuracy of 97.7% was achieved for the training set with an accuracy of 96.6% for the withheld validation dataset. For the FTC vs. benign model, an accuracy of 78.4% was achieved for both the training and validation datasets. We are currently refining statistical workflows to improve the performance of the FTC model and to generate a model discriminating benign thyroid from thyroid cancer (FTC and PTC combined). When testing the prediction of the classifiers built from tissue imaging data on an initial sample set of 19 FNAs, overall per-sample prediction accuracies of 79% and 89% were obtained using the PTC vs. benign thyroid model and the FTC vs. benign thyroid model, respectively. We are currently working to collect, analyze, and validate the

performance of the classification models on additional FNA biopsies, with a focus on samples with indeterminate cytology.

Conclusions Overall prediction accuracies of 79% and 89% were achieved for the benign vs. PTC model and benign vs. FTC model, respectively, for thyroid FNA classification. With the addition of DESI-MS, unnecessary diagnostic surgeries could be prevented by providing improved preoperative specificity.

B-201

Development of a Quantitative Gas Chromatography Tandem Mass Spectrometry Method for Metabolic Profiling of Urine Organic Acids

A. S. Ptolemy, K. Young, C. MacDonald, R. Garrett, M. Kellogg, R. W. Peake. *Boston Children's Hospital, Boston, MA*

Background: Urine organic acids (UOA) analysis is an essential investigation for inborn errors of metabolism (IEMs). The vast majority of methods use gas chromatography mass spectrometry (GC-MS) and are only qualitative. Gas chromatography tandem mass spectrometry (GC-MS/MS) offers greater sensitivity and selectivity, and enables the combination of qualitative screening with quantitative analysis. We describe the development and preliminary validation results of a quantitative GC-MS/MS method. **Methods:** Urine creatinine concentrations were measured (CobasPro, Roche Diagnostics) and normalized to 1.25 mmol/L. After internal standard addition, each urine specimen, calibration standard and QC were incubated with a hydroxylamine solution followed by a liquid-liquid extraction. Further sample concentration permitted the isolated UOA to be derivatized using *N,O*-bis(trimethylsilyl)trifluoroacetamine (BSTFA) with 1% chlorotrimethylsilane (TMCS) prior to injection. GC-MS/MS analysis was performed using a GC-MS-TQ8050 triple quadrupole system (Shimadzu) with a 30 m DB-5MS (Agilent J&W) capillary column (0.25 mm, ID of 0.25 µm). Split injection mode was deployed with an initial column temperature of 70°C. The mass spectrometer was operated in electron ionization (EI) and multiple reaction monitoring (MRM) modes. The linear concentration ranges of the targeted metabolites within the devised GC-MS/MS protocol were determined. **Results:** The optimal temperature gradient for high-resolution UOA separations was derived and their respective retention times determined. MS/MS detection parameters were optimized to permit the identification of precursor and production ions for all (N=38) derivatized UOA. Total analysis time was 33.8 min per injection. The achieved linear concentration ranges for each metabolite was respectively determined. Linear concentration ranges and correlation coefficients of representative UOA: 3-hydroxyisovaleric acid (range=9 to 708 µmol/L, R^2=0.9874); lactic acid (range=15 to 751 µmol/L, R^2=0.9936); 3-hydroxybutyric acid (range=15 to 751 µmol/L, R^2=0.9991); and 2-methyl-3-hydroxybutyric acid (range=10 to 501 µmol/L, R^2=0.9996). The correlation coefficients for all remaining UOA ranged from R^2=0.9458 (4-hydroxyphenylpyruvic acid) to R^2=0.9998 (3-methylglutanoic acid and ethylmalonic acid). **Conclusion:** The devised GC-MS/MS protocol permits the unbiased identification and quantification of UOA used to investigate IEMs. The linear concentration ranges for all analytes were determined and are appropriate for monitoring these markers of inherited enzyme deficiencies. The analytical performance of this UOA GC-MS/MS method must be further validated and its diagnostic performance for selected IEMs characterized.

B-202

Improving performance of an LC-MS C-peptide reference method

S. Connolly, W. Grothoff, R. Little, K. Kabytaev. *University of Missouri, Columbia, MO*

Background:

C-peptide is a useful biomarker for distinguishing among different types of diabetes and is a proxy measure of endogenous insulin production, useful in diabetes management. Additionally, C-peptide tests can be used to diagnose non-diabetic hypoglycemia and insulinoma. However, differences among clinical assays can limit the application of C-peptide testing. To address this issue, efforts are underway to standardize manufacturer assays with serum-based secondary reference materials where values are assigned by a reference LC-MS method. Clinical evidence suggests that patients with diabetes can benefit from residual beta-cell function, yet many clinical assays have high variability at lower levels of C-peptide. Here we explore different approaches for improving sensitivity and selectivity of C-peptide reference measurements with a focus at the lower end of the measurement range.

Methods:

Two methods were developed to evaluate and determine the most effective approach. The first method involved spiking serum samples with isotope-labeled c-peptide (Sigma) and then enriching and enzymatically digesting them. The enrichment process included removing abundant serum proteins by using methanol, followed by centrifugation and filtration through C18 cartridges. The resulting solution was then passed through a strong anion-exchange column after correcting its pH with ammonium formate buffer. The eluent was evaporated, and the residue was dissolved in ammonium carbonate buffer. Enzymatic digestion was performed by adding dithiothreitol and iodoacetamide followed by addition of Glu-C. The digested peptides were then analyzed by LC-MS/MS (QTRAP 6500+) in MRM mode. The second method followed the same steps as the first method, except that the evaporation and digestion step were skipped, and MRM was performed on the intact C-peptide chain. Two separate calibration curves were constructed for each method using isotope labeled c-peptide (Sigma) and certified reference material (National Metrology Institute of Japan) as native c-peptide.

Results:

The first method, which measures intact C-peptide using MRM, had a slope of 1.14 and an intercept of -0.01 nmol/L (Deming regression, R=0.917) when compared to

the reference method. The method demonstrated CV within 0.4%-11.9%. The second method, which quantifies digested peptides, showed a slope of 1.02 and an intercept of -0.21 nmol/L compared to the reference method (Deming regression, R=0.995). The method demonstrated CV within 4.4-20.0%. Controlling the three MRM transitions improved the specificity and accuracy of the results. For the first method two MRM transitions were adequate. Both methods showed no significant matrix effects. The first method is simpler to implement due to a smaller number of separation steps

Conclusion:

We have explored two new alternative workflows for C-peptide quantitation that include first, an enzymatic digestion then MRM quantitation and second, MRM quantitation of intact C-peptide. To improve the analytical performance of the methods, further optimization is still required.

B-203

Development and validation of an LC/MS/MS method to quantify creatinine in dried blood spots

C. Torres, R. Muldrow, D. N. Greene. *LetsGetChecked, Monrovia, CA*

Background: Health disparities in diagnosis and treatment of chronic kidney disease (CKD) are apparent with Black, Brown, and socially disadvantaged people experiencing worse outcomes. Improving access to CKD screening may decrease disparities. Blood creatinine concentrations are a mainstay in CKD screening, as it is commonly used to estimate glomerular filtration rate. Creatinine is classically measured in serum/plasma using high-throughput chemistry analyzers. Dried blood spots (DBS) could offer a solution to under-screening, but require an alternative method since extraction from DBS retrieves minimal sample volume and is difficult to scale using conventional automation. The purpose of this study was to develop an LC/MS/MS DBS method to quantify creatinine and ultimately to increase screening access for underprivileged communities.

Methods: Creatinine was measured using a 100x2.1mm Hypersil Silica column with an ABSciex API4000 MS/MS detector. A 5-point calibration curve was made using creatinine certified reference material (Cerilliant) and blank whole blood (UTAK) (target concentrations: 0.575, 0.975, 4.575, 10.575, 20.575mg/dL). Calibrators were spotted onto Whatman903 cards and allowed to dry for at least 24-hours. A 6mm punch was used for analysis of calibration points and patient samples. Once punched, water (50ul) was added and the samples were incubated at 60℃ for 15 minutes before shaking at 800 RPM. Acetonitrile with 2H-creatinine internal standard (IS; .06 mg/dL) was added and the mixture was shaken and centrifuged. The resulting supernatant was diluted to contain 20% water before injecting 10uL. Creatinine was separated using an isocratic mobile phase (80% Acetonitrile, 20% DI water, 10mM Ammonium Formate) and detected using MRM mode (Q1 114.0 m/z; Q3 86.8 m/z (quantifier) and 44.5 m/z (qualifier)). For the IS a Q1 of 117.0 m/z and Q3 of 89.9 m/z was applied and the extraction ratio of endogenous creatinine to IS was calculated to determine the concentration. Linearity was confirmed by running five levels of DBS calibration standards for five days. Imprecision was established by running 3 control levels five times for five consecutive days. Control material was purchased from BioRad and run as liquid. Accuracy was assessed using residual liquid CAP serum creatinine proficiency testing (PT) material in triplicate. DBS creatinine concentration determined by LC/MS/MS was compared to paired venous serum creatinine concentration determined by the Roche Cobas enzymatic creatinine assay (n=63).

Results: Creatinine concentrations from 0.2-20 mg/dL were within the allowable nonlinearity of 0.075 mg/dL or 5.0% (y=1.00x-0.010;R=0.999). Intraday and interday precision was < 2%and < 3%, respectively for all levels (mean concentrations 1.00, 2.02, and 7.05 mg/dL). Residual CAP PT samples (n=4) were within 2.5% of the peer group mean. Compared to venous results, DBS had a bias of -0.03 mg/dL (-3.573%) and a linear equation of (y=.904x +0.049; R=0.83).

Conclusion: Preliminary results indicate that DBS are a viable sample source for measuring creatinine. Creatinine concentrations measured by DBS compared favorably with CAP PT. A clinically insignificant negative bias was seen when DBS creatinine concentration was compared to venous blood.

B-204

Determination of Lyso-GB3 in dried blood spots: a useful biomarker for Fabry disease

B. F. Paulo, E. M. Novo, D. A. Zauli, A. P. Serra. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Fabry disease, an X-linked inborn error of metabolism, results from pathogenic mutations in the α-galactosidase A gene (GLA). These mutations reduce or abolish the αgalactosidase A activity, which result in accumulation of glycosphingolipids in the lysosomes, including globotriaosyl-sphingosinein (lyso-GB3), throughout the body, in particular the kidney, heart, and brain. Lyso-GB3 may be elevated in symptomatic patients and supports a diagnosis of Fabry disease. It may also be helpful as a tool for monitoring disease progression, as well as determining treatment response in known patients. Moreover, measurement of Lyso-GB3, may provide additional diagnostic information in the evaluation of uncertain cases, such as in asymptomatic heterozygous female patients, individuals with novel GLA variants of unclear clinical significance, as well as asymptomatic patients identified by family screening. This study aimed to determine Lyso-GB3 in dried blood spot (DBS) samples with a simple, rapid and sensitive LC-MS/MS method, and identify its reference interval in the Brazilian population. **Methods:** All experiments were performed on the Waters TQ-S Micro LC-MS/MS system. The gradient chromatographic separation was carried out using a Waters BEH C18 column in 3.5 min. One DBS punch of 3.1 mm diameter was used for analysis in a microtiter plate and then, a solvent extraction containing stable isotope analogue of Lyso-GB3 was added. After incubation time, the supernatant was directly injected into LC-MS/MS system. The method was validated for linearity, carryover, intra and inter-day precision and the limit of quantification was determined. A reference interval was obtained by analyzing 94 healthy volunteers (49 women and 45 men). The difference of Lyso-GB3 concentration between men and women was investigated (Wilcoxon paired test). The reference interval was determined by the superior limit of the confidence interval (90% CI) of a normal distribution of the results. **Results:** The method was linear between 0.2-100.0 $ng.mL^{-1}$. The recovery was between 81.0 and 139.7%, which was acceptable considering CLSI guidelines for DBS validation (CLSI NBS04-A). The intra and inter-day precision were below 12.1%, and the limit of quantification was 0.2 $ng.mL^{-1}$. The reference interval determined was below 0.8 ng/mL. **Conclusion:** In conclusion, the LC-MS/MS method for Lyso-GB3 determination in DBS samples was successfully validated. The sample preparation is simple and the chromatographic method is rapid for routine laboratory. The cutoff determined is comparable with what is available in the literature and adequate for analysis in clinical routine.

B-205

A Simple Method for Opioids, Synthetic Opioids and PCP Detection in hair samples by LC-MS/MS

B. F. Paulo, L. F. Junior, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Hair analysis is useful for monitoring exposure to drugs and is a unique body material for the retrospective detection of drug consumption due to its large detection window, and it is easy to collect, store, and transport. Establishing a LC-MS/MS protocol for opioids determination may be challenging due to similarities among the molecular structures and the MS/MS fragmentation profile. This study aimed to develop and validate a simple, rapid, and sensitive LC-MS/MS method for quantification of 6-acetylcodeine, 6-acetylmorphine, codeine, dihydrocodeine, EDDP, PCP, fentanyl, heroin, hydrocodone, hydromorphone, meperidine, methadone, morphine, oxycodone, oxymorphone and tramadol in hair samples. **Methods:** In this method, 15 mg of hair in a 2.0 mL microtube was decontaminated sequentially with dichloromethane and methanol. The sample was dried, and 0.75 mL of extraction solvent and a labeled internal standards solution were added. The hair sample was pulverized in a FastPrep-24™ 5G from MP-Biomedicals and then sonicated at 60°C for 60 min. The sample was centrifuged and filtered with Captiva filtration plates. Then, 100 µL of supernatant was added to 100 uL of ultrapure water and 5.0 uL was injected directly into the LC-MS/MS system. Chromatographic separation was performed on an Agilent 1290 Infinity II equipped with HALO Biphenyl column, and employing a mobile phase constituted by acetonitrile with 0.1% of formic acid and 5 mM of ammonium formate. The detection was performed with a Sciex 6500+ QTrap mass spectrometer. **Results:** The current method was successfully validated for carryover, linearity, precision, recovery, limits of detection and quantification, and the measurement of uncertainty was calculated. The results were summarized in the Table 1. **Conclusion:** The developed LC-MS/MS method was simple, rapid, and sensitive to detect opioids in hair samples.

Table 1: Results of the validation parameters for determination of opioids and PCP in hair samples.

Analytes	Linearity	LD	LQ	Precision	Recovery	Uncertainty
6-Acetyl-codeine, 6acetyl-morphine, codeine, dihydro-codeine, PCP, fentanyl, heroin, hydro-codone, hydro-morphone, meperidine, methadone, morphine, oxycodone, oxymor-phone, tramadol	80-1,000 pg/mg	0,08-10 pg/mg	80 pg/mg	< 6 %	96,3-113,0 %	± 23-29 %
EDDP	20-250 pg/mg	0,04 pg/mg	20 pg/mg	< 4%	99,5-105,1 %	± 23-29 %

B-206

The determination of Vanillylmandelic acid (VMA), Homovanillic Acid (HVA) and 5-Hydroxyindolacetic Acid (5-HIAA) by LC-Ms/MS assay in urine samples for diagnosis of neuroendocrynes tumors

L. F. Junior, B. F. Paulo, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: The catecholamines dopamine, norepinephrine and epinephrine, as well the neurotransmitter serotonin are biogenic amines that were formed by enzymatic descarboxylation of aminoacids in the body. They realize an important function in central nervous system. Vanillylmandelic acid (VMA) and Homovanillic Acid (HVA) are degradation products of the catecholamines, and 5-Hydroxyindolacetic Acid (5-HIAA) is a serotonine degradation product. They are important to the diagnosis of neuroendocrynes tumors, that produce catecholamines and/or serotonine in excess, resulting in elevated levels of VMA, HVA, and/or 5-HIAA in 24hrs collection urine. **Objective:** This study aimed to validate a simple LC-MS/MS method for quantification of VMA, HVA and 5HIAA in isolated and 24 hrs urine samples for the diagnosis of neuroendocrines tumors. **Methods:** Using a deep-well microtiter plate, 20 µL of isolated or 24hrs urine was diluted in 540 µL of 0.05% of formic acid solution. Then, 20 µL of an internal standard solution containing stable isotopes of the analytes were added (VMA-D_3, HVA-$^{13}C_6$,^{18}O and 5-HIAA-$^{13}C_6$). The plate was sealed, homogenized for 30s at 2,500 rpm, and centrifuged for 15 min at 4,000 rpm and ambient temperature. Then, 5.0 µL of the supernatant was injected into the LC-MS/MS system. Chromatographic separation was performed on the Waters ACQUITY UPLC system equipped with Kinetex XB-C18 1.7µm X 50mm X 2.1mm column (Phenomenex), and methanol and formic acid 0.2% solution as mobile phase in 3.5 min of gradient mode separation. The detection was performed on a Waters XEVO TQ-S Micro mass spectrometer with electrospray ionization (ESI+). The current method was validated for carryover, linearity, precision, recovery, and limit of quantification, and it was evaluated by comparing the analysis in the developed method and a commercial kit by HCPL-ECD, of 15 real samples from volunteers in the laboratory. **Results:** The method was successfully validated for all the parameters. The linearity was between 0.5-100.0 mg/L. The precision was less than 6.3%, the recovery was between 85.4-103.4% and the quantification limit was 0.5 mg/L. The comparison with the reference method showed good coefficient correlations ($R^2 > 0.92$) for the three analytes. **Conclusion:** We validate a simple LC-MS/MS method to quantify three metabolites to help the diagnosis of neuroendocrynes tumors. The method represents a simplification of the extraction compared with the commercial kit employing HPLC-ECD, and an important reduction of the per sample production cost, without loss of quality.

Molecular Diagnostics and Genetics

B-208

Correlation studies of human papillomavirus dna on roche cobas vs human papillomavirus mrna on hologic panther

j. teng. *national healthcare group diagnostics, singapore, Singapore*

Background:

Molecular testing, specifically HPV DNA has revolutionised the screening and management of cervical cancer. WHO in 2021 has recommended HPV DNA as the primary screening test and also the newer technology HPV mRNA as alternative assay. This evaluation aims to study the result concordance between HPV DNA and mRNA assay

Methods:

The evaluation was conducted on Roche Cobas 4800 (HPV DNA) and Hologic Panther (HPV mRNA) at NHGD IMH lab in 2021. Both instruments underwent precision studies and carryover test to ascertain its performance prior to the concordance study. 64 samples were used in the correlation against the reference method- HPV DNA testing onRoche Cobas 6800

Results:

Both Cobas 4800 and Hologic Panther demonstrated acceptable performance in precision, carryover and LoD (for Hologic only). However closer result agreement is observed in Roche 4800 results to the testing laboratory, Quest which is using Roche 6800.

Conclusion:

The disconcordance between cobas 6800 vs cobas 4800 came from the negative samples in cobas 4800 in which their Ct value were found to be near to the detection limit. Similar to Cobas 4800, the discordance observed in Hologic came from the negative samples. However, HPV mRNA screening has been recognized for its higher specificity at baseline in the recent studies which improve clinical outcomes and reduce colposcopy referrals by 14%. Unfortunately, this could not be verified in our evaluation as colposcopy results for those HPV DNA positive sample were not available.

B-210

Spectrum of Germline Mutation with Characteristic MLH1 Hotspots and High Concordance with MMR Deficiency Phenotype in Lynch Syndrome Patients in Taiwan

P. Y. Chang[1], S. C. Chang[1], M. C. Wang[1], C. C. Chen[2], J. M. Chiang[1]. *[1]Chang Gung Memorial Hospital, Taoyuan, Taiwan, [2]Chang Gung University, Taoyuan, Taiwan*

Background: Patients carried defects on cancer susceptibility genes or had cancer family history have at least twice the risk of developing cancer than the general population, with both early onset and multiple cancers features. For elucidating the carrier rate of cancer susceptibility genes in patients with inherited colorectal cancer (CRC) and endometrial cancer (EnCa) in Taiwan, we established a NCCN guided 30-gene panel which included four DNA Mismatch Repair (MMR) genes to detect the germline mutation of patients with Lynch Syndrome. Correlation of genotype and phenotype of MMR enzymes are under investigation as well.

Methods: Sixty-eight blood samples were collected from unrelated cancer patients, 42 of them were from patients with inherited colon cancer, 13 from patients with diagnosis of both CRC and endometrial cancer and 13 from patients with early onset EnCa. 30-gene panel was performed by next generation sequencing (NGS) platform and variants were annotated following ACMG guideline. MMR protein expression in tumor tissue sections were identified by conventional immunohistochemistry (IHC) stain. Loss of nuclear staining were classified as MMR deficiency.

Results: Average age of first tumor onset of 68 patients were 49.2 years old. 54% (37/68) of the subjects had at least one germline mutations with pathogenic effect or deleterious variant predicted by in silico protein function software. Five of them carried two heterozygous mutations. Total 42 mutations appeared majority on three MMR genes, *MLH1* (17/42, 40%), *MSH2* (10/42, 24%) and *MSH6* (6/42,11%) with notified three hotspots on *MLH1* p.R265C (7/17, 41%); p.T117M (2/17, 12%) and p.K618del(2/17, 12%). The rest mutations spread sporadically on nine genes (*PMS2, TP53, CHEK2, POLE, POLD1, ATM, EPCAM, MLH3 and RNF43*). For exploring the effect of germline mutation on MMR protein expression status on tissue, we compared the MMR IHC results and the germline mutation pattern. As high as 57% concordance between phenotype and genotype were found. We also collected six blood samples

from relatives of our CRC cohort to demonstrate the inheritance and pathogenesis of the germline mutation. Exactly the same germline mutations can be found in diseased relatives among different generation or siblings.

Conclusion: In spite of having founder mutations on MLH1 gene in Taiwanese Lynch syndrome, most of the germline mutations randomly occurred in MMR-related genes. Comprehensive cancer susceptible gene screening panel by NGS is a cost-effective way to identify the inherited status of individuals with family cancer history and serves as a surrogate tool to predict MMR protein expression. Genetic counseling and prophylactic therapeutic information can be provided for positive cases for better cancer prevention.

B-212

Application of Annexin A5Coated Beads for Collection of Extracellular Vesicles and TheirContent RNA as Liquid Biopsy for Lung Cancer

W. Mei-Chia[1], G. Guan-Yu[2], W. Chih-Liang[3], C. Pi-Yueh[4], C. Chiuan-Chian[5]. *[1]Department of Medical Laboratory, Chang Gung Memorial Hospital, Taoyuang, Taiwan, [2]Chang-Gung University, Taoyung, Taiwan, [3]Division of Pulmonary Oncology and Interventional Bronchoscopy, Department of Thoracic Medicine, Chang Gung Memorial Hospital, Taoyung, Taiwan, [4]Department of Medical Laboratory, Chang Gung Memorial Hospital, Taoyung, Taiwan, [5]Department of Medical Biotechnology and Laboratory Science, Taoyung, Taiwan*

Background:

Extracellular vesicles (EVs) carry molecules derived from cells, such as RNA. Measurement of tumor-derived extracellular RNA (exRNA) in EVs from plasma can be a promising approach for the development of blood-based cancer detection. However, the lack of an easy method for EVs separation limits its application. The aim of this study is to establish a procedure for EV collection and exRNA detection for non-small-cell lung cancer (NSCLC) diagnosis and monitoring.

Methods: Annexin A5-coated magnetic beads (ANX beads) were developed for EVs isolation. Next, we established a procedure for EV separation and exRNA detection from fluidic specimens using the ANX beads. Then blood samples from 22 NSCLC patients and 20 healthy controls were collected to evaluate the procedure and we detected different RNAs in the samples.

Results:

We evaluated the ability of ANX beads in EVs separation and found that the beads could capture large and small EVs and could increase RNA concentration. Then we detected different RNAs in the samples and found that CK19 mRNA, MALAT1 lncRNA, and miR155 microRNA had good performance to differentiate NSCLC from healthy controls.

Conclusion:

In conclusion, this assay protocol could separate EVs to enrich RNA concentration. The EVs separation employed was captured by magnetic beads which could be adopted into an automatic nucleic acid extract system to conveniently concentrate and extract nucleic acid from EVs.

B-213

Colorimetric LAMP as an Effective Tool for Rapid Molecular Diagnostics

N. A. Tanner, Z. Li, J. Bruce, R. A. Moncion, C. V. Cunningham, B. W. Langhorst, C. K. Carlow. *New England Biolabs, Ipswich, MA*

Background: LAMP has become a standard molecular diagnostic technique, and its robustness and flexibility have enabled rapid, simple tests outside of traditional laboratory settings. The need for additional diagnostic methods became urgent during the SARS-CoV-2 pandemic, and LAMP was extensively evaluated and validated for numerous testing applications. We have extended the utility of LAMP to enable colorimetric detection, multiplex assays, variant identification, and robust direct tests for point of-care and workplace surveillance testing.

Methods: We utilized fluorescent and colorimetric RT-LAMP for assay development and our workplace screening program. A simple lysis buffer was applied to whole saliva samples and added directly to our colorimetric LAMP SARS-CoV-2 tests, with more than 100,000 samples tested. For variant calling we developed novel hybridization probes with single-base sensitivity to regions of sequence changes, and we characterized more than 5,000 LAMP assays internally positioning mismatches in the LAMP primer regions to ensure robustness to and tolerance of mutations.

Results: The colorimetric LAMP assay displayed a sensitivity of 97% and specificity of 100%, and during its utilization in our in-house CLIA-certified workplace screening has identified >300 unique positive cases derived from all variants of concern. 3654 blinded samples were analyzed by 3 operators and colorimetric readout was found to have a strong concordance with real-time fluorescence measurement (kappa=0.999).

Conclusion: RT-LAMP is remarkably tolerant of sequence variation, with no individual positional mutation significantly affecting assay performance. Simple visual readout can be utilized for a reliable diagnostic indicator when sophisticated instrumentation is not available or desired.

B-214

Serial changes of donor-derived cell-free DNA in pancreas transplant recipients

H. Lee[1], K. Shin[2], B. Choi[1], M. Lee[2], k. shin[2]. *[1]Pusan National University Yangsan Hospital, Yangsan, Korea, Republic of, [2]Pusan National University Hospital, busan, Korea, Republic of*

Background: Pancreatic transplant patients need invasive pancreatic biopsies to diagnose graft rejection. Organ damage following organ donation yields circulating tissue-isolated donor-derived cell-free DNA (ddcf-DNA). Based on the ddcf-DNA ratio or quantity in several transplantations, graft rejection is predicted. Thus, we examined a 2-week ddcf-DNA monitoring study after pancreatic transplantation at this medical institution.

Methods: The current prospective observational study examined five pancreas transplant recipients who were sampled on postoperative days (PODs) 4, 7, 14, 30, 60, 90, 120, and 180 following the study protocol. Serum levels were determined for the following: amylase, lipase, and tacrolimus. Cell-free DNA (cf-DNA) was isolated from the plasma using a NucleoSnap cf-DNA extraction kit. The QX200 Droplet Digital PCR System was used to measure ddcf-DNA. To analyze transplant rejection and differentiate between donor and recipient, all selected single nucleotide polymorphisms (SNPs) met the following criteria: known and validated minor allele frequency of 43% in Koreans and reported ethnicities from global populations.

Results: The median patient age was 35 years. All recipients achieved euglycemia without exogenous insulin immediately after the surgery. All 6-month postoperative hemoglobin A1c levels were within the normal range (4-6%). One (20%) of the five patients with pancreas transplant had a T cell-mediated rejection event on POD 531. For PODs 4, 7, 14, 30, 60, 90, and 180, the percentage (median, range) of ddcf-DNA was 2.7 (0.4-5.5), 0.6 (0.2-4.4), 3.7 (0.4-12.7), 7.1 (0.4-7.9), 2.1 (0.1-5.4), 2.0 (0.3-8.5), and 0.3 (0.2-7.6), respectively; and amylase (median, range) was 68.0 (30.0-80.0), 82.0 (57.0-151.0), 74.0 (66.0-157.0), 81.0 (50.0-137.0), 74.0 (39.0-111.0), and 80.0 (27.0-98.0). (43.0-105.0), respectively. The ddcf-DNA value of POD 14 was greater than 10% in individuals who had TCMR on POD 530.

Conclusion: In our study, patients with high ddcf-DNA on POD 14 after transplantation respond poorly to immunosuppressive agents, and rejection was later confirmed.

Baseline characteristics of five pancreatic transplanted recipients

N	1	2	3	4	5
Type of surgery	PTA	PAK	PAK	PTA	PTA
Age	26	38	35	58	27
Sex	F	M	F	M	F
BMI	15.9	25.6	18.4	21.6	23.1
PreOPHbA1c	9.9	8.1	8.2	9.6	5.5
PostOPHbA1c	5.2	5.0	5.5	4.8	5.5
C-peptide	<0.01	0.35	<0.01	<0.01	<0.01
Rejection	TCMR(POD 531)				
Ddcf-DNA(%) at POD 14	12.7	2.1	5.2		0.4
HLA serologic type (Recipient)	A11,A26/ B60,B54/ DR4,DR9	A11,A26/ B60,B54/ DR4,DR9	A02,A33/ B44, B71/ DR4, DR13	A02,A24/ B54,B57/ DR4,DR9	A11,A24/ B07,B35/ DR1,DR4

B-215

Use of whole exome sequencing and T cell receptor β repertoires analysis for identifying potential genetic variants in rheumatoid arthritis

J. Cho[1], H. Lee[2], S. Ahn[3], J. Song[4], J. Kim[1], J. Lee[1]. *[1]Department of Laboratory Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic of, [2]Division of Rheumatology, Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic of, [3]Department of Laboratory Medicine, Gangneung Dongin Hospital, Gangneung, Korea, Republic of, [4]GC Genome, GC labs, Yongin, Korea, Republic of*

Background: Rheumatoid arthritis (RA) is one of the most prevalent autoimmune diseases, which is reported to affect about ~1% of the adult population worldwide. And, it is associated with progressive disease courses, systemic complications, early mortality, and a high socioeconomic burden. The pathogenesis of RA is too complex to be fully understood yet, but abnormal and pathogenic T-cell responses that evade normal immune functions could be considered one of the mechanisms of RA development. Herein, we used whole exome sequencing (WES) and T cell receptor/T cell receptor β repertoires analysis (TCR/TRB) to identify the potential genetic variants in RA.

Methods: Peripheral blood samples from 18 RA patients and 5 healthy controls were analyzed. For RA patients, the baseline naïve samples were collected before disease-modifying anti-rheumatic drugs treatment was started, and both WES and TRB were tested. After 6 months and 12 months of treatment initiation, additional samples were collected and TRB was tested. A total of 18 WES and 27 TRB tests were conducted. WES was done on the MGI-G400 platform (MGI Tech Co., Ltd., Shenzhen, China) and TCR/TRB was done using the LymphoTrack TCR/TRB assay (Invivoscribe Technologies, San Diego, CA, USA) on the MiSeq system (Illumina Inc, San Diego, CA, USA).

Results: In RA patients, deleterious variants that have been reported to be relevant in various diseases were identified in a total of 217 genes. Among 18 RA patients, 5 (27.8%) patients had variants that were suspected to be associated with the pathogenesis of RA, including JAK3, PADI4, TNFSF18, TRAF1, NFKB, etc. Of the 27 TCR/TRB tests, 10 TCR (37.0%) and 14 TRB (51.9%) results had significant changes in RA patients.

Conclusion: The aim of this study is to provide clinical evidence for developing diagnostic and/or prognostic markers for RA. Further analysis whether these variants found in this study are clinically significant and valuable as markers in RA. In addition, it is expected that the results of our study will be used as basic data applied to other rheumatoid diseases such as systemic lupus erythematosus and Bechet's disease.

B-216

Development of a Point-of-care Multiplexed Room Temperature Stable Environmental Toxin Test

S. Aithal, S. Gupta, K. Duong, A. Kumar, N. Ho, S. Guo, Y. Ando, R. Liu, J. Warden, D. H. Ho. *Hememics Biotechnologies Inc, Gaithersburg, MD*

Background: Hememics™ Biosensor System (HBS) is a platform technology enabling antigen and molecular testing in the field. The system consists of a reader, HemBox™, and a biosensing chip, HemChip™. The HemBox™ is a battery-operated, cell phone size with Bluetooth and WIFI module that can be integrated into the Health Management System. The biosensing chips can be programed with bioreceptors to detect pathogens in less than 5 minutes. This study presents performance characteristics of HBS as a rapid POC to detect toxins from environmental samples.

Methods: The testing protocol consists of insert a HemChip™ into the HemBox™ and mixing the sample with the buffer and apply a drop of this mixture to the chip. In less than 5 minutes, results are displayed on the screen of HemBox™ or transmitted to user's cellphone via Bluetooth. Samples were prepared by spiking solutions of mud, serum, vegetable wash, and milk with Staphylococcal enterotoxin B (SEB) and ricin.
Results: HemChip™ was functionalized to recognize Ricin and SEB targets. For testing, these targets were mixed in various matrix. To achieve statistical significance, each sample tested represented a minimum of 10 data points.

Sample	Matrix	LoD
Ricin	PBS	0.1 ng/mL
	Dirt	0.001 ng/mL
	Vegetable Wash	1 ng/mL
	Tap Water	0.1 ng/mL
SEB	PBS	0.1 ng/mL
	Dirt	0.01 ng/mL
	Milk	0.1 ng/mL
	Tap Water	0.1 ng/mL
	Serum	1 ng/mL

Conclusion: Hememics™ Biosensor System (HBS) is designed to be the ultimate Portable Lab Diagnostics for field use. The ability to test for environmental toxins in the field is significant because it allows for quick and on-site detection of potentially harmful substances. Our biosensor system also eliminates the need for sample processing, collection and transportation, which can take significant time and resources. The results of field testing can be available almost immediately, allowing for prompt action to be taken in the case of high toxin levels.

B-217

The study of association of FokI Polymorphism of Vitamin D Receptor (VDR) Gene, Vitamin D and Parathyroid Hormone Levels in Stage 4-5 Chronic Kidney Disease Patients: A cross-sectional study

R. Yadav[1], S. Naagar[2], S. K. Datta[2], A. K. Ahirwar[2], S. Mahajan[2], P. Chopra[2], S. Arulselvi[2]. *[1]AIIMS New Delhi, India, New Delhi, India, [2]AIIMS New Delhi, New Delhi, India*

Background Chronic Kidney Disease (CKD) is an important public health problem globally and almost 35% patients of CKD have deficiency in vitamin D which progresses to 80% in stage 4-5 CKD patients. Active vitamin D binds to Vitamin D Receptor (VDR) to regulate target gene transcription. Various genetic variations (Single Nucleotide Polymorphism) such as FokI polymorphism, which is a T/C transition polymorphism (ACG to ATG) may affect the VDR gene expression and thus may play an important role in pathogenesis of CKD. The aim of the study is to study association between FokI Polymorphism of Vitamin D Receptor (VDR) Gene, serum Vitamin D and PTH levels in Stage 4-5 CKD patients. **Methods** A total of 150 patients, aged 25-60 years, attending the Department of Nephrology AIIMS New Delhi with chronic kidney disease (CKD) stage 4-5, were enrolled for the study. FokI polymorphism was analyzed by using polymerase chain reaction-restriction fragment length Polymorphism (PCR-RFLP) in study subjects. Following DNA isolation and PCR amplification, restriction digestion was done using *FokI* restriction endonuclease and visualised on 1.5 % agarose gel electrophoresis. The prevalence of different genotypes and allelic frequency distribution was compared between CKD patients and healthy controls (HC). Levels of PTH, Vitamin D were estimated by eCLIA. **Results** No significant differences in genotype and in allelic frequencies between CKD patients and HC were observed as shown in table 1. No significant differences in biochemical parameters based on genotypic variations in CKD patients and in HC were observed.

Table **1: Genotype frequency and Allelic frequency of VDR FOK1 gene polymorphism in CKD patients and HC: -**

Genotype (FOK1)	Genotype frequency (%)			p-value	X^2 (chi^2)	Allelic frequency			
	CKD (n=150)	HC (n=150)	Total study group (n=300)	0.770	0.5220	CKD (n=150)		HC (n=150)	
FF	53.33 (n=80)	57.33 (n=86)	55.33 (n=166)			F	f	F	f
Ff	41.33 (n=62)	37.33 (n=56)	39.33 (n=118)			0.74	0.26	0.76	0.24
ff	5.33 (n=8)	5.33 (n=8)	5.33 (n=16)						

Conclusion The present study reveals no association of VDR FokI polymorphism with CKD. Furthermore, no significant differences in biochemical parameters were observed in FF, Ff and ff genotypic subgroups in CKD or HC groups. Further analysis revealed no significant differences in biochemical parameters based on genotypic variations in CKD patients and in HC.

B-218

Role of delta tocotrienol and resveratrol supplementation in regulation of micro RNAs in patients with metabolic syndrome: a randomized controlled trial

D. A. Khan[1], S. Fatima[2]. *[1]National University of medical Sciences, Rawalpindi, Pakistan, [2]NUMS, Rawalpindi, Pakistan*

Background: Micro-RNAs (miRNAs) are involve in the regulation of central obesity, insulin resistance, inflammation and dyslipidemia in development of MetS. δ-tocotrienol and resveratrol improve the insulin resistance, inflammation and clinico-biochemical parameters in the patients with MetS. **Objective:** To determine the effect of δ-tocotrienol and Resveratrol Mixture (TRM) supplementation for 24 weeks in comparison with placebo, on the relative expression of metabolic related miR-130b, miR-221, miR-15b-, miRNA-122, and miR-376b in the patients with MetS. **Methods:** This randomized placebo-controlled trial was conducted at AFIP, NUMS, Pakistan. A total of 82 adult MetS patients, aged 18 to 60 years of both genders, were enrolled and randomly grouped into TRM (n=41) and Placebo (n=41). The TRM group was given 400 mg capsules of (δ-tocotrienol 250 mg; Resveratrol 150 mg), and placebo received 400 mg cellulose capsules twice daily for 24 weeks. miRNA was extracted by Trizole L S reagent with cel-miR-39 (Spike-In control). The cDNA was prepared by Reverse Transcriptase Kit. miRNAs expression profile was done by using qRT-PCR Kit (Qiagen) protocol on the Rotor-Gene. **Results:** The TRM supplementation significantly ($p<0.001$) upregulated miR-130b-5p (3.05fold), miR-221-5p (2.45fold), and downregulated miR-122-5p by 2.22fold as compared to placebo after 24 weeks. No significant difference was observed in miR-15b-5p and miR-376b-5p.

Table: MiRNAs Relative Fold-changes expression in study groups (n=82)

Parameter	Delta-delta Cq (ΔΔCq)		Relative Fold-Change (FC)				
	TRM (n=41)	Placebo (n=41)	TRM (n=41)		Placebo(n=41)		p-value
			FC	-1/FC*	FC	-1/FC*	
miRNA-130b-5p	-1.57 ± 0.35	-0.05 ± 0.24	3.05 ± 0.78	+3.05	1.04 ± 0.17	+1.04	<0.001
miRNA-221-5p	-1.18 ± 0.57	0.03 ± 0.33	2.45 ± 0.94	+2.45	1.05 ± 0.24	+1.05	<0.001
miRNA-15b-5p	-0.06 ± 0.26	-0.04 ± 0.25	1.06 ± 0.20	+1.06	1.04 ± 0.18	+1.04	0.670
miRNA-122-5p	1.13 ± 0.12	0.03 ± 0.29	0.45 ± 0.04	-2.22	0.99 ± 0.20	-1.01	<0.001
miRNA-376b-5p	0.06 ± 0.18	0.04 ± 0.19	0.96 ± 0.12	-1.04	0.97 ± 0.13	-1.03	0.746

.FC<1 calculated by [-1/(FC)]

Furthermore, patients in the TRM group had significant ($p<0.05$) correlation in improvement of MetS biomarkers and miRNAs including hs-CRP, miR-130b; miR-221, TNF-α; miR-221, fasting plasma glucose, miR-122, triglyceride and miR-130b with waist circumference. No significant side effect was reported. **Conclusion:** TRM daily supplementation in addition to dietary restriction, is effective in the improvement of the metabolic syndrome components by upregulation of miR-130b-5p involve in central obesity, inflammation; miR-221-5p by decreasing insulin resistance and down-regulation of miRNA 122 to improve dyslipidemia in the patients with MetS.

B-220

Development of Reference Materials for detection of Monkeypox

C. Huang, R. Raman, E. Morreale, N. Truong, B. Anekella. *LGC Clinical Diagnostics, Gaithersburg, MD*

Background: Monkeypox virus (MPXV) is a member of *Poxviridae* family and belongs to the genus of *orthopoxvirus*. The Monkeypox virus has a double stranded DNA genome of approximately 197 kb with almost 190 nonoverlapping ORFs. Monkeypox virus encodes all the enzymes needed for transcription and replication process of the viral genome. It has been hypothesized that progressive gene loss, primarily at the terminal end of the genome, has been the justification behind evolution of a new adaptive virus capable of attacking humans and causing serious illness. Monkeypox virus infection in human can lead to a smallpox-like illness with almost 11% fatality rate in individuals who are non-vaccinated. Monkeypox virus infects a wide range of mammalian cells without the need for specific host receptors, cell entry molecules and replication machinery. No specific approved treatment exists for Monkeypox. The symptoms could be managed by treating secondary bacterial infections, reducing symptoms, and providing supportive care. ST-246 (tecovirimat), FDA approved drug against Smallpox virus, has shown some efficacy against Monkeypox, and therefore, accurate diagnosis, usually through PCR-based testing is critical. LGC Clinical Diagnostics has developed AccuPlex Monkeypox Reference Material, which is a non-infectious, stable, and reproducible standard to aid nucleic acid testing for Monkeypox virus.

Methods: AccuPlex™ Monkeypox Reference Material consists of recombinant Adeno virus bearing the complete Monkeypox genes: J2L (TNF receptor gene), F8L (DNA polymerase gene), F3L (Double-stranded RNA-binding protein, inhibitor of IFN signaling), and N3R (orthopoxvirus MHC class I-like protein -OMCP). The design of the recombinant virus is based on the sequence of ON585038.1 USA strain from the recent outbreak. A digital PCR assay was developed using the E9L Non-variola (NVAR) Orthopox Generic Assay design described in Y. Li et al. /Journal of Clinical Virology 36 (2006) 194-203. Digital PCR testing using the BioRad QX-200 droplet digital PCR system was used to quantitate the viral copies/mL and guide formulation. The final product is targeted as a low positive control, approximately 2-3 times the detection limit of commercial PCR testing methods and is formulated in defibrinated human plasma.

Results: AccuPlex™ Monkeypox Reference Material gave an average value of 5.33E+03 copies/mL ± 6.34E+02 copies/mL by digital PCR. Along with the undiluted sample, 1:2 and 1:4 dilutions were made which were then tested on Quest Diagnostics Qualitative RT-PCR platform to verify performance and compatibility with the assay detection limits. All three samples were positively detected.

Conclusion: LGC Clinical Diagnostics has developed a stable, non-infectious reference material using recombinant Adenovirus technology for assays that detect Monkeypox DNA. Our study helps ensure that the control will be low positive for Monkeypox virus and could be used for monitoring the sensitivity of the assay. Data presented indicates the compatibility of the reference material with commercially available real time PCR assays. These materials may be used by labs for development, validation, training, and ongoing QC of Monkeypox detection assays and workflows.

B-221

Quantitation of DNA in Antibody-based and RNA-based Drugs by Quantitative Polymerase Chain Reaction (qPCR)

J. Su, J. Uriu, E. N. Aguilar, R. Tobias, G. Marr. *Nitto Avecia Pharma Services, Irvine, CA*

Background: Current FDA regulation defined acceptance level of 10 ng per dose of residual DNA in drug products poses great challenges for accurate quantification of residual DNA, particularly for the products used in high doses or formulated in complex matrixes. The purpose of this study is to develop and qualify a qPCR method for the quantification of Chinese Hamster Ovary (CHO) DNA in antibody-based drugs and for quantitation of human DNA in RNA-based drugs.

Methods: An 8-point standard curve consisting of 0.005, 0.05, 0.5, 5, 50, 500, 1000 and 5000 pg CHO DNA and a 5-point standard curve consisting of 0.3, 3, 30, 300 and 3000 pg human DNA was generated by serial dilutions. The antibody sample (300 µg) was spiked with 2.5, 10, and 50 pg CHO DNA. The RNA sample (1.6 µg) was spiked with 0.3, 3 and 30 pg human DNA. The samples were analyzed by qPCR using a Real-Time PCR instrument. Commercial PCR test kits (TaqMan™ Probe and TaqMan™ Universal Master Mix), and sequence specific forward and reverse primers were used.

Results: For CHO DNA in antibody product, the linearity slope and R2 are -3.5 and 1.00, respectively, which met system suitability criteria. The accuracy by spiked recovery of 2.5 pg, 10 pg and 50 pg CHO DNA in 300 µg antibody, 150 pg CHO DNA in formulation buffer, and 150 pg CHO DNA in 1000 pg human DNA are 70%, 76%, 86%, 93% and 95%, respectively. The precision of the assay for 2.5 pg, 10 pg, and 50 pg of CHO DNA spiked in 300 µg antibody are 6%, 7% and 2%, respectively. The QL of the assay is 2.5 pg CHO DNA per 300 µg of antibody product.For human DNA in RNA product, the linearity slope and R2 are -3.3 and 1.00, respectively, which met system suitability criteria. The accuracy by spiked recovery of 0.3 pg, 3pg, and 30 pg human DNA in 1.6 µg RNA, 3 pg human DNA in formulation buffer, 1.5 pg human DNA in 150 pg CHO DNA are 113%, 104%, 102%, 97% and 88%, respectively. The precision of the assay for 0.3 pg, 3 pg and 30 pg human DNA spiked in 1.6 µg RNA are 6%, 5% and 2%, respectively. The QL of the assay is 0.3 pg human DNA per 1.6 µg of RNA product.

Conclusion: Two qPCR methods were developed to quantify residual DNA in drug products. One method is specific to quantitation of low levels of CHO DNA in antibody product. Another method is specific to quantitation of low levels of human DNA in RNA product.

B-222

Validation of a qPCR Method for Qualitative Detection of Monkeypox Virus

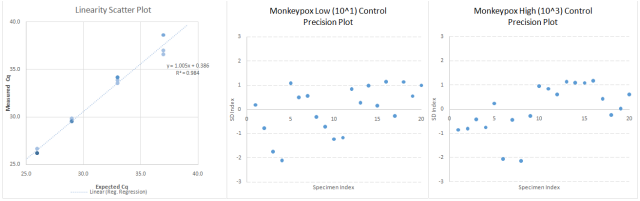
A. Chan, K. Amaro, C. Bui, O. Camarena, A. Dang, D. Herron, J. Lara, L. Lim, P. Lopez, M. Mayoralgo, A. Nguyen, J. Nguyen, B. Ortega, A. Pham, K. Tran, D. Bach. *Bach Diagnostics, Irvine, CA*

Background: The 2022 Monkeypox virus (MPXV) outbreak became a global concern, prompting a multidisciplinary response to increase surveillance and isolate suspected and confirmed cases to reduce disease spread. Quantitative PCR (qPCR) is capable of detecting small amounts of DNA or RNA, and can be used to quickly and accurately confirm MPXV cases, even at early stages of infection, which is crucial for outbreak control and management.

Methods: qPCR was performed on a 384-well plate with a total reaction volume of 20 µl consisting of 9µL Acrometrix Monkeypox DNA Positive Control at serial dilutions, 10µL BactoPure Master Mix, and 1µL TaqMan Monkeypox Virus Microbe Detection Assay. Positive control dilutions 103, 102, 101, and 100 were diluted with TE Buffer. qPCR was performed on two different Quantstudio 12K Flex instruments.

Results: The qPCR method validation was performed by calculating precision, accuracy, and linearity on serial dilutions of Acrometrix Monkeypox Virus DNA Positive Control. Precision testing was performed on 2 levels of control: low (101) and high (103) over 5 days with 4 repeats per day. Accuracy and linearity testing were performed on 4 levels with 3 repeats. Coefficient of variation (CV) was less than 5%, specifically 0.9% for the low control and 4.3% for the high control, indicating an acceptable level of precision. Percent recovery fell close to 100%, specifically within 101%-103% for all controls, indicating the results fell close to the expected value. Linearity R2 was close to 1, specifically 0.984, demonstrating the results are proportional to the concentration analyzed.

Conclusion: Validation of MPXV detection through qPCR was successful. Development and validation of qPCR assays for the detection of positive MPXV cases is essential for managing future outbreaks and combatting future pathogenic strains.



B-223

Validation of a Non-invasive DNA NGS Assay for Detecting Somatic Mutations in Normal Appearing Sun Exposed Skin

K. Kaur, B. Riley, R. Ai, E. Roberts, J. Whitaker, C. Ibarra. *DermTech, San Diego, CA*

Background: Ultraviolet radiation (UVR) exposure is a major risk factor for developing nonmelanoma skin cancer and somatic mutations caused by UVR exposure can arise even in normal-appearing skin. Non-invasive identification of UVR induced somatic mutations can provide a quantitative evaluation of the extent of UVR damage. This study describes the development and analytical validation of a noninvasive method for identifying low level somatic variants in sun exposed peripheral skin by ultra-deep Next Generation Sequencing, in specimens collected using adhesive tape designed to non-invasively collect skin samples.**Methods:** Five genomic regions with the highest density of known UVR induced somatic mutations across *TP53* and *NOTCH1* were amplified by 5-plex PCR primers affixed with Illumina NGS adapters. These amplicons were purified, size selected and then indexed using Unique Dual Indexes. The NGS libraries were pooled to 96-plex and bi-directionally sequenced with 2x150 chemistry on the Illumina iSeq100. Data analysis was performed using the Illumina DRAGEN Somatic Variant Calling pipeline. Titrations between 0.01 and 1 of synthetic controls comprising plasmids containing amplimer inserts with 19 of the most well characterized UVR-induced C>T driver mutations spiked into a wildtype insert were sequenced in duplicate. In addition, 3 cell line controls at input concentrations of a 2-fold serial dilution from 13.2ng – 0.4ng were spiked into a mutation negative cell line at variant allele frequencies between 0.005 and 1 and sequenced in triplicate across three NGS runs. Finally, 178 specimens collected from the central forehead, nose and cheeks of human donors were sequenced and their cumulative mutation frequency analyzed by linear regression against the age of the participants. **Results:** All samples were sequenced to a mean duplex coverage of >10000x. All expected mutations in the plasmid control titrations were identified for each titration. Both reproducibility and specificity in these samples were 1.0. The cell line controls were sequenced in a matrix of DNA input concentration vs. Variant Allele Frequency (VAF). The Limit of Detection for the assay was a function of DNA concentration, with 0.4ng, 0.8ng and 3.3ng DNA required to identify mutations accurately and reproducibly at 0.05, 0.02 and 0.01 VAF respectively. The study found that the mutations detected among the human donor samples strongly correlated cumulative VAF with age (p value = $2.56x10^{-7}$). **Conclusion:** This study describes the validation of a method for identifying low frequency variants in sun exposed skin. We demonstrate that the assay is a robust and reliable tool for detecting *TP53* and *NOTCH1* somatic variants associated with UVR exposure in normal-appearing skin down to 0.01 VAF with >0.8 sensitivity, specificity, and reproducibility. Damage from UV exposure is cumulative and therefore it is expected that older individuals will be more likely to have UVR-induced mutations in their skin, as we observed in this analysis. The assay can detect somatic DNA variations in normal-appearing skin for markers that are associated with UV damage and increased overall risk for certain nonmelanoma skin cancers.

B-224

Comparison of an unlabeled probe High Resolution Melting Analysis (HRMA) assay for Factor V Leiden 1691 G>A mutation to a TaqMan hydrolysis assay.

H. H. Han, S. Lewis, A. Hoang, K. Badri. *Tarleton State University, Fort Worth, TX*

Background: The clinically significant Factor V Leiden (FVL) mutation (1691 G>A) causes replacement of Arg with Gln, preventing activated protein C from inactivating Factor V leading to a lengthened clotting process. Individuals with the Factor II and Factor V Leiden mutations have an increased risk for venous thrombosis. The aim of this study is to compare an unlabeled probe High Resolution Melting Analysis (HRMA) assay for Factor V Leiden mutation to a TaqMan hydrolysis assay. High Resolution Melting Analysis (HRMA) is a post-PCR, homogenous, closed tube system for the detection of sequence variants. Post-PCR, the amplicons are heated gradually until the melting temperature is reached and the fluorescent dye unbinds from the amplicon and exhibits low fluorescence. A melt-curve analysis is generated that is characteristic of a particular sequence variant, therefore HRMA allows for comparison of one base changes in genetic sequences based on their differences in melting rate. **Methods:** HRMA Assay: Blood samples were collected in EDTA tubes and DNA extracted using the Roche MagnaPure. DNA content was determined to supply 66 ng of genomic DNA to each reaction mixture. Factor Vc.1601 G>A Novallele Genotyping Assay by Canon Biosciences was used. The following primer and probe sequences were used: forward primer sequence: 5'-CCCATTATTTAGCCAGGAGA-3', reverse primer sequence: 5'-GCCTCTGGGCTAATAGGACT-3', unlabeled probe sequence: 5'-TTCAAGGACAAAATACCTGTATTCCTCGCCT/3AmM/3'. PCR was performed in Roche Light Cycler 480 with 11 uL volumes per reaction of 66.6 ng of DNA. Forward primer, reverse primer and probe concentration was 100 uM and a 5X volume excess amount of forward primer and probe was used. A 151 bp amplicon was generated. Three control references obtained from Coriell Institute were used including wild type (WT), mutant (MUT) and heterozygote (HET). The HRMA FVL assay was performed on a total of seventeen samples. The TaqMan hydrolysis FVL assay uses the following DNA primers and probe: forward primer: GCCTCTGGGCTAATAGGACTACTTC; reverse primer: TTTCTGAAAGGTTACTTCAAGGACAA; WT FVL probe: HEX-ACC TGT ATT CCT CGC CT-BHQ-2; MUT FVL probe: FAM-ACC TGT ATT CCT TGC CT-BHQ-2. Reactions were performed in a 20 ul volume with 50 ng of DNA per reaction. The same sets of reference DNA and 17 samples of DNA as the high-resolution melting analysis were used. **Results:** For FVL, the Tm was 65.58° C and 69.75° C respectively for the WT and MUT. Tm for all 17 samples was 69.75° C respectively indicating all 17 samples were WT FVL. This TaqMan FVL assay confirmed the FVL genotype of the 17 samples and reference samples. **Conclusion:** Comparing the results of the unlabeled probe, HRMA FVL assay with a real-time TaqMan probe endpoint genotyping assay resulted in 100% sensitivity and 100% specificity for both assays.

B-225

Assessment of the Genotype Frequency of Thiopurine Methyltransferase (TPMT) Deficiency in a Large Cohort of Patients With Immune Mediated Inflammatory Disease and Cancer.

J. Stachelski, D. Pham, P. Hughes, O. McLachlan, T. Dervieux. *Prometheus Laboratories, San Diego, CA*

Background: Thiopurine methyltransferase (TPMT) alleles (*1/*2/*3A/*3B/*3C) are routinely genotyped to determine the metabolizer status of patients that plan to undergo thiopurine therapies (6-Mercaptopurine and Azathioprine). Screening of these alleles allows for personalized patient dosing and reduces the risk of severe or even fatal myelosuppression. The objective of this study was to determine the genotype frequency of TPMT deficiency in a large clinical cohort of patients with immune mediated inflammatory diseases and cancer. **Methods:** All genotyping was performed in a reference clinical laboratory. Patient specimens were tested using genomic DNA isolated from whole blood. Clinical patient diagnoses were physician provided (international classification of disease, ICD-10). The genomic DNA was tested for the presence of three single nucleotide polymorphisms (SNPs) in the TPMT gene coding region and known to associate with reduced TPMT activity (rs1800462-Exon 5, rs1800460-Exon 7 and rs1142345-Exon 10). These polymorphisms determine the presence of *2/*3A/*3B/*3C alleles. Testing occurred from 10/26/2000 to 9/9/2022. Three different testing technologies were used during the 22 years of testing (PCR-RFLP from 2000 to 2003, Taqman™ chemistries from 2004 to 2015, and Luminex™ xMAP™ bead-based since 2016). **Results:** A total of 219,129 patients were tested (mean age: 38yrs, 56% female). As presented in the Table the genotype frequency for the wild type *1/*1 was 90.8% (95%CI: 90.7-90.9%), it was 9.0% (95%CI: 8.8- 9.1%) for the heterozygous and 0.22% (95%CI: 0.020-0.024%) for homozygous variant, with similar frequency across disease states. The TPMT *1/*3B genotype frequency was very rare (<0.1%, n=5) and the *3B/*3C homozygous variant (which cannot be distinguished from the *1/*3A) was calculated to have a frequency of 1:7,706,230 (95%CI: 1:7,043,228-1:8,369,232) and lower than initial estimates. **Conclusion:** We estimated the frequency of TPMT genotypes in the largest cohort reported to date. The occurrence of the *3B/*3C genotype may be rarer than initially estimated.

TPMT Genotype Frequency

	Diagnosis	UC	CD	RA	SLE	Cancer	Unclassified	Total
Genotype	N	23698	35018	1601	2374	3117	153321	219129 (95% CI)
Wild Type	*1/*1	0.910	0.909	0.902	0.899	0.899	0.908	0.908 (0.907-0.909)
Heterozygous variant	*1/*2	0.006	0.004	0.004	0.004	0.004	0.005	0.005 (0.0045-0.0051)
	*1/*3A	0.070	0.068	0.071	0.057	0.089	0.066	0.067 (0.066-0.068)
	*1/*3B	0.000	0.0057	0.000	0.000	0.000	0.002	0.0023 (0.00028-0.0034)
	*1/*3C	0.013	0.016	0.021	0.036	0.016	0.019	0.018 (0.0173-0.0184)
Homozygous variant	*2/*2	0.000	0.000	0.000	0.000	0.000	0.0013	0.00091 (0.0004-0.0022)
	*2/*3A	0.034	0.020	0.001	0.000	0.000	0.021	0.022 (0.02-0.03)
	*2/*3C	0.000	0.000	0.000	0.042	0.000	0.046	0.0037 (0.0004-0.0022)
	*3C/*3C	0.0084	0.020	0.000	0.002	0.0032	0.033	0.029 (0.022-0.036)
	*3A/*3A	0.001	0.002	0.002	0.002	0.002	0.002	0.002 (0.0015-0.0019)
	*3A/*3B	0.000	0.000	0.000	0.000	0.000	0.000	0.00065 (0.00044-0.0014)

Key: UC- Ulcerative Colitis, CD- Crohn's Disease, RA- Rheumatoid Arthritis, SLE-Systemic Lupus Erythematosus

B-226

Clinical evaluation of ElsiQ™ STI-12 Detection Kit 3/4 for detection of human sexual transmitted infection pathogens in human vaginal swab and/or urine specimens

J. Ahn[1], D. Kim[1], W. Lee[2], S. Yoon[3], S. Cho[4]. *[1]Gachon University Gil Medical Center, Incheon, Korea, Republic of, [2]Kyung Hee University Hospital at Gangdong, Seoul, Korea, Republic of, [3]Korea University College of Medicine, Seoul, Korea, Republic of, [4]Ajou University Hospital, Suwon, Korea, Republic of*

Background: Nucleic acid amplification tests are a highly sensitive method for detecting sexual transmitted infection (STI) pathogens, and multiplex molecular assays are even more advantageous in screening and diagnosing multiple STI pathogens simultaneously. The ElsiQ™ STI-12 Detection Kit 3/4 (ElsiQ) is in vitro diagnostic marked multiplex real-time PCR assay that targets 6 STI pathogens, including human herpes simplex virus 1 (HSV1), human herpes simplex virus 2 (HSV2), *Gardnerella vaginalis* (GV), *Treponema pallidum* (TP), *Candida albicans* (CA), and *Ureaplasma parvum* (UP) in human vaginal swab and/or urine specimens.

Methods: In this retrospective study, 575 clinical specimens suspected of having STI pathogen were enrolled; 280 genital swabs and 295 urine samples from April to July 2021. The comparison tests were performed using both the ElsiQ and PANA RealTyper™ STD Kit (PANA). Clinical sensitivity and clinical specificity were evaluated by comparing the patient's clinical features.

Results: The comparison results between ElsiQ and PANA were all consistency in 5 strains (HSV1, HSV2, GV, TP, UP), and 96.4% positive percent agreement value with 0.97 kappa value in CA. (Table 1.) The clinical sensitivity and clinical specificity showed 100% in 5 strains (HSV1, HSV2, GV, TP, UP). Only CA has 97.0% sensitivity, and 100% specificity.

Conclusion: The ElsiQ assay demonstrated excellent analytical performance for detecting six STD pathogens, with high sensitivity and specificity. The results of this study support the use of the ElsiQ assay as a reliable and efficient tool for the simultaneous diagnosis of multiple STIs.

Table 1. The percent agreement results of human sexual transmitted infection pathgens including human herpes simplex virus 1, human herpes simplex virus 2 (HSV2), *Gardnerella vaginalis* (GV), *Treponema pallidum* (TP), *Candida albicans* (CA), and *Ureaplasma parvum* (UP) between ElsiQ™ STI-12 Detection Kit 3/4 and PANA RealTyper™ STD Kit with 575 specimens in human vaginal swab and/or urine specimens.

		Percent agreement					
	Total	Positive	Negative				
Strain	n	%		95% Cl	%		95% Cl
HSV1	575	100.0	(177/177)	97.9-100.0	100.0	(398/398)	99.0-100.0
HSV2	575	100.0	(164/164)	97.7-100.0	100.0	(411/411)	99.1-100.0
GV	575	100.0	(262/262)	98.6-100.0	100.0	(313/313)	98.8-100.0
TP	575	100.0	(183/183)	97.9-100.0	100.0	(392/392)	99.0-100.0
CA	575	96.4	(161/167)	92.4-98.3	100.0	(408/408)	99.1-100.0
UP	575	100.0	(170/170)	97.8-100.0	100.0	(405/405)	99.3-100.0

B-228

Partition-free digital polymerase chain reaction: a novel approach to enable rapid turnaround time with digital accuracy

P. Erb, J. Myrick, M. Hensley, J. Schreiber, S. Sundberg. *Canon Virginia, Inc., Newport News, VA*

Background: Partition-free digital PCR (dPCR) is a new approach developed to provide absolute quantification in less than 10 minutes. Partition-free eliminates the need for droplets or physical wells that need to be filled and sealed. The technique relies on rapid thermal cycling and microfluidics to limit diffusion and enable the identification of individual amplicon clusters originating from a single target nucleotide template. Rapid heating and cooling were achieved with a thermal cycling engine comprised of a broadband LED with a sapphire block that is both a light guide and heatsink. Sample is distributed through a custom microfluidic cartridge containing 64 microchannels for optical quantification.

Methods: The feasibility of partition-free dPCR was tested with primers targeting *MTHFR* c.665. A titration was performed on three 4X serial dilutions of cell line genomic DNA in triplicate. Fluorescent profiles were generated for all 64 microchannels from an image captured after 35 PCR cycles, where amplicon clusters were identified. Fluorescent peaks of the amplicon clusters were counted and quantified via a custom analysis algorithm, which uses Poisson statistics to calculate concentration.

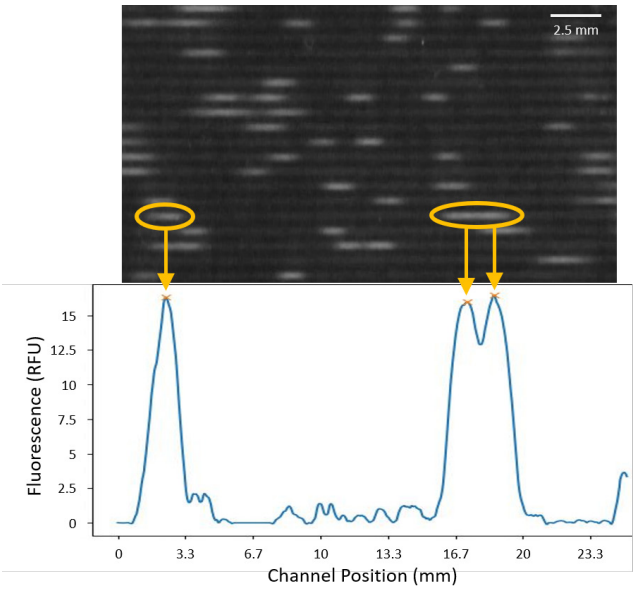


Figure 1: Grayscale image of cartridge (Top) with a single microchannel's fluorescent profile (Bottom). The image, which shows only 20 of 64 microchannels, was taken from PCR cycle 35. The fluorescent profile of a single microchannel identified three amplicon clusters (circled on image).

Results: The quantification results obtained from the partition-free dPCR system were compared to concentration measurements taken via a Bio-Rad QX200 platform. Concentrations of 69.4 +/- 0.8, 15.4 +/- 1.5, and 3.3 +/- 0.6 copies/µL (R^2=0.9997) were measured on the partition-free system, and concentrations of 67.8 +/- 3.6, 17.2 +/- 1.1, and 2.8 +/- 0.2 copies/µL (R^2=0.9995) were measured on the Bio-Rad QX200.

Conclusion: It has been demonstrated that absolute quantification can be achieved in less than 10 minutes with a partition-free dPCR platform, and shows results comparable to a QX200 droplet dPCR system. The partition-free rapid dPCR technology described herein allows for the exploration of new avenues in molecular diagnostics, with the potential to bring absolute quantification of dPCR to a Point-of-Care setting.

B-229

Benchmarking of bioinformatics and molecular tools for copy number variants calling from human genome: a detailed look on single exons

G. Barra, A. Coqueiro, N. Pessoa, S. Correia, R. Benício, P. Mesquita, I. Sgardioli, A. Lamas, A. Andrade, R. Jácomo, L. Nery. *Sabin diagnóstico e saúde, Brasília, Brazil*

Background Copy Number Variants (CNV) are deletions, duplications or insertions larger than 50 base pairs. They account for a large percentage of the normal genome variation and play major roles in human pathology. NGS bears the promise to allow concomitant exploration of CNV and smaller variants. However, accurately calling CNV from NGS data, especially targeted-NGS, remains a difficult computational task, for which a consensus is still lacking. Here, we aim to compare two bioinformatics approaches for CNV calling based on reads count [Dragen CNV caller and Normalized Reads Counts algorithm (NRC)] with legacy molecular tools for CNV detection such as MLPA and qPCR on a set of 20 polymorphic CNV of human genome.

Methods DNA extracted from whole blood of 20 participants were enrolled in this validation. All samples were submitted to a clinical exome NGS targeted sequencing performed with lotus DNA library prep, xGEN hybridization capture, xGEN inherited disease panel (all from Integrated DNA Technologies) and sequenced using NextSeq-500 (Illumina). Twenty polymorphic CNV sites of human genome which overlap the exons included in the clinical exome were selected from NCBI dbVar curated common structural variants. Their median (min-max) minor allele frequency was 5% (1-63%). When intersected with regions included in clinical exome these CNV matched regions with 1 to 17 consecutive exons. One exon per CNV was the most prevalent condition (n=10), followed by 2 exons (n=4), 3 exons (n=1), 4 exons (n=3), 6 exons (n=1), 8 exons (n=1), 11 exons (n=1), and 17 exons (n=1). A single exon (region) of each included CNV were selected to be genotyped by the four tested methods: MLPA, qPCR, CNV caller implemented on Dragen enrichment v.4.0.3 (Illumina) and a laboratory-developed algorithm which detects CNV based on reads depth count of single exons normalized between samples from the same batch. Dragen was the exception for analysis of only single exons; its segmentation algorithm was not disable. The agreement between each compared method was calculated using absolute and relative frequency and measured using kappa statistics. MLPA was considered the reference method because of its known higher performance over qPCR for CNV calling. MLPA results were presented as copy numbers (CN). **Results** MLPA and qPCR fail to return results from 1 region each. MLPA called 24 losses with CN=0, 71 losses with CN=1, 260 no-CNV (CN=2), 25 gains with CN=3, and 0 gains with CN=4. The CNV calling agreement of MLPA with other tested methods where: MLPA *versus* Dragen - 341/380 (89.7%), Kappa=0.66 (substantial agreement); MLPA *versus* NRC 366/380 (96.3%), Kappa=0.92 (almost perfect agreement); MLPA *versus* qPCR 329/360 (91.4%), Kappa=0.82 (almost perfect agreement). **Conclusion** We observed an overall good concordance between all tested methods when applied to a large number CNV to be interrogated (360-380 CNV callings). qPCR could validate most MLPA calls. Dragen was challenged by the fact that only polymorphic CNV were considered and showed substantial agreement with MLPA. MLPA and NRC showed the highest agreement suggesting that NRC could reliability confirm single exons CNV callings, as MLPA is extensively used to.

B-230

Monitoring SARS-CoV-2 Subvariants for Evaluation of the Diagnostic Kit's Annealing Site using Nanopore Sequencing

Y. Kim[1], T. Noh[1], S. Lee[1], Y. Kim[1], D. Prakash[2], K. Lee[1]. [1]*Seoul Clinical Laboratory (SCL), Yongin city, Gyeonggi-do, KOREA, Korea, Republic of,* [2]*HANARO medical foundation, Seoul, Korea, Republic of*

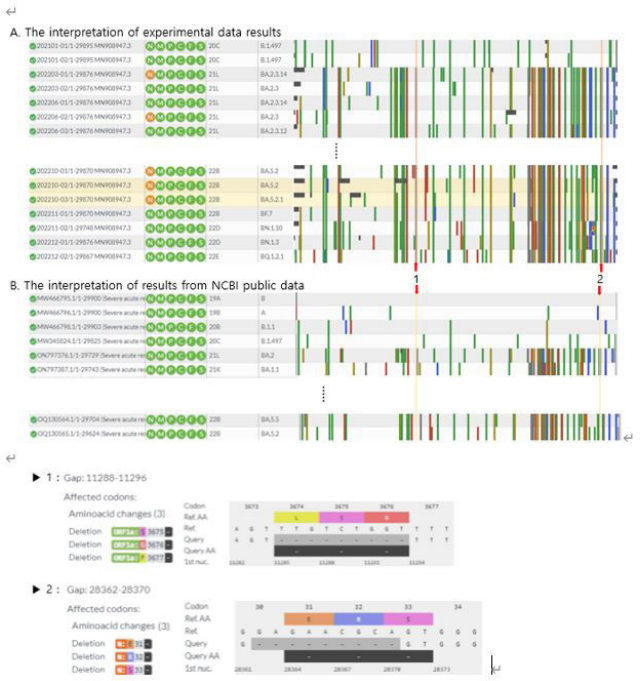
Background: Currently, various diagnostic kits are being developed to aid in the rapid diagnosis or detection of SARS-CoV-2, but viruses continue to evolve through mutations resulting in phenotypic changes such as increased infectivity and pathogenicity, or vaccine evasion. Over time, such alterations in sequence may also occur at the annealing sites of the primers and probes in the diagnostic kit, leading to false-negative results. To prevent this, a continuous mutation monitoring is required. The authors performed SARS-CoV-2 Whole Genome Sequencing (WGS) and then compared and monitored the difference between actual experimental and public data from the National Center for Biotechnology Information (NCBI) datasets for each mutant strain.

Methods: WGS was performed by using GridION (Oxford Nanopore Technologies, UK) on 1,500 nasopharyngeal swab SARS-CoV-2 positive samples requested for testing at SCL from January 2020 to December 2022. Experimental data were aligned through epi2me and analyzed using the Nextclade platform (Figure 1A). For public data, 119 sequences reported in Korea (Figure 1B) were retrieved from the NCBI database, reanalyzed using Nextclade platform, and compared.

Results: In numerous samples tested, mutation, insertion, and deletion were found to occur over time. In particular, in the case of Omicron subvariants, a common Gap (region 1; Gap: 11288-11296, region 2; Gap:28362-28370) were identified in orf1ab (RdRP), the target gene for the majority of existing diagnostic kits, and in N gene (Figure 1). Therefore, while designing a diagnostic kit, this region should not be included in the annealing site.

Conclusion: In order to maintain an effective diagnosis and quarantine system for COVID-19, mutations affecting the annealing site of primers and probes must be identified through a continuous genome monitoring. The results obtained from the continuous genome monitoring can be utilized to upgrade the existing commercially available kits as well as to develop new kits.

Figure. 1: WGS results of experimental data and NCBI public data



B-231

Development and Evaluation of a Lyophilized Mpox Virus qPCR Assay

X. Li[1], D. Chen[1], Y. Zeng[1], H. Chen[1], C. Zhang[2], H. Xu[2], B. Lam[2], J. Dinh[2], B. Turng[1]. *[1]Jiangsu Medomics Medical Technology Co.,Ltd., Nanjing, China, [2]XYZ Laboratory, Lakeway, TX*

Background Increased cases of monkeypox virus (MPXV) have been reported to WHO from 110 countries. As of January 16, 2023, a total of 84,733 laboratory confirmed and 1,354 probable cases, including 80 deaths, have been reported to WHO. The US is assessed as high-risk region. Real-time PCR, with its sensitivity and specificity, is the preferred lab test technique. Since gene mutation has been observed in MPXV, developing assays with more than one target was recommended by the FDA. **Methods** A lyophilized qPCR multiplex assay was developed for MPXV detection. This assay contains primers and (1) MGB probe targeting highly conserved MPXV sequence (GeneBank AF380138 F3L), (2) BHQ probe targeting non-variola orthopoxvirus (OPX) sequence, and (3) a probe targeting a selected human house-keeping gene as an internal control. Furthermore, external positive and negative controls were included. A MPXV positive clinical sample from human skin lesions was used for the assay validation. The viral copy number of the sample was determined as $1x10^7$ copies/mL using the standard curve method. Two hundred µL of samples were processed in the KingFisher™ Flex system. The PCR reaction consisted of 5 µL of DNA sample and 20 µL reaction mix and was run on 7500 Fast Dx Real-Time PCR System (Life Technologies). The limit of detection (LoD) was determined as the concentration at which at least 95% of samples were tested positive. A total of 230 contrived samples with concentrations lower than LoD were used to determine cut-off value. Inclusivity and exclusivity studies were conducted by *in silico* analysis of 80 sequences of MPXV (clade I), 3026 of MPXV (clade II), 10 of camelpox virus, 93 of cowpox virus, 6 of ectromelia virus, and 116 of vaccinia virus (NCBI and GISAID accessed on 10/03/22).

Clinical evaluation of the assay was conducted using 80 contrived clinical samples in unique negative skin lesion matrix, including 40 positives (five samples at 5X LOD, five at 2.5X, ten at 1.5X, and twenty at 1X), along with 40 negatives. The positive (PPA) and negative percent agreement (NPA) of the assay were calculated. The study was approved by BRANY IRB. **Results** The qPCR assay had a cut-off Ct value of 40, and a LoD of 500 copies/mL for both MPXV and OPX with good inclusivity and exclusivity. Clinical evaluation for MPXV yielded a PPA and NPA of 94.9% (95% CI: 83.1-98.6%) and 100% (89.9%-100%), respectively. For OPX, the PPA and NPA were 92.3% (79.7- 97.4%) and 100% (89.9-100%), respectively. **Conclusions** Both analytical and clinical performances of the developed MPXV qPCR assay were good. Lyophilized reagents contain all required components, providing longer shelf-life and easier transportation. MPXV is the only member of the OPX genus known to be circulating among humans in the US currently. This assay as designed could potentially cross-detect different non-variola OPX, such as vaccinia virus and cowpox virus. Healthcare providers should always contact the local public health authorities for more guidance. Furthermore, this study was limited to lesion swab specimens and performance evaluation with additional specimen types is warranted.

B-232

Versatile, Extraction-Free Nucleic Acid Sample Preparation in 15 Seconds via Hyperbaric Heating

J. A. Hambalek, R. L. Martin, E. Alvarado, P. W. Halle, R. E. Davis, H. Janwari, H. Kido, R. Peytavi. *Autonomous Medical Devices Incorporated, Santa Ana, CA*

Nucleic acid extraction-based PCR sample prep is currently limited by long processing times, liquid transfers, kit cost, and lab storage requirements. Extraction-free approaches enable streamlined sample prep, but lack sensitivity compared to extraction-based methods. We studied several sample types to demonstrate the effectiveness of a novel 15-second Hyperbaric Heating (HBH) process to prepare nucleic acids for PCR. HBH exposes the sample to a short duration, high temperature environment to effectively lyse organisms and denature PCR inhibitors such as nucleases in seconds. Samples are introduced and sealed in a pressure-tight metallic vessel, which is then heated in seconds using magnetic induction. Within the sealed metallic vessel, the vaporization of the aqueous sample raises the internal pressure, enabling an internal temperature higher than 100°C to be achieved. Chelating agents and other proteins in HBH also protect the target nucleic acids from fragmentation. Post HBH, the sample is ready for direct PCR amplification without further dilution or extraction. Efficacy of HBH for efficient PCR amplification was tested against commercial kits. HBH was applied to viral, bacterial, eukaryotic, and fungal organisms. For SARS-CoV-2, HBH detected down to 250 viral copies/mL. Additionally, HBH outperformed commercial kits for PCR sample prep of *Candida albicans* and *Bacillus subtilis*. HBH is a generalizable sample prep technology with sensitivity comparable to that of current extraction kits. We have applied HBH to a multitude of organisms and have demonstrated that HBH can be employed as a rapid and comprehensive nucleic acid prep approach for applications that involve PCR amplification post sample prep. Future integration of HBH in devices suitable for point of care molecular testing are underway. We also envision HBH to be a useful, generalizable sample prep method for the molecular lab in applications where cost, simplicity, and speed are essential for preparing nucleic acids for amplification.

Target Organism (Concentration)	Commercial Kit		Hyperbaric Heating Threshold Cycle	Ct Differential
	Manufacturer Kit (Time in min.)	Nucleic Acid Extraction Threshold Cycle		
SARS-CoV-2 (500 copies/mL)	Qiagen QIAamp Viral RNA Mini Kit (50)	34.4±1.8	33.6±0.5	-0.8
HeLa Mammalian Cells (1.5E3 cells/mL)	Qiagen DNeasy UltraClean Microbe DNA Kit (75)	23.5±0.1	22.1±0.1	-1.4
S. cerevisiae Yeast (3.4E4 CFU/mL)	Qiagen DNeasy UltraClean Microbe DNA Kit (75)	35.5±1.1	29.4±0.1	-6.1
B. cereus Bacterial Spore (6E5 CFU/mL)	Qiagen DNeasy UltraClean Microbe DNA Kit (75)	37.9±2.9	27.1±0.2	-10.8
B. subtilis Bacteria (4E5 CFU/mL)	Qiagen DNeasy UltraClean Microbe DNA Kit (75)	25.0±0.1	25.1±0.1	0.1
C. albicans Fungi (1E6 CFU/mL)	Qiagen DNeasy UltraClean Microbe DNA Kit (75)	25.1±1.2	16.9±0.1	-8.2

B-233

Chromatin accessibility uncovers KRAS-driven FOSL2 promoting pancreatic ductal adenocarcinoma progression via upregulating CCL28

C. Wang[1], J. Li[1], S. Zhang[2]. *[1]The Second Hospital of Shandong University, Jinan, China, [2]The Second Hospital of Shandong University, jinan, China*

BACKGROUND: Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal human cancer with 5-year survival rate less than 10%. Pancreatic cancer remains a malignancy of poor prognosis and effective therapies are lacking, therefore, studying the

pathogenesis and identifying more therapeutic targets are urgently needed for PDAC treatment. The epigenetic mechanisms of PDAC progression are poorly understood. This study aims to identify key transcription factor (TF) by multiomics sequencing to explore the molecular mechanisms of TF with critical roles in PDAC.**METHODS:** we used ATAC-seq, H3K27ac ChIP-seq, and RNA-seq to characterized the epigenetic landscape by genetically engineered mouse models (GEMMs) of PDAC with or without KRAS and/or TP53 mutations. Survival of FOSL2 was assessed using Kaplan-Meier method and multivariate Cox regression analysis for patients with PDAC. We performed Cleavage Under Targets and Tagmentation (CUT&Tag) to study the potential targets of FOSL2. CCK8, transwell migration and invasion, RT-qPCR, western blot, IHC, ChIP-qPCR, dual-luciferase reporter, and xenograft model assays were used to explore the functions and underlying mechanisms of FOSL2 in PDAC progression. **RESULTS:** We found that the epigenetic changes were involved in some immuno-suppressed signaling with PDAC progression. Also, we identified a critical regulator FOSL2 which showed enriched open chromatin, H3K27ac signal, and higher RNA expression in KC and KPC than WT. The high expression of FOSL2 was associated with poor prognosis of patients with PDAC. Single-cell RNA-seq data showed that FOSL2 expression was very low in T, B, and acinar cells, and enriched in endothelial, ductal cells and upregulated in cancerous ductal cells compared with normal pancreatic ductal cells. functionally, FOSL2 can not only promote the proliferation, migration and invasion of pancreatic cancer, but also inhibit tumor immunity such as reducing the infiltration of CD8+T cells and increasing the infiltration of regulatory T (Treg) cells.In terms of mechanism, we found that CCL28 was a key target gene of FOSL2 by CUT&Tag sequencing. We confirmed that FOSL2 can regulate the expression of CCL28 by knockdown or overexpression of FOSL2 in vitro. ChIP-qPCR and dual luciferase reporter assays confirmed that FOSL2 activated the transcription of CCL28 by binding to the upstream of CCL28. In vivo mouse assay showed that FOSL2 promoted tumor growth and Treg cell recruitment through CCL28. We also explored the mechanism by which FOSL2 was upregulated in pancreatic cancer and found that KRAS mutation mediated FOSL2 transcription and subsequent CCL28 expression through the MAPK/ERK pathway, which implicated an immuno-suppressed regulatory axis of KRAS/MAPK-FOSL2-CCL28-Treg cells in PDAC development. **CONCLUSION:** We described the changes of epigenetic landscape during PDAC progression and found that KRAS mutation promoted FOSL2 expression, which recruited more Treg cells by upregulating CCL28 and ultimately led to immunosuppression in pancreatic cancer. Targeting CCL28 with CCL28 antibody can reduce the invasion of Treg cells, which provides a new way to overcome the Treg cell-mediated tumor immune escape in patients with PDAC.

B-234

The Quality in NGS: Do you know how much we lost?

T. Chao, P. Chen, W. Huang. *Chang Gung Memorial Hospital-Kaohsiung Medical Center, Kaohsiung, Taiwan*

Background Next generation sequencing (NGS) is a new high throughput approach for genomic sequencing and variations detection. The consumption of NGS is numerous, such as times, money and human resource, therefore, some hospitals provided the service but delivered the specimen to other laboratories. Hence, we aimed to establish a procedure to monitor the quality and integrity of report. **Method** Here we used one clinical case to elucidate the whole process of raw sequence data analysis by free resource, a website usegalaxy.org and the IGV visualization software, with flowchart and quality control (QC) items. A delivered case will be an example to show how to monitor the QC. **Results** The flow chart showed the whole steps of analysis of NGS, including evaluation genetic variants in the report from individual laboratory. We processed the FASTQ file and analyzed the quality with three parameters. An overall picture of bases showed the phred score (QV) less than 28. Distribution of sequence lengths over all sequences highlighted a prominent peak at 110-119bp. The mean QV of reads was around 26 with low quality (QV less than 20) in some reads. We then filtered the FASTQ file and the reads with length less than 45bp or greater than 150bp and QV less than 20 were filtered out. The data of the BAM file mapped to the designed panel were output. There were 8252 genome regions designed over 23 chromosomes (chr). A total number of reads was 1,085,653 with the average 131.6 reads (range: 0-31034; quartiles, IQ25: 1; IQ50: 27; IQ75: 152). 7746 of designed genome regions (93.9%) had reads less than 500 (quartiles, IQ25: 0; IQ50: 20; IQ75: 118). We further used IGV to check one of mutations which showed *KRAS* G12V in the report. *KRAS* G12V (chr.12: 25245350) was read 25 times with the variant allele frequency (C to A) of 24%. **Conclusion** We showed how to monitor the NGS quality by free resource. It is important to ensure high-quality data after we received the individual laboratory reports. That may help increase the quality of patient care and clinical service.

B-236

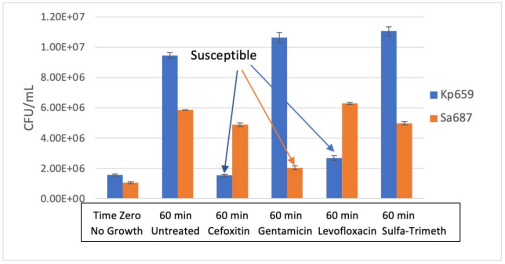
Portable 2 hour Sample-to-Answer Multi-pathogen Phenotypic Antibiotic Susceptibility Test (AST)

M. E. Roegner. *GeneCapture, Inc, Huntsville, AL*

Background: Antimicrobial Resistance is a rapidly growing issue worldwide, arising from the overuse and misuse of antibiotics. The strongest tool the medical community has available to combat AMR is rapid, correct application of antibiotics to identified bacterial infections. Clinics and physician offices seek on-site rapid solutions to speed the pathway to timely and appropriate antibiotic therapies. GeneCapture is prototyping a rapid, sample-to-answer product to provide antibiotic susceptibility results directly from infected urine or wound samples. The test provides a susceptible versus non-susceptible call in under two hours and can be applied to samples with single or mixed pathogens. With support from the Defense Health Agency, a portable table-top instrument and disposable cartridge are being engineered to run this test at the point of care, allowing same-day prescriptions. The process is currently operational on the lab bench. **Methods:** Fluorescent labeling of bacterial species, followed by optical counting, determines population growth with/without a suite of antibiotics. An on-board algorithm instantly converts raw cell numbers into a differential call of Susceptible or Non-susceptible for each bug/drug pair. A disposable cartridge with on-board antibiotics has been designed and is being finalized for fabrication. The optical counting is operating in a desktop device. **Results:** Testing to date includes 124 strains from 7 of the most common Urinary Tract Infection and wound pathogens against a panel of six antibiotics. A total of 541 antibiotic/bacterial strain pairs in single or multiple pathogen antibiotic susceptibility testing (AST), are producing an initial 88.7% agreement to gold standard culture results. These results are all generated in two hours or less of assay time and represent a strong preliminary data set. **Conclusion:** This 2-hour portable, novel, phenotypic AST profiling will produce informed and effective treatment options for medics, clinics and physician offices long before existing AST methods could return results.

Sample 60-Minute Growth Results for Mixed Pathogen Mock Wound Sample

Figure 1. *Mixed culture AST data from Klebsiella pneumoniae strain Kp659 and Staphylococcus aureus strain Sa687 in a mock wound sample produced AST results consistent with gold standard. The K. pneumoniae was susceptible to both Cefoxitin and Levofloxacin; the S. aureus was only susceptible to Gentamicin.*



B-237

Analytical and Clinical Performance Evaluation of the Panther Fusion® GI Parasite Assay (In Development)

J. Moberly, V. Dong, B. Dockter, Z. Marsh, M. Higgins, D. Miller, K. Laffer, D. Lopez, B. Kirkconnell, M. Carvallo Pinto. *Hologic, San Diego, CA*

Background: Acute diarrhea from gastrointestinal (GI) infections is the leading cause of outpatient visits, hospitalizations, and loss of quality of life, with an estimated global impact of 500 million illnesses and 230,000 deaths annually. Most GI infections from bacteria, viruses, and parasites present similar symptoms, but successful treatment is dependent on accurate pathogen identification. For identification of parasites, microscopic testing is often used, but is laborious and often results in inconclusive or inaccurate diagnoses. Clinicians now rely on rapid and accurate molecular diagnostics to correctly identify the causative organism, which leads to optimal infection control and appropriate treatment. The Panther Fusion (PF) GI Parasite Assay is a multiplex qualitative real-time PCR assay that addresses the need for accurate and sensitive identification and detection of the most common parasites that are known to cause gastroenteritis: *Cryptosporidium*, *Entamoeba histolytica*, *Giardia lamblia* and *Cyclospora cayetanensis*.

Methods: Stool specimens collected in Cary-Blair (CB) are transferred to the Aptima™ Multitest collection tube (Hologic). Multitest tubes are loaded on Panther Fusion where nucleic acid isolation, amplification, and detection are performed. Analytical sensitivity was assessed using cell suspension of *Cryptosporidium*, *Giardia*

or *E. histolytica* spiked in negative CB Stool (CBS) matrix and the limit of detection (LoD) was determined using probit. Since *Cyclospora cayetanensis* cannot be cultured, in-vitro transcribed RNA (IVT) was spiked in CBS matrix for LoD evaluation. Inclusivity was evaluated using cell suspensions for 20 *Giardia* and 15 *E. histolytica* strains spiked in CBS matrix. *Cryptosporidium* inclusivity was evaluated with synthetic nucleic acid. Specificity was evaluated by spiking cells suspensions or synthetic nucleic acid of various microbes in CBS in the presence or absence of GI targets. For clinical performance, 119 archived frozen stool specimens in CB and 40 contrived specimens were tested on Film Array GI Panel (BioFire) and PF GI Parasite Assay and results were compared.

Results: The LoD for *Cryptosporidium*, *Giardia* and *E. histolytica* was determined to be < 1 cell/mL in the Aptima Multitest tube. For *C. cayetanensis*, the LoD was found to be 1032 copies/mL. The PF GI Parasite Assay detected 20 strains of *Giardia lamblia* and 15 *E. histolytica* strains. In-silico and initial testing of synthetic nucleic acid demonstrated detection of 10 *Cryptosporidium* species. No cross-reactivity to closely related organisms or other microorganisms commonly found in the gastrointestinal tract was observed, except for cross-reactivity to *Entamoeba nuttalli,* which rarely infects humans. In testing of clinical remnant specimens, the PF GI Parasite Assay had a 97% PPA (35/36) for *Cryptosporidium*, 100% PPA (58/58) for *Giardia lamblia*, 100% PPA (42/42) for *E. histolytica* (included 40 contrived specimens) and 100% PPA (8/8) for *Cyclospora* with the Film Array GI Panel.

Conclusion: The PF GI Parasite Assay demonstrated excellent analytical and clinical performance in the detection and differentiation of *Cryptosporidium*, *Entamoeba histolytica*, *Giardia lamblia* and *Cyclospora cayetanensis*. Together with its companion Panther Fusion assays that detect bacterial and viral GI pathogens (also in development), this assay will provide a sensitive and accurate molecular tool for the automated detection of parasites in human stool.

B-239

Long-read Nanopore Sequencing is a Practicable Method of Targeted or Untargeted Microbial Contamination Detection

R. Suhandynata[1], S. Maheu[2], S. Safiari[2], N. Bevins[2]. *[1]University of California at San Diego, San Diego, CA, [2]Sequence Sciences, San Diego, CA*

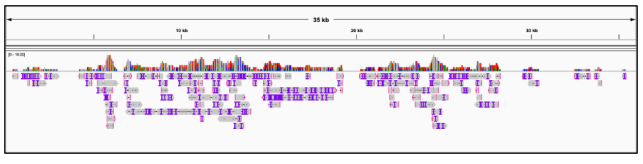
Background: Sequencing-based methods for microbe detection are poised to replace both *in vivo* and PCR-based detection methods due to advantages in speed, sensitivity, specificity, and multiplexing. Here we describe pilot experiments to assess the practicability of nanopore sequencing for microbe detection.

Methods: Quantitative genomic Human Adenovirus 5 or *Mycoplasma hominis* DNA was spiked into remnant human serum and nucleic acids were extracted. 16S rRNA PCR amplification and barcoding was performed for *Mycoplasma hominis* identification. Sequencing was performed with a R9.4.1 flowcell on the MinION sequencer. Basecalling and genome alignment was performed with Guppy v.6.4.2.

Results: The performance of untargeted sequencing was evaluated by spiking quantitative Adenovirus DNA at 10^4 genome copies per microliter. Illustrative data from a single run is presented. Replicates yielded similar results. 1,520,991 reads between 31 bases and 970 kilobases were generated. 162 reads between 700 bases and 11.3 kilobases aligned to the Adenovirus genome (Image 1).

To evaluate the performance of PCR amplified sequencing, we spiked *Mycoplasma* genomic DNA into serum or water. The entire 16S rRNA gene (1.5kb) was PCR amplified and genomic DNA was spiked in at 10^4, 10^2, 10, and 0 (control) copies per microliter. A 10% signal: noise ratio cutoff yielded 100% specificity between spiked samples and negative controls.

Conclusion: Short read sequencing techniques are adequate for microbial detection but are capital intensive and prone to false positive results. We demonstrate that nanopore sequencing can generate long reads (>1.5 kb) with high-confidence microbe genome alignment. Our methods were sensitive to 10^4 and 10 copies per microliter utilizing untargeted and targeted methods, respectively. Further method optimization and validation will need to be performed, but these results demonstrate that nanopore sequencing is a practicable approach for microbe detection.



B-240

Development of AcroMetrix™ Human Microbiome Quality Control Materials

Q. Gan[1], B. Drews[2], W. Tom[2], M. G. Torralba[3], L. Liu[1]. *[1]Thermo Fisher Scientific, Fremont, CA, [2]Thermo Fisher Scientific, South San Francisco, CA, [3]Thermo Fisher Scientific, Frederick, MD*

Background: The human microbiome is receiving significant attention given its critical roles in the maintenance of human health. The compositional and functional alterations of the human microbiota have been associated with various pathological conditions including colorectal cancer (CRC). As the number of human metagenomic data increases, the interest to utilize these metagenomic biomarkers for diagnosis of colorectal cancer and other diseases has also elevated. Studies that evaluated the defined fecal microbial markers for CRC detection showed the test accuracy could vary from 73% to 98% depending on the detection panels of microbiome biomarkers. The measurement inconsistencies between laboratories due to the use of different detection platforms, the coverage of various assay panels, the biases in different steps of microbiome workflow all indicated the critical need for reliable quality control materials. Currently, the commercially available microbial standards usually contain limited species and also mixed with fixed ratios which may not reflect the microbial composition in the clinical samples. There is no commutable reference material that recapitulates the dysbiotic microbial composition under healthy or pathological conditions (e.g. CRC). Therefore, in this study, we developed DNA quality control materials (QCMs) that recapitulate the dysbiotic microbial composition for colorectal cancer to assess the technical and non-technical variation for part of the microbiome workflow.

Methods: We first identified the specific bacterial species that either are enriched in CRC patients or healthy human gut based on comprehensive literature search and the other available dataset. We then created microbiome QCMs to recapitulate their dysbiotic microbial composition in comparison to the compositions in healthy condition. The QCM consists of a mixture of genomic DNA from the selected bacterial species, the copy number of DNA was determined by Bio-Rad™ droplet digital PCR (ddPCR) with the assays that target to species-specific regions to reflect the microbial composition. In addition, the microbiome controls are further evaluated and validated by Microbiome Health Research Assay on Ion Ampliseq and GeneStudio S5 platforms.

Results: The AcroMetrix™ human microbiome QCMs have been carefully formulated to mimic dysbiotic microbial composition for colorectal cancer or healthy human gut. This CRC QCM covers 29 different species with a wide range of guanine-cytosine content sequences, belonging to 10 phyla and 20 genera. 25 out of these 29 species (86.2%) were covered by Microbiome Health Research Assay. To mimic the trends of dysbiotic microbial composition, the DNA frequency among these 29 species was targeted from 0% to 15% according to the microbial composition. Droplet digital PCR results confirmed the target frequencies within the accepted tolerance range and the results were compared with the data from the Next Generation Sequencing platform.

Conclusions: The human microbiome QCMs that cover 29 bacterial species and mimic dysbiotic microbial composition for colorectal cancer or healthy human gut have been designed and developed. These QCMs could provide essential elements to verify, validate and monitor test performance, technical and non-technical variation of microbiome workflows, as well as product QC releases and method development for ddPCR & NGS assays.

B-241

A Novel Approach to Develop Multiplex Loop Mediated Amplification (LAMP) Assays

y. chander, D. Mead. *Varizymes, Middleton, WI*

Background: Loop mediated amplification (LAMP) technology is a sensitive, accurate and rapid method for point-of-care (POC) molecular diagnostic detection. One disadvantage is that current LAMP methods can detect only one target per reaction whereas in a standard real-time PCR assay, multiple targets (3 - 4) can be detected in a single reaction. This is because current methods of detection for amplification products in a LAMP assay are not sequence-specific. Common detection methods include turbidity, use of color-changing dye or intercalating dye in the reaction mix, gel electrophoresis, etc. These methods are not sequence-specific and, thus, unable to differentiate between targets if amplified in a single tube, with the exception of lateral flow devices or melt analysis, which add time, complexity, and cost. To overcome this challenge, we have developed a novel method which simplifies development of multiplex LAMP without use of any additional primers/probes/reagents or steps. **Methods:** Triplex RT-LAMP assay was developed for rapid detection of foot-and-mouth disease virus (FMDV) at POC. For this, LAMP primers targeting two different regions of

3D pol gene of FMDV were designed. For use as internal control (IC), LAMP primers were also designed targeting a conserved region of the bovine 18S rRNA gene. For multiplexing, best performing primer sets for each target were labelled with a unique fluorophore at 5' end and a quencher (BHQ-1) at 3' end. FMDV-1 primers were labeled with Cy5 fluorophore, FMDV-2 primers were labelled with Texas Red (TxR) fluorophore, and 18S rRNA with FAM fluorophore. Triplex RT-LAMP assay was performed using a specially formulated Multiplex Isothermal Master Mix but without any intercalating dye added to the mix. All 3 primer mixes were added to the Master Mix and reactions were carried out in a real-time thermocycler at 72°C for 30 minutes, with data collected every 30 seconds. Reaction kinetics were monitored as an increase in fluorescence associated with the accumulation of double-stranded DNA in respective channels (FAM for 18S rRNA, Cy5 for FMDV-1, and TxR for FMDV-2).

Assay sensitivity was determined by testing 10-fold serial dilution of respective template, individually as well as mixed together.

Results: Results obtained showed feasibility of triplex RT-LAMP assay for simultaneous detection of 3 targets in a single reaction. Sensitivity and specificity of triplex RT-LAMP was comparable to control reactions (RT-LAMP assay with intercalating dye) for each target. No cross reactivity or non-specific amplification was observed with any of the primer designs.

Conclusions: This newly developed method allows detection of up to 3 targets in a single reaction without affecting the assay performance. This method is much easier to develop and optimize than previously reported methods as it uses standard LAMP primers without need of any additional primers/probes.

B-242

Fast and cost-effective RNA extraction in onco-hematology laboratories

C. Pugliese, L. Dame, S. Lanes, A. Marinato, R. Proto-Siqueira. *Flow Diagnosticos, Sao Paulo, Brazil*

Background: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by the presence of a *BCR-ABL1* rearrangement; its detection and quantification by real-time quantitative PCR (RQ-PCR) plays a central role in CML diagnosis, therapy monitoring, and sequencing to identify resistance mutations in case of therapy failure. To evaluate the molecular response of CML, *BCR-ABL1* quantification must be calculated on the international scale (IS%) for which RQ-PCR has the necessary sensitivity (up to 10^{-5}). Currently, *BCR-ABL1* quantifications are ubiquitous in onco-hematology laboratories (OHLs; ~ 70% of tests performed in our OHL), whereby quality, cost, and turn-around-time are of critical importance. Today, the majority of OHLs in Brazil rely on laborious manual RNA-extraction methods (e.g., Trizol) that usually require 8 mL of peripheral blood (PB) to monitor CML. Here, we report a strategy that requires only 2 mL of PB in combination with the automated magnetic DNA/RNA-extraction kit Extracta-MPTA (Loccus do Brasil) for CML diagnosis and monitoring. Methods: BCR:ABL1 was quantified and its IS% determined using our developed kit, which was calibrated using ERM-AD263 and UK-NEQAS (external quality assessment). Total RNA was extracted from 40 PB samples, after erythrocyte lysis using two different methods: 1) Leukocytes from 8 mL of PB were extracted manually using Trizol; 2) leukocytes from 2 mL of PB were automatically extracted using Extracta-MPTA (Loccus do Brasil). *BCR-ABL1* quantification, *BCR-ABL1* IS%, and BCR quantification (internal control) were compared statistically (paired t-test). The reproducibility was evaluated by analyzing 20 replicates; the low limit of the leucocyte number to *BCR:ABL1* quantification using Extracta-MPTA were also evaluated. Median and moving means of 135 unpaired samples were used to monitor *BCR-ABL1* IS% between Trizol, Extracta-MPTA, while EQA from UK-NEQAS was also evaluated using both methods. In parallel, RNA was extracted by both methods from 20 samples for the diagnosis of acute lymphoblastic leukemia (ALL) (BCR-ABL p190 transcript) and acute promyelocytic leukemia (APL) (PML-RARA transcript). Results: A comparison of *BRC-ABL1*, BCR, and %IS in 40 paired samples showed that the results obtained from Trizol or Extracta-MPTA are comparable (paired t-test: p = 0,4222, p = 0,9263, and p = 0,071, respectively). The reproducibility was evaluated by BCR quantification (24%).. A comparison of the medians from 135 unpaired samples extracted using Trizol and Extracta-MPTA revealed that *BCR-ABL1* IS% distribution among our patients is stable over time (p = 0,7704; Mann Whitney: r^2 = 0,0012). EQA was extracted using both methods and the results were identical. A comparison between Trizol and EXTRACTA_MPTA for ALL and APL was 100% identical (Fischer; p = 1,000). **Conclusion**: Both RNA extraction methods are suitable for OHLs for PML-RARA and BCR-ABL (p190 and p210) detection and CML diagnosis/monitoring by BCR-ABL1 detection/quantification. By using Extracta-MPTA the amount of PB used to obtain RNA can be reduced by 75%, test results can be obtained within 4 h, and direct reagent costs are reduced by 25% with a minimum of hands-on time.

B-243

Utilizing Lyophilized LAMP Reagents in Rapid Molecular Assays

E. Alpaslan, Y. Zhang, N. A. Tanner, G. C. Patton. *New England Biolabs, Ipswich, MA*

Background: Loop Mediated Isothermal Amplification (LAMP) has become a widely used method for detection of target nucleic acids (DNA and RNA) as it offers a robust and simple alternative to PCR. LAMP is particularly well suited to point of care (POC) applications such as COVID-19 diagnostics because only a single incubation temperature is required for nucleic acid amplification and the technology is compatible with simple detection strategies, including colorimetric or lateral flow readouts. To further increase the utility of LAMP in POC molecular diagnostic workflows, elimination of cold chain requirements for reagent shipment and storage is desired. In this study, we investigate single and multiplex detection of several viral infectious diseases using lyophilized RT-LAMP reagents.

Methods: Assays targeting SARS-CoV-2, FluA, FluB and a host control nucleic acid were evaluated using various detection methods with lyophilized RT-LAMP reagents. Compatibility was established with endpoint LAMP detection strategies, such as colorimetric dyes, and real time multiplex LAMP detection, which is increasingly finding utility in POC testing for common respiratory infections that present with similar symptoms.

Results: Assays employing lyophilized RT-LAMP reagents maintained robust detection of viral respiratory targets within 30 minutes at 65°C using various detection strategies. Colorimetric dyes such as eriochrome back T and calcein resulted in successful visual readout upon amplification while, one prominent colorimetric dye, hydroxy naphthol blue, was found to be incompatible with the lyophilized reagents used in the study. Additionally, simple hybridization probes permitted simultaneous detection of SARS-CoV-2, FluA and FluB in a single reaction.

Conclusion: LAMP is a robust, simple, isothermal amplification strategy that continues to enable testing beyond traditional laboratory settings. Lyophilized reagents extend the utility of LAMP in POC settings regardless of the detection strategy and will be important factors in the extension of this powerful molecular diagnostic tool to decentralized, field, and at-home testing applications.

B-244

DNA library amplification to produce archived reference samples for R&D and clinical assay development, optimization, and validation

M. Williams, C. Gilley, K. Gowen, Z. Zhang, L. Elagina, J. Charlton, R. Witkowski, A. Shuber. *Harbinger Health, Cambridge, MA*

Background Rapid advances in clinical diagnostic testing are constrained by finite specimen availability, technical and financial burdens incurred by patient recruitment, and sample collection. Expanding the availability of patient-derived analyte for experimentation could accelerate assay development and regulatory submissions, while reducing demand for additional resource-intensive clinical studies. We developed the AReS (Archived Reference Sample) platform to utilize genomic libraries as templates for PCR amplification. The resulting PCR product, referred to as an AReS library, serves as an alternative sample type from which development, optimization, and validation studies can be iteratively performed.

Methods PCR conditions were optimized to maximize yield while minimizing amplification bias, generating approximately 50X more mass than the starting input. To evaluate the AReS process, aliquots of bisulfite converted original DNA libraries were further amplified under optimized conditions to produce AReS libraries. Both the original and AReS libraries were then hybrid captured using Harbinger Health's proprietary 8.4 Mb panel and sequenced to ≥100X unique median target coverage (MTC) depth. Original libraries were compared to AReS libraries across Picard sequencing metrics by quantifying methylation across our regions of interest and by classification as determined by our cancer yes/no (CYN) determining algorithm. Harbinger Health's CYN algorithm was developed using a multi-layered logistic regression-based machine learning approach trained on a separate patient cohort and locked prior to being used in this study. In total, we generated 528 AReS libraries from 321 unique patient-derived DNA samples, of which 124 were from patients diagnosed with cancer and 197 from patients with no cancer diagnosis. An additional sub-study was performed on 16 paired original and AReS libraries containing unique molecular identifiers (UMIs). The UMIs allowed for the comparison of individual cfDNA molecules between the two sample types.

Results All AReS samples, including both intra- and inter- batch replicates, were highly concordant to the original library. There was no significant difference across sequencing metrics (e.g. conversion efficiency or %CC and MTC). All AReS libraries

had similar methylation values to original libraries; Pearson correlation was greater than 98%. Our data also indicated that greater than 97% of AReS libraries were concordant with the original library by our CYN algorithm classification. Read-level UMI analysis identified that approximately 77% of reads were common between the original and AReS libraries. As both the UMI and sequence insert used were identical, these common reads were derived from the same cfDNA molecule. As a frame of reference, sequencing replicates of the original library similarly shared approximately 77% of common reads. In addition, the Pearson correlation of read frequency compared between AReS

and original libraries were within 4% difference of the correlation between original library sequencing replicates. These results showed no indication of AReS-derived amplification bias.

Conclusion Taken together, the AReS process produces excess libraries that highly reproducible. AReS libraries are functionally and analytically identical to original libraries and can be applied to both research and clinical use.

B-245

Applicability of metagenomic Next-Generation Sequencing (mNGS) in challenging infectious diseases in a tertiary hospital

L. F. Chebabo[1], F. M. Conti[1], S. P. Bandeira[2], M. C. Castelo[2], A. C. Lopes[3]. *[1]Dasa, Rio de Janeiro, Brazil, [2]Dasa, Fortaleza, Brazil, [3]Dasa, Florianópolis, Brazil*

Background Infectious diseases caused by rare pathogens or with unusual presentations pose a diagnostic challenge. Traditional microbiologic tests can be time-consuming, labor-intensive and have limited sensitivity for some organisms. Plasma metagenomic Next-Generation Sequencing (mNGS) detects whole microbial genetic material and detects even unsuspected organisms. We present three cases of challenging infectious diseases and discuss the applicability of mNGS in different scenarios **Methods** Plasma NGS was performed at Karius, Inc. (Redwood City, CA, USA). Patient data was collected from hospital and laboratory electronic records **Results - Case 1:** An 8-year-old female with sickle-cell anemia developed a persistent daily fever two weeks after recovery from sepsis due to *Streptococcus pyogenes*. Clinical exam was normal and investigation was negative (blood cultures, echocardiogram, thoracic and abdominal CT scans and bone scintigraphy). Two weeks later mNGS was performed, which came out positive for *Cytomegalovirus*. As patient became afebrile in 48 hours and the viral burden was low, she could be safely discharged. **Case 2**: A 43-year-old male with prosthetic aortic valve and an intraluminal aortic ring had a year-long history of fatigue, weight loss and hypoalbuminemia. CT scans showed ground-glass infiltrates and enlarged mediastinal lymph nodes. Empirical treatment for tuberculosis was ineffective. A year later, he developed hepatosplenomegaly, fever and pancytopenia. Blood cultures, bone marrow biopsy and serologies (HIV, hepatitis B/C, syphilis, brucellosis, bartonellosis, leishmaniasis) were negative. Cardiac surgery found no apparent signs of endocarditis and the prosthetic aortic valve biopsy revealed only an inflammatory infiltrate. A new CT scan showed thickened intraluminal aortic ring with aortic pseudoaneurysms and splenomegaly with splenic infarction. A blood sample was then collected for mNGS and liver biopsy and splenectomy were performed. However, surgery complicated with hemodynamic instability, renal and liver failure and the patient died in less than 24 hours. Two days later, mNGS came out positive for *Coxiella burnetti*. **Case 3:** A 45-year-old female with relapsed AML treating a chronic disseminated candidiasis with micafungin for 3 months had persistent liver and splenic nodules and new pulmonary nodules on the CT scan. In the following three months after chemotherapy (Gilteritinib), she developed multiple episodes of febrile neutropenia, some without microbiological documentation, leading to multiple courses of antibiotic treatment and a shift to liposomal amphotericin B. After clinical improvement and bone marrow recovery, liposomal amphotericin B was replaced by isavuconazole. A negative mNGS result allowed antifungal treatment to be safely discontinued, avoiding six months of unnecessary antifungal treatment **Conclusion:** We summarize three different scenarios of mNGS applications. In common, patients were submitted to multiple laboratory and image testing and received large doses of empirical antimicrobials due to lack of a precise diagnosis. In two cases, mNGS was diagnostic, shortened hospital stay and reduced overall costs. In case 2, the patient's death might have been prevented if mNGS had been performed earlier. Vulnerable hospitalized patients with a broad spectrum of diagnostic possibilities, in whom sequential diagnostic tests could delay adequate therapy and cause significant adverse effects, could greatly benefit from mNGS when performed in a timely manner.

B-246

The Evaluation of Actionable Mutations By Multiplex Analysis Using IntelliPlex® Lung Cancer Panel for Non-Small Cell Cancer Management

W. Cheng. *PlexBio Co., Ltd., Taipei City, Taiwan*

Background: Understanding the genetic alterations which occur in genes associated with various cancers can improve therapy outcomes by promoting tailored treatments for each patient. Molecular profiling is therefore a crucial tool for precision medicine and enables for a personalized approach to cancer management. Current guidelines for molecular testing of Non-Small Cell Lung Cancer (NSCLC) patients involves treatment selection of a targeted Tyrosine Kinase Inhibitor based on the minimal testing of EGFR, ALK and ROS1 genes and strongly recommending the additional testing of KRAS, BRAF, MET, RET, NTRK and ERBB2 (Her2) genes to fully evaluate the patient's mutational status. **Method:** The IntelliPlex Lung Cancer Panel, based on PlexBio's multiplexing πCode® technology, is an in vitro molecular amplification assay intended for the qualitative identification of 74 DNA mutations in the KRAS, NRAS, PIK3CA, BRAF, EGFR, HER2, MEK1 and AKT1 genes and 28 gene variants of the ALK, ROS1, RET, NTRK1 and MET genes. The assay is suitable for the use with formalin-fixed paraffin-embedded (FFPE) tumor tissues from NSCLC patients. The performance of IntelliPlex Lung Cancer Panel was evaluated on FFPE specimens by comparing the results with two commercially available molecular amplification assays. Discrepant samples were resolved by a corresponding digital PCR method kit. **Results:** The IntelliPlex Lung Cancer Panel can detect DNA mutations at very low allele frequencies (as low as 0.1%) while only requiring DNA quantities of 10ng. Most RNA rearrangements are detected with 10 or more copies present in the sample while only low RNA input quantities (50ng) are required. The IntelliPlex Lung Cancer Panel can analyze the mutational status of 102 targets in less than 5.5 hours from sample to results and allows screening of up to 48 samples in parallel. Among 50 FFPE DNA specimens tested, the overall agreement for EGFR and KRAS mutations between IntelliPlex system and the comparison method were both found to be 98%. And the overall agreement after resolved the discrepancies by digital PCR was 100%. **Conclusion:** The multiplex amplification and detection capabilities of the IntelliPlex Lung Cancer Panel provides a comprehensive and efficient way to evaluate the individual mutational status from lung cancer patients with high sensitivity and specificity. Accurate genetic analysis provides valuable information which can support precision medicine decisions for better cancer management. This can also be used further with non-invasive whole blood sampling (i.e., 'liquid biopsy') for subsequent treatment monitoring.

B-247

Rapid and Field-deployable Microchip-based Real-time PCR for Hepatitis B Virus DNA Detection

Y. LIU, Y. GAO, Q. SONG, Y. Hong, W. WEN. *Hongkong University of Science and Technology, Hong Kong SAR, Hong Kong*

Background: Hepatitis B is one of the most common infectious diseases with global significance, seriously causing chronic liver complications and a heavy health burden in developing countries. There is no doubt that real-time polymerase chain reaction (RT-PCR) has been a golden standard for monitoring the Hepatitis B virus (HBV) level in clinical health management. However, the conventional PCR protocol cannot fulfill the field test requirement in some resource-poor areas due to its drawbacks, such as huge size, long turnaround time, and demand for professional laboratory facilities. For solving these problems, a portable microfluidic chip-based PCR detection system was developed to enable the nucleic acid test in a flexible, rapid, and economical way. With the efficient temperature control module and advanced optical path design, this novel panel can achieve fast thermal circulation with a 30℃/s heating rate and multiple fluorescent detection with a throughput of 6 samples at a time. **Methods:** In this study, we assessed the detection performance of the system using a commercial HBV nucleic acid detection kit. The reproducibility, limit-of-detection (LoD), and linearity were validated by reference samples with gradient concentrations. We evaluated the clinical sensitivity and specificity of the microdevice by a total of 200 extracted serum samples (100 positive and 100 negative cases). The analytical results were compared with a traditional PCR device with FDA certification. **Results:** The variation coefficients of the microdevice for reference samples with different concentrations are 1.78%,1.98%, and 1.50%(n=10), respectively. The field-deployable PCR system can provide the HBV DNA assay with a detection limit of 300 IU/ml, whose reaction time was approximately 67 mins rather than the 108 mins performed by the comparator. During the establishment of the standard curve, this fast PCR test shows a strong linear correlation (R^2=0.9919) between targeted gene concentration and dilution factor.

Additionally, among the 200 clinical samples, we used the microchip-based system to rapidly complete quantitative detection for each sample with high sensitivity of 100% (100/100) and specificity of 100% (100/100), which is critically consistent with the traditional PCR method. **Conclusion:** Hence, the point-of-care microfluidic PCR system exhibits great feasibility in offering timely, accurate, and reliable molecular diagnosis for early epidemic disease screening and therapeutic guidance of chronic infections.

B-248

Rapid PCR Method for Sexually Transmitted Infection (STI) multiplex testing based on novel microfluidic chip

S. Lee, J. Park, H. Koo, D. Kim, J. Park, D. Lee. *Genesystem, Daejeon, Korea, Republic of*

Background: qPCR is being widely used for STI(Sexually Transmitted Infection) testing as the most sensitive and accurate diagnostic method. Genesystem developed a novel qPCR-based diagnostic platform, called Genoplex chip, which enables the simultaneous detection of multiple sexually transmitted pathogens with a single test. Genoplex chip realized high multiplex detection (up to 14 pathogens by a single pipetting), making it easy and fast to process more targets than conventional qPCR. In this study, Genoplex chip was used for diagnosing sexually transmitted infection(STI) to verify the usefulness in diagnostics.

Methods: Genoplex chip is a flat-shaped microfluidic chip where target-specific primers and probes are prelabeled free from contamination. This chip has 1 well for negative control and 9 connected wells for target detection. By a single pipetting into the inlet hole, samples are loaded and spread into every well of this microfluidic chip since all the wells are connected to each other. We completed performance validation of this new chip with STI diagnosis. Primers and probes for identifying STI pathogens are prelabeled on the inner surface of the Genoplex chip. We can detect and identify 12 major pathogens of STI, which are *Chlamydia trachomatis* (CT), *Ureaplasma urealyticum* (UU), *Mycoplasma hominis* (MH), *Mycoplasma genitalium* (MG), *Neisseria gonorrhoeae* (NG), *Trichomanas vaginalis* (TV), *Ureaplasma parvum* (UP), *Gardnerella vaginalis* (GV), *Candida albicans* (CA), *Treponema pallidum* (TP), Herpes simplex virus type 1 (HSV1) and Herpes simplex virus type 2 (HSV2) by using these probes. Amplification of the target genes was performed by Genechecker UF-300 PCR System.

Results: We estimated 50 copies of 12 STI pathogens by qPCR process in 40 minutes. We also confirmed that there was no contamination and no interference in 12 targets

Conclusion: : With Genoplex chip, various targets can be quantitatively detected in one sample and more accurate results can be provided in efficient way. This novel system gives an opportunity to overcome the limitations of current molecular diagnostics by innovative multiplex detection technology. Therefore, it is expected that the Genoplex chip system will attract attention in the molecular diagnostics market.

B-249

Usefulness of simultaneous detection of germline and somatic variants from different specimen types by next-generation sequencing

M. Han, J. Kim. *Kangdong Sacred Heart Hospital, Seoul, Korea, Republic of*

Background: Genetic variants detection by next-generation sequencing (NGS) from tumor specimen only is considered limited in diagnosis due to difficulties in discriminating somatic variants from germline ones. It will be even more helpful in diagnosing patients with cancer if we perform NGS analysis from specimen of both tumor and blood. This study shows the usefulness of simultaneous detection of germline and somatic variants from different specimen types by NGS.

Methods: NGS panel assay designed for solid tumor composed of 80 genes was conducted for detection of both somatic and germline variants. DNA samples extracted from both buffy coat of whole blood and formalin-fixed paraffin-embedded (FFPE) specimen from 4 patients with renal cancer and 3 patients with ovarian cancer were used in assay. We compared results from both specimen and finalized somatic and germline variants detected.

Results: In 4 patients specimen with renal cell carcinoma, we were able to rule out germline variants from somatic variants by analysis of NGS assay from both specimen types. Genes ruled out for germline variants of uncertain significance (tier 3) or higher levels are ARID1B: c.1031C>T, p.A344V, ERBB2: c.3430G>C, p.D1144H, BRCA2: c.3220A>T, p.S1074C, POLE: c.2974G>A, p.A992T, EP300: c.1666A>G, p.M556V, EP300: c.2539C>T, p.P847S, ERBB3: c.1115C>G, p.P372R, BAP1: c.1124A>T, p.E375V. In 3 patients with ovarian cancer, genes ruled out are EGFR: c.739G>C, p.D247H, MET: c.4172A>T, p.D1391V, PBRM1: c.2678A>G, p.Y893C, SETD2: c.1208G>A, p.S403N, AKT1: c.1373T>C, p.M458T, FGFR3: c.160G>A, p.G54R, KMT2C: c.9530G>A, p.R3177H, SMARCB1: c.1116G>A, p.T372T. All variants confirmed are tier 3 level of clinical significance. **Conclusion**: According to the results of this study, we were able to exclude germline variants form somatic ones in NGS assay reports for cancer patients. This study shows the usefulness of simultaneous detection of germline and somatic variants from different specimen types by NGS.

B-250

Simultaneous Detection of Twelve Pathogens of Sexually Transmitted Infections Using an Innovative All-in-One PCR Instrument

S. Nam, J. Lee, M. Song, H. Heo, Y. Jeong, D. Kim, H. Park. *BIONEER, Daejeon, Korea, Republic of*

Background: Every day, nearly 1 million people are infected with sexually transmitted infections (STI) that are transmitted through sexual contact. Early diagnosis and treatment of STI are critical in reducing their transmission, and PCR is the most sensitive method among diagnostic tests. To address this need, we have developed a new STI Real-Time PCR-based diagnostic kit, which is capable of detecting twelve STI pathogens simultaneously in up to 94 specimens at once. To evaluate the analytical performance, we used the Existation™ FA System, which automatically performs from decapping primary sample tubes followed by nucleic acid extraction to PCR analysis within 2 hours. Twelve (12) pathogens of STI are described below: *Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Ureaplasma urealyticum (UU), Mycoplasma genitalium (MG), Trichomonas vaginalis (TV), Mycoplasma hominis (MH)*, Herpes simplex virus type1 (HSV1), Herpes simplex virus type2 (HSV2), *Ureaplasma parvum (UP), Treponema pallidum (TP), Candida albicans (CA), Gardnerella vaginalis (GV),* from male urine and female vaginal swabs.

Methods: The analytical performance evaluation was performed using the new STI diagnostic kit to detect twelve pathogens of STI. The analysis was performed using culture fluids (CT, NG, UU, MG, TV, MH, HSV1, HSV2, UP, CA, GV; ATCC, USA) and TP genomic DNA (Vircell, Spain), which were spiked in urine and vaginal swab media. The limit of detection (LoD) was determined by testing multiple replicates of serial dilutions of the spiked sample around the expected LoD. The LoD was defined as the lowest dilution at which the assay detected ≥95% of 24 replicates. To evaluate precision, each sample was tested 2 times a day in duplicate per run for 5 days. To assess cross-reactivity, twenty-two bacteria and viruses related to STI were used.

Results: The LoD was determined 60.26 IFU/mL, 35.48 cfu/mL, 467.74 copy/mL, 45.71 cell/mL, 19.5 cells/mL, 316.23 copy/mL, 199.53 $TCID_{50}$/mL, 13.18 $TCID_{50}$/mL, 588.84 copy/mL, 993.25 copy/ml, 660.69 copy/mL, 389.05 copy/mL in Urine and 47.86 IFU/mL, 27.54 cfu/mL, 363.08 copy/mL, 38.90 cell/mL, 15.14 cells/mL, 245.47 copy/mL, 181.97 $TCID_{50}$/mL, 8.71 $TCID_{50}$/mL, 575.44 copy/mL, 741.31 copy/ml, 524.81 copy/mL, 288.40 copy/mL in vaginal swab media for CT, NG, UU, MG, TV, MH, HSV1, HSV2, UP, CA, GV, and TP, respectively. For repeatability assay, the 12 STI samples showed similar Ct value in each run, with all standard deviation (SD) were less than 2.0. In terms of cross-reactivity, there were no positive results obtained from testing bacteria and viruses using the new STI diagnostic kit.

Conclusions: The Existation™ FA system is highly convenient platform for Real-Time PCR-based diagnostics. Particularly, our new STI diagnostic kit provides an accurate and efficient method for simultaneous detection of 12 STI pathogens.

B-251

Nmr lipoproteins help to reveal subphenotypes of phenoreversion from sars-cov-2 infection

J. Wist[1], S. Lodge[1], R. Masuda[1], P. Nitschke[1], C. Cannet[2], H. Schaefer[2], H. Ruffieux[3], G. Bantug[4], C. Hess[5], E. Holmes[1], J. K. Nicholson[1]. *[1]Murdoch University, Australian National Phenome Center, Perth, Australia, [2]Bruker, Rheinstetten, Australia, [3]University of Cambridge, Biostatistics Unit, Cambridge, United Kingdom, [4]University and University Hospital Basel, Department of Biomedicine, Basel, Switzerland, [5]University of Cambridge, Cambridge Institute of Therapeutic Immunology and Infectious Disease, Cambridge, United Kingdom*

Background:

It is estimated that 10% of patients that suffered from severe acute SARS-CoV-2 infection experience symptoms beyond 3 months post-disease onset. Long COVID is a multisystemic condition that comprises more than 200 symptoms. Common new-onset medical conditions include cardiovascular, type-2 diabetes and chronic fatigue

syndrome. Whilst progress has been made in characterising the mechanisms underlying long COVID, supported by similarities with other viral infections, available diagnostics are still insufficient. Molecular phenotyping is an established systems medicine tool that provides an integrative profile of an individual's biological status that results from the cooperative genomic, transcriptomic and proteomic response to environmental stimuli. It exploits spectroscopic platforms based on nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) to generate precise information on a plethora of molecules that can be modelled to explain specific physiological and pathological conditions.

Methods:

A well characterised longitudinal cohort with > 200 SARS-CoV-2 positive individuals was collected in Cambridge Hospitals (2020, Wuhan strain). Each individual was classified according to the severity of its respiratory symptoms ranging from A (asymptomatic) to E (external ventilation). For each sample, cellular and immunological parameters, NMR lipoproteins, amino acid, tryptophan and lipidomics assays were measured. Functional PCA models captured latent dynamics in individual trajectories for C-reactive protein, an established marker of inflammation, and metabolic parameters found to be associated with it. Based on these trends, patient heterogeneity was explored using Gaussian mixture modelling and the resulting stratification was used to train personalised predictors of disease outcome using the first time point after onset as input. The addition of a penalty function during training ensured a parsimonious selection of parameters. Patients were asked to complete surveys months (2-6) after infection. Abundance and co-occurrence of symptoms was assessed using latent factor analysis.

Results:

Linear mixed model regression revealed strong associations between C-reactive protein abundance and molecular parameters. Quinolinic acid, VLDL triglycerides and phospholipids, VLDL and IDL cholesterol and glycoprotein related GlycA are positively correlated, while tryptophan, taurine, indole-3-acetic acid, HDL cholesterol, and phospholipids and the supramolecular phospholipid composite (SPC) peak are strongly anti-correlated. Those findings are congruent with prior work on cohorts from Western Australia and Spain. In both cases, strong lipoprotein signatures were observed; GlycA is increased during acute phase, while SPC and HDL subfraction 4 are strongly depleted. The analysis of individual trajectories measured by CRP and associated parameters revealed 3 groups, mildly affected, good recovery and poor prospect. Patients from the last category showed profoundly altered metabolic profiles even weeks after onset, and a higher co-occurrence of neurological related symptoms. Furthermore, disease outcome scores were predicted for each patient in the early stage of infection. The training of these predictors also distilled a panel of most relevant parameters for that purpose. NMR lipoproteins featured excellent predictive capabilities.

Conclusion:

Broad phenotyping, combined with multi-view multivariate analysis, allowed for robust stratification of COVID-19 patients and accurate personalised prediction of disease outcome. It also confirms the critical role played by lipoprotein metabolism in the immune response that is successfully captured by NMR-base lipoprotein parameters.

B-252

In Vitro Expression Analysis of Variants in the Upstream Region of Genes Related to Familial Hypercholesterolemia

J. G. de Araujo[1], L. Tamborlin[2], A. D. Luchessi[3], R. D. Hirata[4], M. H. Hirata[4], A. D. Luchessi[5], V. N. Silbiger[5]. *[1]Northeast Biotechnology Network (RENORBIO), Graduate Program in Biotechnology, Federal University of Rio Grande do Norte, Natal, Brazil, [2]Institute of Biosciences, São Paulo State University (UNESP), Rio Claro, Brazil, [3]Laboratory of Biotechnology, School of Applied Sciences, University of Campinas, Limeira, Brazil, [4]Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, Brazil, [5]Department of Clinical and Toxicological Analyses, Federal University of Rio Grande do Norte, Natal, Brazil*

Background: Familial hypercholesterolemia (FH) is commonly described as an autosomal dominant disorder caused mainly by variants in genes *LDLR*, *APOB*, and *PCSK9*, resulting in severe hypercholesterolemia. However, the substantial number of individuals who are clinically diagnosed but negative for known FH-causing variants indicates that relevant variants outside of frequently analyzed regions might exist. Considering the increasing number of promoter variants reported as likely to cause FH and the relevance of in vitro expression assays in the pathogenicity evaluation of these variants, this work aimed to examine the functionality of variants *LDLR* rs36218923-T and *APOB* rs934197-T, identified in a Brazilian FH cohort.

Methods: Upstream fragments of *LDLR* (-861 bp to +85 bp) and *APOB* (-1460 bp to +127 bp) were PCR amplified from genomic DNA of two FH patients carrying *LDLR* rs36218923-T and *APOB* rs934197-T in heterozygosis and homozygosis, respectively. After linearization and PGK promoter removal of the pGL4.53 vector using Kpn I and Hind III restriction enzymes, fragments were cloned upstream of the Firefly Luciferase (luc2) coding region using the NEBuilder HiFi DNA Assembly Cloning Kit. Site-directed mutagenesis was also performed using partially overlapping primers with 3′-overhangs and Platinum SuperFi II DNA Polymerase to produce different constructs, including one containing *LDLR* rs879254375-G, which is a known FH-causing variant. All constructs were confirmed by Sanger sequencing. pGL4.53 constructs containing either wild type or variant alleles and pNL1.1.TK (control of transfection variation, expressing NanoLuc Luciferase) were co-transfected into HepG2 cells. Wild type constructs were considered positive controls, while promoterless pGL4.53 was considered negative control. Luciferase assays were carried out 48 h after transfection using the Nano-Glo Dual-Luciferase Reporter Assay System. After normalization of luminescence units of each assay (Firefly/Nanoluc), luciferase activity was determined as the mean of four transfections with the assay performed in triplicate.

Results: The construct carrying *LDLR* rs36218923-T variant caused a significant mean reduction of luciferase activity (75,4% ± 2,9% SEM) over the promoterless construct relative to *LDLR* wild type construct. However, the construct harboring the *LDLR* rs879254375-G variant caused a more substantial reduction of luciferase activity relative to the wild type construct (91,2% ± 0,9% SEM). This reduction was consistent with shown by the previous study that reported *LDLR* rs879254375-G as a functional variant, which corroborates the methods adopted in this study. APOB rs934197-T was previously reported as functional based on a CAT assay using constructs of two tandemly arranged 30 bp fragments of *APOB* promoter, but in our study, the construct carrying the entire *APOB* promoter sequence and harboring rs934197-T variant did not cause a significant difference in mean luciferase activity compared to *APOB* wild type construct ($p = 0.564$). It is important to mention that our *APOB* upstream constructs had the additional variants rs617314-G, rs1560357-T, rs1625764-A, and rs1800481-G, which are considered benign because they occur at high frequency in the general population (0.82-1).

Conclusion: Our results suggest that *LDLR* rs36218923-T is likely to contribute to FH phenotype and emphasize the importance of performing in vitro expression assays in determining relevant upstream variants for FH molecular characterization.

B-253

Performance Characteristics and Validation of Next-Generation Sequencing for Human Leucocyte Antigen Typing

C. Yang, M. Wang, S. Chang, C. Huang, P. Chang, T. Chueh. *Department of Laboratory Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan*

Background:

High-resolution human leukocyte antigen (HLA) typing is a crucial process in transplant matching. Sequencing-based typing (SBT) has been the gold standard method used in HLA-based clinical applications. Recently, next-generation sequencing (NGS) has been adopted in clinical laboratories for HLA typing. NGS HLA typing assays cover whole or near-whole target HLA genes, allowing the detection of genetic variants outside of key exons. Multiplex amplicon-based NGS is an efficient high-resolution HLA typing method with minimal ambiguity and a high throughput. Here, we report the performance and validation of NGS for HLA typing and compare the results obtained using NGS with those of SBT to identify any discrepancies between the 2 methods.

Methods:

A total of 237 samples from 2019 to July 2022 were collected from Chang Gung Memorial Hospital, including 42 samples previously subjected to SBT sequencing for 4-digit HLA analysis. NGS was performed for 5 whole genes (HLA-A/B/C/DQA1/DPA1) and 3 near-whole genes (HLA-DRB1/DQB1/DPB1; excluding exon 1 and part of intron 1) by using the AllType NGS kit (One Lambda), Illumina MiSeq platform, and Type Steam Visual NGS analysis software (One Lambda).

Results:

Our validation process comprised 34 NGS runs and 237 samples. In total, 42 samples were analyzed with an overall accuracy and precision of 100% and 100% reproducibility. The overall ambiguity rate was 0% in HLA-A/B/C compared with 56.9% for SBT. Two discrepant alleles of HLA-A and DRB1 were noted in NGS and Sanger sequencing. In accordance with additional SBT exon analyses, one of the results was corrected according to the NGS results.

Conclusion:

NGS HLA typing is a high-throughput approach with high accuracy and precision and a low ambiguity and is promising for use in clinical HLA laboratories.

B-255

Engineered Reverse Transcriptases and Taq DNA Polymerases deliver robust yield in the presence of clinically relevant inhibitors

J. Walker[1], P. Venter[2], B. Faurholm[2], R. Wadsworth[2], J. Pavlica[1], C. Marshall[1], C. Ross[1]. *[1]Watchmaker Genomics, Boulder, CO, [2]Watchmaker Genomics, Cape Town, South Africa*

Objective & Background: All biological information is read, written and edited by enzymes. If you improve the enzymes, you improve data quality for precision diagnostics. Reverse transcriptase (RT) and Taq DNA Polymerase (Taq DNAP) are commonly used to detect RNA and/or DNA viruses in patient samples for point of care (POC) diagnostics. However, crude patient samples bring over inhibitors that inhibit these enzymes leading to a decrease in enzymatic activity and an increase in false-negative results in POC testing. In addition, there is a need for faster turn-around time for patient diagnosis which is enabled by engineering more active and sensitive Taq variants that work in fast PCR set-ups. At Watchmaker Genomics, our vision is to overcome hurdles in clinical medicine by engineering enzymes that confer desired phenotypes (i.e., greater activity, thermostability, and inhibitor tolerance) that will enable more sensitive and rapid detection of diseases in clinical medicine.

Methods: We take a 3-pronged approach to protein engineering: rational design, computational design and directed evolution. The aforementioned engineering approaches were used to rapidly identify and characterize > 20 RT and Taq DNAP variants. First, we deeply characterized each RT variant by measuring processivity via cDNA length and by measuring inhibitor tolerance and thermostability via RT-qPCR. In RT-qPCR, first strand synthesis (FSS) was done at temperatures from 42-65°C in the presence and absence of various clinically relevant inhibitors. For measuring inhibitor tolerance the ΔCt was calculated to determine the level of inhibition (ΔCt = Ct (with inhibitor) - Ct (without inhibitor)). Next, we characterized the inhibitor tolerance of the Taq variants by performing qPCR reactions in the presence of clinically relevant inhibitors. To determine if the Taq variant would be an appropriate fit for fast PCR, the polymerization activity of the Taq DNAP variants were characterized by annealing a single primer to ssDNA and allowing isothermal extension of the primer while monitoring the SYBR green fluorescence increase.

Results & Conclusions: Reverse transcriptases termed; StellarScript, StellarScript HT and StellarScript HT+ exhibit high processivity at 42°C. StellarScript HT+ exhibited the highest inhibitor tolerance, processivity and thermostability at temperatures up to 65°C. Based on these key attributes, StellarScript HT+ is well suited to give robust yields with clinical samples. Our Taq DNAP variants exhibited a 2.26 fold increase in polymerase activity over native Taq DNAP. Taq variants were identified that efficiently and robustly (i.e., high yield) amplify DNA in the presence of inhibitors when compared to native Taq DNAP. Based on the increased polymerase activity and inhibitor tolerance, WMG Taq variants are well suited to give robust DNA yields with clinical samples. We were successful in engineering RT and Taq DNAP variants that are highly thermostable, active and inhibitor tolerant and thus will enable breakthroughs in existing POC diagnostics.

B-256

Genotype distribution of the hepatitis C virus: 10 years data of a clinical laboratory in Brazil

J. M. Nurmberger, G. C. de Carvalho, B. B. Perez, J. G. Borges, D. R. Ramadan, S. Tufik. *Associação Fundo de Incentivo a Pesquisa (Afip - Medicina Diagnóstica), São Paulo, Brazil*

Background: Hepatitis C virus (HCV) infection is a significant health problem worldwide and is a major cause of severe liver disease including chronic hepatitis, cirrhosis, and hepatocellular carcinoma. HCV is divided into six genotypes with several subgenotypes depending on the geographic region, some genotypes are endemic. The purpose of this study was to describe the frequency of the HCV genotype (G) profile by gender and age using a database of a large Brazilian clinical laboratory.

Methods: This was a retrospective analysis from January 2012 to December 2022 based on molecular data of 3.566 isolated from clinical samples. The automated extraction and purification of nucleic acids were conducted from EDTA plasma samples of individuals suspected of HCV infection. Then, nucleic acid amplification was performed using oligonucleotide probes labeled with fluorescent dyes specific for the qualitative identification of HCV genotypes 1 to 6 and subtypes A and B of genotype 1.

Results: A total of 3.566 HCV genotype cases were analyzed during the period. The genotype distribution was (2.653/74.4%) G1 (48.1% G1A and 46.4% G1B), (733/ 20.5%) G3, (133/3.7%) G2, (35/0.9%) G4, (11/0.3%) G5 and (1/0.02%) G6. Twelve patients had multiple genotypes over the last ten years. At least one individual changed the genotype over a 3-year evaluation period, and 11 (0.30%) changed it twice. The genotypes G1 and G3 were predominant in all years analyzed. The prevalence of G1 and G3 was 75% and 19.6% among females and 74% and 21.2% among male patients, respectively. The analysis was conducted on individuals aged 6 to 99 years, with an overall average age of 52.6±13 years. The average age among men was 51.1±12 years and among women, it was 54.7±13 years. According to the age groups evaluated, genotype 1 was the most frequent, ranging from 69.3% (21 to 30 years old) to 75.8% (over 60 years old). The main subtype in the over 60 years old group was 1B. The highest frequency of genotype 2 was observed in the group over 60 years old (6.1%). The patient's symptoms, clinical history, or further diagnostic procedures were not accessible.

Conclusion: The study found that genotypes 1 and 3 were the most prevalent in Brazil over the ten-year period, with genotype 1 being the most frequent among all age groups. Genotype 1B was most prevalent in individuals above 60 years old. The change of genotype per individual was less than 1%, but further attention is required for potential virus tropism.

B-257

Rapid implementation of Monkeypox detection in a Brazilian diagnostic laboratory

M. S. Andrade[1], L. Silva[1], F. A. Hurtado[1], A. Belmok[1], A. Ferreira[1], C. Sousa[1], A. F. Vieira[1], R. Correa[1], G. Gil[1], T. Sena[1], M. Soares[1], M. Santos[1], R. Jácomo[1], L. Velasco[1], L. Nery[1], R. Andrade[2]. *[1]Sabin Diagnóstico e Saúde, Brasília, Brazil, [2]Universidade Católica de Brasília, Brasília, Brazil*

Background: Monkeypox is a double-stranded DNA virus (approximately 197 kbp) belonging to the genus Orthopoxivirus. It is responsible for sporadic outbreaks in countries in Africa. Recently, in May 2022 a patient with a recent travel history to Nigeria tested positive for monkeypox in the UK. After that, people with symptoms (usually epithelial pustular lesions) were diagnosed with the virus in several countries, such as Portugal and the United States. In Brazil, the first case was confirmed in June 2022, the patient had traveled to Spain and Portugal. It was in this context that the Technical Operational Nucleus of our laboratory, Sabin Diagnosis and Health, located in Brasilia, capital of Brazil, validated and implemented a test to detect the genetic material of Monkeypox. Thus, in this work we present the implementation of this test. **Method:** For the detection of Monkeypox viral DNA we chose to implement a real-time PCR (qPCR) methodology. The validated samples were swabs and wound scabs. The nucleic acid extraction chosen was using the Virus Pathogen kit from (QIAGEN), 'midi' protocol in the qiasymphony equipment (QIAGEN). Later we validated the extraction with the Maxwell RSC Viral Total Nucleic Acid Purification Kit extraction kit (Promega). For qPCR, we selected monkeypox-specific primers and probes, which were not able to recognize other Orthopoxiviruses (such as human pox) to ensure specificity. We used RNASEP-specific primers as an endogenous extraction control. As a positive control (PC) we used a synthetic double-stranded DNA fragment with the target sequence flanked by 10 bp. The limit of detection of the test was performed using serial dilutions of the positive control. Furthermore, given the initial absence of a positive sample for validation, we designed four pairs of primers that amplified four different regions of the viral genome for sequencing by the Sanger technique. The first positive samples were confirmed by sequencing these four regions. **Results:** The protocol was designed in the first week after the confirmation of the first case in Brazil. The first tests with the PC showed the effectiveness of the primers. After adjusting the reaction conditions to assess the specificity of the test, we tested the protocol with samples known to be positive for other viral pathogens and obtained negative results for all of them, confirming the high specificity. After that we experimentally determined the limit of detection (25 copies per reaction). On July 8, 2022, one month after the first confirmed case in Brazil in the state of São Paulo, we released the Monkeypox detection test with the methodology described above. Since then, we have performed 193 tests, of which 59 (31%) were positive, most of them (56 - 95%) in men. The first 10 positive results were confirmed by genetic sequencing (Sanger). **Discussion:** The implementation of the Monkeypox detection test by our laboratory allowed a quick response for Brazilian patients who needed the test after the virus entered the country.

B-259

Exocas-2 rapid and pure isolation of exosomes by anionic exchange using magnetic beads

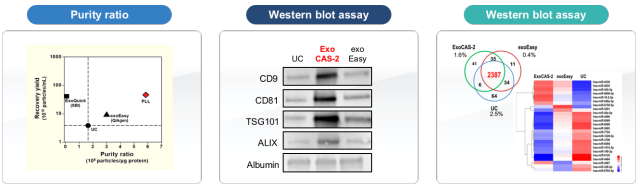
M. Bae, S. SHIN. *Korea University, Seoul, Korea, Republic of*

Background: Extracellular vesicles (EVs) are considered essential biomarkers in liquid biopsies. Despite intensive efforts aimed at employing EVs in a clinical setting, workable approaches are currently limited owing to the fact that EV-isolation technologies are still in a nascent stage. This study introduces a magnetic bead-based ion exchange platform for isolating EVs called ExoCAS-2 (exosome clustering and scattering). Owing to their negative charge, exosomes can easily adhere to magnetic beads coated with a polycationic polymer. Owing to the features of magnetic beads, exosomes can be easily processed via washing and elution steps and isolated with high purity and yield within 40 min.

Methods: Cationic salt is coated on the surface of magnetic beads with carboxyl group surface residues. Inject the cation coated magnetic bead (ExoCAS) into the plasma solution and incubate in 4℃ for 30 minutes. By charge interaction, negative charged EVs are attached to the cationic beads. And then, pH 6 washing buffer and 1M NaCl elution buffer were used for EV isolation.

Results: The present results confirmed the isolation of exosomes through analyses of size distribution, morphology, surface and internal protein markers, and exosomal RNA. Compared with the commercially available methods, the proposed method showed superior performance in terms of key aspects, including operation time, purity, and recovery rate.

Conclusion: This highlights the potential of this magnetic bead-based ion exchange platform for isolating exosomes present in blood plasma.



B-260

Innovative one-step extraction of exosomal micro RNA without isolating exosomes

M. Bae, S. SHIN. *Korea University, Seoul, Korea, Republic of*

Background: Extracellular vesicles (EVs) are receiving great attention as biomarkers in liquid biopsies since they are involved in physiological and pathological processes. It is important to obtain a high-yield of nucleic acids because it is possible to prediagnose through the exosome.

Methods: Here, we report an efficient one-step method for extraction of miRNAs by clustering EVs. When cationic salt is added to samples, anionic EVs tend to form clusters and precipitate within a short time. After centrifugation and adding lysis buffer, miRNA can be extracted with a spin column. Commercial methods were compared to determine the yield of the precipitation method using cationic salt. In addition, we compared with two reference methods to determine the miRNA extraction efficiency. The first one is consisted of two-step method of extracting miRNA from isolated exosome (2 step), which is a general method, where as the second reference method is a commercial product consisting of 1-step extracting miRNA from biofluids.

Results: Cluster precipitation efficiency using cationic salt was identified through cluster image, SEM, and Western blot. The present method for extract exosomal miRNA has an EV isolation efficiency of more than 3 times compared to the commercial EV isolation methods. In addition, it has excellent exosomal miRNA extraction efficiency from various biofluids such as Saliva, Urine, and Plasma, and can be used as a sample of breast cancer patients.

Conclusion: These results might be contributed by the sequence of precipitation of exosomes, exosome lysis, and nuleic acid extraction. The rapid and efficient method for extracting exosomal nucleic acids will make an innovative contribution to clinical applications.

B-261

Identification of HLA-DQ2.5 haplotype using a Real-Time PCR method

A. H. de Carvalho, D. A. Zauli, J. d. Silva. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Celiac disease is an autoimmune pathology triggered by the ingestion of gluten in genetically susceptible patients. The disease presents a clinical heterogeneity that makes the combined use of laboratory and histopathological approaches necessary. The presence of the DQ2.5 haplotype (DQA1*0501/DQB1*0201) is associated with celiac disease. For example, in Caucasian patients, approximately 90% of patients with celiac disease carry the DQ2.5 haplotype. However, the DQ2.5 haplotype is present in only 15 to 30% of the general population. Due to this, the presence of the haplotype cannot be considered decisive for the diagnosis of celiac disease. On the other hand, the identification of the haplotype is useful in excluding the possibility of the disease, since the absence of the marker makes the hypothesis that a patient has celiac disease very unlikely. One of the main approaches for identifying the DQ2 haplotype is the use of PCR with specific primer sequences. Although this technique has great sensitivity and specificity, the process is time-consuming and costly. Thus, in order to improve the cost-effectiveness of the diagnosis we investigated if the use of SNP tag approach is able to correctly identify HLA-DQ2.5 genotype. **Methods**: We selected 235 samples from patients genotyped for HLA-DQ2.5 by PCR followed by electrophoresis detection. 94 samples were positive for the HLA-DQ2.5 haplotype. The 235 samples were genotyped using TaqMan probe assay (ThermoFisher Scientific) for the SNP rs2187668 according with the manufacter instructions. The SNP rs2187668 has a C/T substitution. The homozygous C genotype is associated with the absence of the HLA-DQ2.5 haplotype, while the presence of at least one T allele is associated with the presence of the HLA-DQ2.5 haplotype. **Results**: Of the 94 positive samples for the HLA-DQ2.5 haplotype, Real-Time PCR genotyping was able to correctly identify 74 (70 C/T samples and 4 T/T samples). The other 20 positive samples were identified with the C/C genotype. Among the negative samples, Real-Time PCR genotyping correctly identified the genotype of 139 of the 141 samples. These results indicate a sensitivity of 82.4% and a specificity of 98.6%. On the other hand, the positive predictive value is 97.4% and the negative predictive value is 89.7%. **Conclusion**: The results observed suggest that the use of the SNP rs2187668 is effective for detecting the presence of the HLA-DQ2.5 haplotype, given the low rate of false-positive individuals and the high positive predictive value. On the other hand, the exclusion of the presence of the haplotype did not obtain the same effectiveness, since a high frequency of false-negative results was observed. One of the possibilities for this high frequency of false-negatives may be due to the high number of polymorphisms in the SNP region or the low frequency of the T allele in the population. Thus, other approaches are necessary to establish the diagnosis of the HLA-DQ2.5 genotype by Real-Time PCR, such as, for example, the adoption of other SNPs in the region.

B-262

Evaluation of the genotypic profile of the c.202 G>A mutation in the G6PD gene in Brazil

J. d. Silva, J. S. Rios, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Introduction Glucose 6 phosphate dehydrogenase (G6PD) is an enzyme found in the cytoplasm of all human cells and plays an important role in preventing cellular damage to reactive oxygen species (ROS). G6PD deficiency is inherited in an X-linked recessive genetic disorder that can destroy red blood cells (hemolysis) leading to acute hemolytic anemia during periods of increased ROS production. The disease prevalence is more frequent in male individuals. To date, more than 400 G6PD variants have been identified, and molecular studies have shown that G6PD deficiency can be associated with different mutations. The c.202 G>A mutation (rs1050828; NM_000402.4) was described as the most frequent. The rapid and accurate diagnosis of this condition is extremely important in neonatal screening and cases of diagnostic suspicion. Therefore, this study aims to describe the genotypic profile of the c.202 G>A mutation in the G6PD gene in Brazilian samples by two molecular assays. **Methods:** Genomic DNA samples from 130 individuals previously genotyped by Restriction Fragment Length Polymorphism PCR were used for validation. DNA isolation was performed in MagNA Pure 24 Total NA Isolation Kit at the automated platform MagNA Pure 24 System (Roche Diagnostics, Basel, Switzerland), according to manufacturer instructions. Genotyping for rs1050828 was performed using TaqMan SNP Genotyping Assays (Thermo Fisher, Foster City, CA) at 7500 Fast Real-Time PCR System (Thermo Fisher, Foster City, CA), according to manufacturer instructions. The results were analyzed using TaqMan Genotyper Software version 1.0.1. **Results:** This study evalu-

ated 130 blood samples, of which 81 were men (62.3%) and 49 were women (37.7%). Male carriers of rs1050828 A genotype (hemizygotes) predominated (56/81, 69.1%) and the remaining carriers for allele G (wild type) were 25/81 (30.9%). While female carriers of rs1050828 GG (wild type) predominated (39/49, 79.6%), heterozygotes were 5/49 (10.2%), and homozygotes also were 5/49 (10.2%). The number of male individuals with the presence of the mutation in hemi/homozygotes was considerably higher when compared to female individuals. This is explained by the fact that the mutation is inherited in an X-linked recessive manner. The results showed agreement and reproducibility of 100% for both methodologies. However, the qPCR assay sensibility and efficiency (>99%) were higher than the RFLP PCR assay. **Conclusions:** Here, we described an efficient molecular method to detect the mutation c.202 G>A in the G6PD gene. The frequency of c.202 G>A described in this study was similar to that observed in others population studies in Brazil. Detection of the mutation target requests an additional evaluation of the G6PD activity level. Obtained results should be evaluated in association with the clinical aspects. The absence of the c.202 G>A in the G6PD gene does not exclude the existence of other mutations in the same region.

B-263

Molecular Assay Evaluation to SMA and SCID Diagnosis in Newborn Dried Blood Spots (DBS)

J. d. Silva, C. M. da Silva, D. A. Zauli, A. P. Serra. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by insufficient levels of the survival motor neuron (SMN) protein. Almost 95% of SMA cases are caused by the homozygous deletion involving exon 7 of the SMN1 gene. Its incidence is estimated in 1:6000-11,800 live births and it is the most common genetic cause of death in infancy. Symptoms of SMA include several cognitive disabilities leading to death, due to loss of motor functions, rapid denervation, and progressive weakening. In addition, another very common syndrome among newborns is Severe Combined Immunodeficiency (SCID). SCID is a genetic disease that may compromise the Lymphocytes T, B, and/or Natural Killers cells functions causing repeated and persistent infections. Low levels of the recombination excision circles of T and B cells (TREC and KREC, respectively) are indicative of SCID or other lymphopenias. Due to the early onset and rapid progression of SMA and SCID, accurate diagnosis of these syndromes is of utmost importance to perform therapeutic intervention before the onset of symptoms. Therefore, this study aims to describe the analytical and clinical validation of a kit for the implementation of a multiplex SCID/SMA real-time diagnoses routine in dried blood spots (DBS). **Methods:** Commercial quantified positive control and samples were used for the analytical and clinical validation, respectively. The Clinical validation included 67 samples with the SMN1 gene and 7 samples with gene deletion. For the SCID evaluation, 60 samples with normal levels of TREC and KREC and 11 samples with reduced levels were included. DNA isolation was performed with the DNA Extract All Reagents Kit (Thermo Fisher, Massachusetts, EUA) from a 3.2mm disc of newborn DBS. The qPCR assay was performed by the TaqMan SCID/SMA Plus Assay (Thermo Fisher, Massachusetts, EUA) to target TREC, KREC, and SMN1 according to the manufacturer's instructions. The analysis parameters included: (i) Determination of assay efficiency; (ii) Analytical sensitivity (Limit of detection); (iii) Intra-assay and inter-assay precision; and (iv) Clinical validation. All reactions included the endogenous control RNase P. **Results:** The assay demonstrated reaction efficiency >98% and the limit of detection was 30 copies per reaction for TREC, KREC, and SMN1 targets with a 95% confidence interval. The regression equations demonstrated good amplification conditions with a coefficient of determination (r2) = 0.99. The precision experiments demonstrated optimal repeatability and reproducibility. The results obtained in the clinical validation showed 100% agreement with the expected results for the SMN1 target. To the TREC and KREC targets, the assay demonstrated 95% and 100% agreement for normal and reduced levels, respectively. **Conclusion:** An accurate and rapid diagnosis is essential to guide the clinical management of SCID and SMA in newborns. Here, we evaluated and described the TaqMan SCID/SMA Plus Assay performance, a molecular test for detecting SCID and SMA by Quantitative Real-time PCR (qPCR). This assay was highly efficient due offer of a multiplex detection method, allowing the rapid release of results. The use of this tool can help clinicians in rapid newborn screening, providing agility in clinical conduct.

B-264

Identification of potential biomarkers related to Major Depressive Disorder using a meta-analysis approach

A. H. de Carvalho[1], A. B. de Lima[1], H. D. Dias[2], D. A. Zauli[1]. *[1]Instituto Hermes Pardini, Vespasiano, Brazil, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Background: Major depressive disorder (MDD), also known as depression, has become the most prevalent mood disorder today. Currently, the diagnosis remains a major challenge given the disease heterogeneity and have been made by associating signs and symptoms with other diagnostic criteria. Several treatment options are available for MDD. However, less than 35% of patients achieve remission while 60% may show some degree of resistance to treatment even when receiving antidepressant treatment according to consensus guidelines and using measurement-based care. Between difficulties associated with the management of MDD, the lack of consensus on classification, diagnosis, and treatment stands out. Because of the incomplete understanding of MDD pathogenesis, it is urgent to develop studies that can help in the elucidation of the mechanisms related to the disease and the identification of biomarkers that allow its correct classification. Thus, this work aimed to perform a meta-analysis study to identify potential molecular biomarkers from different NGS databases. **Methods:** Datasets from seven association studies stored in the IEU Open GWAS Project Database were selected. The meta-analysis was performed using the METAL tool (https://genome.sph.umich.edu/wiki/METAL_Documentation), which combines the relevance of the *p-values*, sample size, and the direction of the effect size. The criteria adopted for selecting the datasets were: 1) Specification of "depression", "major depressive disorder" or "major depression" as the only phenotype; and 2) description of allele frequency data. The threshold for results significance was defined as *p-value* <= 5 x 10^{-8}. After performing the analyses, only the SNPs whose direction of effect was the same in all original studies were evaluated, considering only the set of SNPs that were not significant in the original studies. **Results:** After performing the meta-analysis, 348 new SNPs with p-value <= 5 x 10^{-8} were identified and, of those, 133 SNPs were mapped next to 17 genes. None of these genes was previously associated with depression. However, the *GABBR1* gene, which encodes a type B GABA receptor subunit, has been associated with alcoholism, while the *SORCS3* gene has been associated with attention deficit/hyperactivity disorder and Alzheimer's disease. **Conclusions:** The meta-analysis findings could help elucidate genes and polymorphisms involved in MDD pathogenesis. Due to MDD heterogeneity is nearly impossible to restrict and select a single disease biomarker. It is worth noting that only the identification of genetic polymorphisms involved in MDD development cannot be the only source of potential biomarkers for MDD. Indeed, it is necessary to extend the analysis to other components, such as regulatory regions, epigenetics, metabolites, etc. However, this work provide new insights into the identification of MDD potential biomarkers that can be better investigated in association to others factors related to the disease.

B-265

Prevalence of 844ins68 polymorphism in the cystathionine β-synthase gene in Brazilian individuals

J. S. Rios, J. d. Silva, F. S. Malta, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Deficiency of Cystathionine β-Synthase (CBS) is the most common cause of the homocysteine increase in blood and urine. This is an important autosomal recessive disorder with pleiotropic clinical manifestations in the vascular system, the skeletal structure, the ocular lens, and the central nervous system. Several polymorphisms in CBS gene have been associated with homocystinuria, with consequent premature arterial and/or venous occlusive diseases. The thymine-to-cytosine substitution at nucleotide 833 of the CBS gene (T833C) is the majority predominant mutation in individuals with homocystinuria. Several studies of T833C have been performed on patients where venous or arterial occlusive diseases are an important risk factor. The T833C causes a substitution of hydrophobic isoleucine by a more hydrophilic threonine at codon 278 (I278T) and affects the CBS conformation or the interaction of the CBS subunits. Most occurrences of T833C are known to segregate in cis with 844ins68 insertion polymorphism in the exon 8, duplicating the intron 7 splice acceptor. At the mRNA level, polymorphisms T833C and 844ins68 are skipped next to the use of this alternative splicing site. Therefore, previous studies about 844ins68 produced conflicting results that show a neutral or even effect protective of the insertion against occlusive venous and arterial occlusive disease. Thus, this study aims to describe the genotypic profile of the 844ins68 mutation in CBS gene in Brazilian individuals affect with vascular diseases or patients at risk for genetic variants segregating in the family. **Methods**: The study group consisted of 1,301 individuals

submitted to the identification of the CBS 844ins68 in Grupo Pardini Laboratory. Genomic DNA was isolated from EDTA blood using QIAcard FTA Classic (Qiagen). To detect the insertion, the genomic fragments comprising the intron 7 and exon 8 in the coding region and splicing junctions of the CBS gene were amplified by Polymerase Chain Reaction (PCR). **Results**: Our sampling included 214 men's blood samples (16.4%) and 1,087 women's blood samples (83.5%). We found a prevalence of 21.6% individuals with the insertion. Males without the 844ins68 mutation (wild type) predominated 161/214, (75.2%), carriers of 844ins68 heterozygotes were 45/214 (21%), and homozygotes were 8/214 (3.7%). In female individuals, was found 859/1,087 (79%) allele without 844ins68 (wild type), 214/1,087 (19.7%) carriers of 844ins68 heterozygotes and 14/1,087 (1.3%) homozygotes. The number of solicitations for the exam in women is greater compared to men. **Conclusions**: Our findings show that the prevalence of 844ins68 in Brazil is compatible with others studies involving Brazilian women with cardiovascular defects. According to the literature, the insertion is most frequent in females than in males. Considering the possible restoration of non-mutated RNA through preferential splicing of the 844ins68, our study suggest that mutated individuals have the insertion as a protective factor for the development of venous or arterial occlusive diseases. The search of 844ins68 should be evaluated in association with the clinical aspects.

B-266

Analytical performance comparison of two genotyping assays for HIV-1 drug resistance using Sanger sequencing

F. L. Marinho, J. d. Silva, L. B. Alvim, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: The first case of Acquired Immunodeficiency Syndrome (AIDS) was registered in the United States in early 1980. Two years later, was identified the Human Immunodeficiency Virus (HIV-1), which causes AIDS. Compared to other diseases, the treatment of AIDS achieved an important advance in just over 30 years, in which several drugs were used and replaced. However, resistance to antiretrovirals (ARVs) remains a problem in clinical practice. There are about 20 approved HIV-1 treatment drugs belonging to four different classes: Protease Inhibitors (PI), Nucleoside Reverse Transcriptase Inhibitors (NRTI), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI), and Integrase Strand Transfer Inhibitor (INSTI). ARVs resistance is caused by mutations in molecular targets of these drugs: protease, reverse transcriptase, and integrase enzymes. Virus genotyping that includes these regions is extremely important to identify the resistance mutations to ARVs therapies, being able to direct treatments. Thus, this study aimed to evaluate the analytical performance of the Applied Biosystems HIV-1 Genotyping Kit with Integrase compared to in-house Sanger genotyping method. **Methods:** Viral RNA isolation from 20 individuals was performed by QIAamp Viral RNA Kit (Qiagen), according to the manufacturer's instructions. HIV-1 genotyping was performed using both methods: in-house Sanger genotyping and Applied Biosystems HIV-1 Genotyping Kit with Integrase (Thermo Fisher), according to the manufacturer's instructions. All results were submitted to SeqScape software to evaluate sequencing metrics. The consensus sequence was generated for each sample and analyzed at the online Stanford HIVdb Program (version 9.4) for drug resistance evaluation. **Results:** The 54 mutations analyzed were distributed among the targets: PI (27.8%; 15/54), NRTI (24.1%; 13/54), NNRTI (22.2%; 12/54), and INSTI (25.9%; 14/54). The in-house method evaluated the susceptibility to PI drugs (ATV, DRV, FPV, IDV, LPV, NFV, SQV, and TPV), NRTI (ABC, AZT, D4T, DDI, FTC, 3TC, and TDF), and NNRTI (DOR, EFV, ETR, NVP, and RPV). In addition to protease and reverse transcriptase targets, the commercial kit also evaluated INSTI (BIC, CAB, DTG, EVG, and RAL) drugs. Four of 20 individuals evaluated had viral loads below 1000 copies/mL and have no results in both methods, as expected due to the detection limit of both assays. Furthermore, due to sequencing limitations using the in-house methodology, three samples were not included in the analysis as it didn't show any results. It was observed 92.3% agreement (n=12/13) in the analyzed samples. A sensitivity of 100% and precision of 99% were found in the commercial kit using the in-house kit as a reference. The evaluation of the intra-assay (repeatability) and inter-assay (reproducibility) indicated 100% of agreement. **Conclusions:** The Applied Biosystems HIV-1 Genotyping Kit with Integrase showed satisfactory performance in the detection of the main antiretroviral resistance mutations.

B-267

Validation of a New Molecular Test to Quantify Plasma Cytomegalovirus Load

B. Dehesa-García, A. García-Murillo, I. Cortés-Portero, V. Pérez-Grijalba, B. García-Manrique. *CerTest Biotec, San Mateo de Gállego, Spain*

Background:

Cytomegalovirus (CMV) is a DNA virus that often causes severe disease in immunosuppressed or immunocompromised patients. With the expanding indications for immune-modulating agents and transplant patients, the number of patients at risk for developing CMV disease is increasing. Test results are used to determine active CMV disease, risk of developing active disease, response to therapy, risk of relapsed infection, and/or appropriate time to discontinue therapy. For this purpose, molecular assays are attractive because of their broad linear range and low limits of detection. CerTest Biotec has developed a quantitative real time PCR assay for the detection and quantification of cytomegalovirus in plasma samples.

Methods:

Plasma-EDTA samples characterized as positive or negative and quantified by IU/ml were selected. The selected samples were collected from both male and female adults (except 1 sample of an infant) between July 2016 and December 2020. Two CE-marked molecular reference assays were used for the comparative analysis: RealStar® CMV PCR Kit 1.0 (Altona), using the CFX96™ Real-Time PCR Detection System (Bio-Rad) and extracted with the QIAamp® MinElute® Virus Spin Kit (QIAGEN); and cobas® CMV (Roche Diagnostics), using the cobas® 8800 system. VIASURE analysis was performed using the following workflow: the automated extraction method MagDEA® Dx SV with magLEAD® 12gC and CFX96™ Real-Time PCR Detection System (Bio-Rad).

Results:

A total of 273 EDTA-plasma clinical samples were analysed, 120 samples were reported as CMV positive samples and quantified and 153 samples were reported as CMV negative samples. Comparative analysis determined >99% sensitivity and specificity. Regarding quantification, all methods obtained a correlation value of $R^2 \geq 0.77$.

Conclusion:

With this study it was possible to observe that the VIASURE *CMV q* Real Time PCR Detection Kit present good clinical sensitivity and specificity compared to reference molecular assays results. No false positive results were observed. However, all variables employed on the workflow contribute to final quantification results and therefore should be taken into account as accumulative error in the calculation.

B-268

Validation of a New Real-Time PCR Test for the Detection of Mpox Virus on the BD MAX™ System

G. Marín-Royo, C. Palacios-Pérez, A. Aznar-García, L. Llobet-Sesé, B. García-Manrique. *CerTest Biotec, San Mateo de Gállego, Spain*

Background:

Enabling labs to rapidly respond to unexpected healthcare challenges is the kind of situations for which the BD MAX™ open system reagent suite is designed. Therefore, after the monkeypox outbreak in non-endemic countries on May 2022, development of a molecular assay for monkeypox detection on the BD MAX™ System was initiated.

Methods:

VIASURE *Monkeypox virus* Real Time PCR Reagents for BD MAX™ System was designed for the qualitative detection of DNA from monkeypox virus in human skin lesion swabs in VTM/UTM obtained from individuals suspected of monkeypox infection.

The Limit of Detection (LoD) was determined by testing a 3-fold dilution series across 5 concentrations. The lowest concentration with 100% detection was tested with an additional 20 replicates. The preliminary LoD was confirmed with a ≥95% detection with 20 replicates. Then, a fully contrived clinical evaluation was performed. Each contrived clinical specimen was prepared using a unique natural clinical specimen matrix (lesion swabs). Twenty (20) of the clinical specimens were prepared at 1-2X LoD, ten (10) were prepared at 3-5X LoD, and thirty (30) were tested as negative samples.

Results:

The lowest detectable concentration of virus at which >95% of all replicates test positive (LoD) was 288 copies/mL. The results for the contrived clinical evaluation are

shown in Table 1. 19/20 (≥95%) low positive specimens (1-2xLoD) and 20/20 (100%) moderate positive specimens (3-5x LoD) were detected demonstrating the functionality of the test.

Conclusion:

VIASURE *Monkeypox virus* Real Time PCR Reagents for BD MAX™ System provides results in a short time and requires less than one minute of hands-on time per sample due to integration of fully automated extraction and thermocycling steps.

An additional clinical testing of positive natural clinical specimens must be performed to complete evidence that this product is for the purpose and the population for which it is intended.

Table 1. Monkeypox contrived clinical evaluation results.

Concentration	Total Valid Results	MPXV Positive	Mean Ct[a]	
			MPXV	HBB
Moderate Positive ~3-5x LoD	10	100% (10/10)	31.7	25.7
Low Positive ~1-2x LoD	20	95% (19/20)	33.2	26.5
Negative	30	0% (0/30)	n/a	27.2

[a] – only positive replicates were used for mean Ct calculations. MPXV, monkeypox virus; HBB, haemoglobin subunit beta gene.

B-269

Plasmodium Detection: Sera, Blood, Do They All Work?

E. Franco-Marín[1], G. Ulloa[2], B. Dehesa-García[1], M. Santolalla[3], A. Lescano[3], B. García-Manrique[1], P. Mayor[4], A. Mayor[5], H. Alonso[6]. *[1]CerTest Biotec, San Mateo de Gállego, Spain, [2]Universidade Federal Rural da Amazônia, Belém, Brazil, [3]Emerge, Emerging Diseases, and Climate Change Research Unit, School of Public Health and Administration, Universidad Peruana Cayetano Heredia, Lima, Peru, [4]Department of Health and Animal Anatomy, Autonomous University of Barcelona, Barcelona, Spain, [5]Instituto Salud Global (ISGlobal), Barcelona, Spain, [6]Department of Microbiology, Paediatrics, Radiology, and Public Health, Faculty of Medicine, University of Zaragoza, Zaragoza, Spain*

Background:

When diagnosing malaria, testing employs blood samples using either microscopy, the current gold standard or molecular diagnostic tests (more sensitive but significantly more expensive). In Plasmodium spp. screening programs in at-risk populations where there are not many resources available, dried blood on filter paper is used to ease transport, storage and preservation of the sample. Although *Plasmodium* detection studies using blood serum as a sample are available, it isn´t always as sensitive as using whole blood-based sampling, as the concentration of DNA in plasma is proportional to the level of parasitaemia. This study aims to confirm the differences in sensitivity using both samples, demonstrating the importance of choosing the right type of sample to use for diagnosis.

Methods:

The study was carried out using the VIASURE *Malaria* Real Time PCR Detection Kit with sera and blood samples from 94 inhabitants from Nueva Esperanza community in the Peruvian Amazon collected between 2007 and 2020, establishing a comparative analysis between matrixes. Nucleic acid extraction was performed using the QIAamp DNA Mini Kit from QIAGEN and the VIASURE assay was performed using QuantStudio®6 Flex Real-Time PCR System thermocycler (Thermo Fisher Scientific) and the BioRad CFX96™ Real-Time PCR Detection System for analysing peripheral blood samples and the BioRad CFX96™ Real-Time PCR Detection System for analysing sera.

Results:

From the total of samples, only 5 sera (5.31%) showed amplification of *Plasmodium spp*., whereas from blood samples, 30 resulted to be *Plasmodium spp*-positive. Therefore, 26.59% of detection is lost when serum is used as a sample, as opposed to blood.

Conclusion:

After this analysis it could be concluded that malaria detection sensitivity is appreciably higher for blood samples than for sera samples. Thus, serum samples aren´t the best choice for *Plasmodium spp*. testing and whole blood-based matrixes are preferable for this diagnosis. These results reinforce the idea of the importance of the sample type when diagnosing different pathogens, as well as the relevance of a confirming comparative analysis when similar sample types are involved.

B-270

Validation of a New Molecular Assay for the Detection and Quantification of Hepatitis B Load in Plasma

I. Cortés-Portero, B. Dehesa-García, A. García-Murillo, B. García-Manrique. *CerTest Biotec, San Mateo de Gállego, Spain*

Background:

Hepatitis B virus (HBV) is an enveloped DNA virus and is the most common worldwide cause of viral hepatitis in humans. It is primarily transmitted through blood or blood products. Although the diagnosis of hepatitis viral infections is usually performed with serological markers in blood, this technique has limitations and therefore the diagnosis of the disease by qPCR allows an improvement in the diagnosis of the infection, therapeutic decision making and evaluation of the response to treatment. Therefore, CerTest Biotec has developed a quantitative real-time PCR assay for the detection and quantification of hepatitis B virus in plasma samples.

Methods:

Plasma-EDTA samples characterized as positive or negative and quantified in IU/ml were analyzed. The selected samples were collected from infants and adults of both sexes between September 2019 and August 2020. The origin of the population is diverse, with the majority being European. For comparative analysis, the cobas® HBV molecular assay (Roche Diagnostics) using the cobas® 8800 system was used. VIASURE analysis was performed using the following workflow: the MagDEA® Dx SV automated extraction method with magLEAD® 12gC and CFX96™ Opus Real-Time PCR Detection System (Bio-Rad).

Results:

A total of 210 clinical EDTA plasma samples were tested, of which 110 were positive for hepatitis B virus and 100 were negative. The comparative analysis determined a sensitivity and specificity >99%. In addition, the positive predictive value (PPV) and negative predictive value were >99%. As for quantification, an R^2 correlation value of 0.9095 was obtained.

Conclusion:

This clinical evaluation demonstrated that the VIASURE *HBV q* Real Time PCR Detection Kit is a good tool for the clinical diagnosis of the virus as it has good sensitivity and specificity. No false positive or false negative results were observed. Moreover, the correlation of the quantification with the reference method gives a value of R^2>0.9. Therefore, clinical diagnosis of hepatitis B virus by real-time PCR can encompass the limitations of the current diagnostic technique by serology.

B-271

Molecular EQA Programs Verify the Use of a Quick Lysis Reagent in a Wide Range of Matrices and Pathogens

M. Martínez-Santolaria[1], C. Sota-Diez[2], B. Gaston-Galvez[1], B. García-Manrique[2], E. Machetti-Mareca[2], C. Gil-Rodríguez[2]. *[1]Facultad de Ciencias, Universidad de Zaragoza, Zaragoza, Spain, [2]CerTest Biotec, San Mateo de Gállego, Spain*

Background:

External Quality Assessment (EQA) programmes, like Quality Control for Molecular Diagnostics (QCMD), allow laboratories to verify the correct functionality of the molecular workflows used in the diagnosis of different pathogens. The aim of this study was to evaluate whether a quick molecular workflow had a good functionality for the diagnosis of different pathogens.

Methods:

A total of 90 frozen samples from 2022 QCMD programmes were analysed. 20 transport medium (TM), 9 TM or saline and 1 saline samples from respiratory panels (QAV164188, QAV164189, QAM174193), 20 synthetic faecal matrix samples from gastrointestinal panels (QAP124154, QAB124153), 13 TM, 4 simulated swabs and 3 urines from sexual transmitted infections (STI) panels (QAB154177, QAM174201), and 18 TM and 2 synthetic cerebrospinal fluid from central nervous system (CNS) panels (QAV174195, QAM174196). Samples were processed using an in-house processing method called quick lysis reagent (QLR) (Certest Biotec S. L.). In parallel, samples were also extracted using the automatic robot KingFisher (Thermo Fisher Scientific). Nucleic acids were analysed at least in duplicate with qPCRs from VIASURE using the CFX96™ Real-Time PCR Detection System (Bio-Rad). Results were compared to the final diagnosis provided by QCMD and with the automatic extractor results.

Results:

The in-house processing method QLR, in combination with VIASURE qPCRs, could process and identify bacteria, parasites and viruses from respiratory, gastrointestinal, STI and CNS panels. Negative samples were correctly detected. This workflow obtained a 100% of sensitive and specificity, same as the automatic extractor.

Conclusion:

These EQA programmes tested the correct functionality of a quick molecular workflow, based on the VIASURE diagnostic products, that could detect different pathogens (bacteria, viruses, and parasites) from different origins (respiratory, gastrointestinal, STI and CNS).

B-272

Interference Assays: How Different Substances can Affect the Detection of Respiratory Viruses Using a Quick Molecular Solution

M. Martínez-Santolaria[1], B. García-Manrique[2], E. Machetti-Mareca[2], C. Gil-Rodríguez[2]. *[1]Facultad de Ciencias, Universidad de Zaragoza, Zaragoza, Spain, [2]CerTest Biotec, San Mateo de Gállego, Spain*

Background:

Some substances present in clinical samples could affect the performance of qPCR and significantly impact its results. If nucleic acids are obtained using quick lysis reagents, the inhibition effect could be even worse since no washing steps are included. Thence, the aim of this study was to evaluate some potentially interfering substances that may be present in respiratory samples and their effect on the diagnosis using a quick molecular solution.

Methods:

Ten negative nasopharyngeal swab samples in Viral Transport Medium (VTM, Vircell) were pooled and enriched with a known concentration of viral cultures from ATCC (Influenza A: VR-95PQ™ A/Puerto Rico/8/34; Human Respiratory Syncytial virus (RSV): VR26PQ™; and SARS-CoV-2: VR-1986HK™ SARS-Related Coronavirus 2, Isolate USA-WA1/2020) to obtain positive samples around the limit of detection. The pool was divided into seven aliquots. Six of them were spiked (each) with a known concentration of potentially respiratory interfering substances, according to FDA recommendations (Table 1). Another aliquot did not contain any substance and was considered as the positive control. Each aliquot was processed in triplicate using VIASURE Resp. viruses Quick Lysis Reagent (Certest Biotec S. L), following the manufacturer's instructions. Nucleic acids were analysed with VIASURE *SARS-CoV-2, Flu & RSV* Real Time PCR Detection Kit (Certest Biotec S.L) in triplicate on CFX96™ Real-Time PCR Detection System (Bio-Rad).

Results:

Almost all substances did not interfere throughout the workflow at the concentrations tested. Only benzocaine had a negative effect on the three pathogens analysed (SARS-CoV-2, Influenza A and RSV), detecting less than 8/9 replicates. This problem was resolved when the substance was tested at 0.15 mg/ml.

Conclusion:

Evaluation of potentially interfering substances in quick molecular solutions is important, especially if nucleic acids processing methods do not have many purification steps. Thus, the study confirmed that the diagnosis using this solution is not easily compromised.

Table 1. Potentially interfering substances and their concentrations in the sample analyzed

Potentially Interfering Substances (Category, Brand name)	Concentration in sample tested
Oxymetazoline hydrochloride (Nasal spray, Respibien)	0.05 mg/ml
Benzocaine (Sore throat and cough lozenges, Angileptol®)	0.30 mg/ml
Becydamine hydrochloride (anti-inflammatory sore throat, Tantum Verde)	0.30 mg/ml
Cloperastine fendizoate (Cough syrup, Flutox®)	0.35 mg/ml
Mucin (Endogenous protein)	1% (v/v)
Nicotine (Organic compound, Nicotinell Mint)	0.01 mg/ml

B-273

Lymphogranuloma Venereum: Validation of a Real-Time PCR Kit for its Rapid Diagnosis

M. Peris[1], A. Milagro[2], N. F. Martínez[2], O. Algara[2], D. Martínez-Mateos[2], B. Dehesa-García[3], B. García-Manrique[3], A. Rezusta[2]. *[1]Instituto de Investigación Sanitaria de Aragón, Zaragoza, Spain, [2]Hospital Universitario Miguel Servet, Zaragoza, Spain, [3]CerTest Biotec, San Mateo de Gállego, Spain*

Background:

Chlamydia trachomatis serovars L1, L2 and L3 are responsible for lymphogranuloma venereum (LGV), a sexually transmitted infection that causes ulcerated lesions and adenopathy. Following the procedures in Clinical Microbiology recommendations, rectal samples positive for *C. trachomatis* are processed to detect the LGV-producing serovars in order to provide appropriate treatment. Since European regulations require for CE and IVD certification that an in vitro diagnostic product must be validated by an external body, the aim of this study was to validate the VIASURE *C. trachomatis (LGV)* Real Time PCR Detection Kit from Certest Biotec SL, using DNAs characterized as positive or negative for LGV extracted from clinical samples previously analyzed in the Laboratory.

Methods:

A total of 214 DNAs from clinical samples collected between January 2021 and November 2022 were analyzed. Based on the initial diagnosis performed with the Allplex™ Genital ulcer Assay (Seegene®), 37 samples were positive for LGV and 177 were negative. The use of all data and samples was approved by the Aragon research ethics committee (CEICA) (PI20/426). The clinical sensitivity and specificity of the kit and the positive and negative predictive values (95% CI) were calculated with MetaDisc 1.4 software.

Results:

Results are summarized in table 1.

Conclusion:

The comparative analysis of the "VIASURE *C. trachomatis (LGV)* Real Time PCR Detection Kit" proved to be as sensitive and specific as the reference molecular assay. The main advantage is that VIASURE products feature a stabilized, ready-to-use format, reducing the number of time-consuming laboratory steps, avoiding possible contamination and allowing transport and storage at room temperature.

Table 1. Summary of obtained results

True positive results	True negative results	False positive results	False negative results	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
37	177	0	0	1(0.90-1)	1(0.98-1)	1(0.88-1)	1(0.97-1)

B-274

One-step detection of *candida albicans, trichomonas vaginalis* and *gardnerella vaginalis*

M. Peris[1], A. Milagro[2], B. Gilaberte[2], N. Gamadiel[2], M. López[2], B. Dehesa-García[3], B. García-Manrique[3], C. Mendoza[2], P. Aabad[2], A. Rezusta[2]. *[1]Instituto de Investigaciones Sanitarias de Aragón (IISA), Zaragoza, Spain, [2]Hospital Universitario Miguel Servet, Zaragoza, Spain, [3]CerTest Biotec, San Mateo de Gállego, Spain*

Background:

Trichomonas vaginalis and *Candida albicans* infections and bacterial vaginosis (BV) are among the three main causes of vaginitis.The most widespread method for diagnosis is still Gram staining and culture. The disadvantages are the microscopy time required, as cultures are examined after 24-48 hours. Different commercial molecular biology kits are currently available on the market to detect these pathogens in a sensitive, specific and rapid way.In vitro diagnostic products must be marketed with CE and IVD certification for marketing in the European Union and must be externally validated with clinical samples. For this reason, the company Certest Biotec proposes a collaborative study to evaluate the product VIASURE *C. albicans, G. vaginalis & T. vaginalis* Real Time PCR Detection Kit, in the Microbiology Laboratory of the Miguel Servet University Hospital (HUMS) in Zaragoza (Spain) using samples characterised by Gram and culture as positive or negative for *C. albicans, G. vaginalis* and *T. vaginalis*.

Methods:

240 DNA extracts of clinical samples from vaginal swabs of patients with suspected vaginitis were analysed. For the validation of the kit under study, the Segene® AllplexTM Vaginitis Screening Assay was used as a reference. Discordant results will be resolved by Sanger sequencing. Preliminary results are therefore expressed as true positives, true negatives, false positives and false negatives. The use of clinical samples was approved by CEIC Aragón (CEICA): PI20/426.

Results:

In the absence of resolution of the discordances by sequencing, the results were as indicated in table 1.

Conclusion:

The main advantage of using these techniques is speed, as they can provide results on the same day and reduce the time spent on microscopy and culture reading. VIASURE products are in a stabilised, ready-to-use format, which facilitates handling, avoids possible contamination, allows storage at room temperature and transport.

		Allplex™ Vaginitis Screening Assay					
		Trichomonas vaginalis		*Candida albicans*		*Gardnerella vaginalis*	
VIASURE *C. albicans, G. vaginalis & T. vaginalis* Real Time PCR Detection Kit		+	-	+	-	+	-
	+	46	0	69	10	164	20
	-	0	194	0	161	13	43

B-275

Retrospective Study for Mpox Detection: Validation of a Real Time PCR Kit for its Fast Diagnosis

M. Peris[1], A. Milagro[2], B. Fortuño[2], D. Martínez-Mateos[2], B. Gilaberte[2], B. Dehesa-García[3], B. García-Manrique[3], L. Laborda[2], M. Latorre-Millán[2], C. Aspiroz-Sancho[4]. *[1]Instituto de Investigaciones Sanitarias de Aragón (IISA), Zaragoza, Spain, [2]Hospital Universitario Miguel Servet, Zaragoza, Spain, [3]CerTest Biotec, San Mateo de Gállego, Spain, [4]Hospital Royo Villanova, Zaragoza, Spain*

Background:

In an increasingly globalised and interconnected world, a small outbreak of an infectious disease (such as monkeypox in 2022) can turn into a global health emergency. In this context, global health systems must develop effective strategies to interrupt transmission as early as possible by identifying cases and sources of infection. Since clinical diagnostic products must be externally validated to comply with current regulations, the aim of this collaborative and retrospective study was to evaluate the clinical sensitivity and specificity of the VIASURE *Monkey pox* Real time PCR detection kit (CerTest Biotec) for the detection of monkeypox by Real time PCR.

Methods:

This is a retrospective and collaborative study conducted at the Microbiology Department of the Miguel Servet University Hospital at the request of the company Certest Biotec SL with the approvement of the Aragon research ethics committee (PI22/412; 5 Oct 2022). A total of 192 suspected MPX samples were collected in 2022-2023. RealStar Orthopoxvirus PCR kit 1.0, Altona Diagnostics and Sanger sequencing were used as reference techniques. Clinical sensitivity and specificity (95% CI) were calculated with MetaDisc 1.4 software, while overall concordance and quantitative correlation between both real-time PCR kits was checked with the statistical package SPSS v24 (IBM Corporation).

Results:

All the results obtained by the kit under study coincided with the reference kit ones (being 69 of them positives for monkeypox). Therefore, clinical sensitivity and specificity values are 100%. Furthermore, the overall agreement was almost perfect (100, 98-100) as was the Spearman correlation test (rs=0.78; P<0.001). All positive samples were sequenced and confirmed the presence of the virus.

Conclusion:

This retrospective study demonstrates the good clinical parameters and strong overall agreement of the VIASURE® Monkey pox virus Real Time PCR detection kit (CerTest Biotec) compared to both the RealStar Orthopoxvirus PCR 1.0 kit and Sanger sequencing results. In addition, the stabilised, ready-to-use format allows storage at room temperature, and facilitates transport, as it does not need to be refrigerated. This makes it environmentally friendly and reduces additional costs.

Proteomics and Protein Markers

B-276

Preservation of Proteomic Inflammatory Biomarkers signal in a Saliva Multiomic Buffer at Room Temperature

M. M. Jensen[1], H. Maecker[2], T. Nguyen[3], D. Vu[4], D. Vigerust[4], R. Gupta[4]. *[1]Spectrum Solutions, Inc., Draper, UT, [2]Stanford University, Stanford, CA, [3]Stanford University, Palo Alto, CA, [4]Spectrum Solutions, LLC., Draper, UT*

Background

Saliva represents an attractive biospecimen as it is pain-free, simple for at home collections, and patients can provide multiple samples over time to provide time resolved data. However, few diagnostic tests have been developed using saliva due to poor stability and comparatively low concentration of analytes. Stabilization of biomarkers at ambient temperature in saliva would enable labs to take advantage of the benefits of saliva sample collections, while minimizing the risks posed by the degradation of already scarce analytes.

Methods

Matched samples of neat serum, saliva, and saliva collected with Saliva Multi-omic Device (SMD) preservative were collected from 30 volunteers (15 male & 15 female) ages 18-64 with no known morbidities. The Olink Inflammation panel was used to examine 92 inflammation-related proteins. The method utilized proximity extension assay technology, wherein antibody probe pairs reacted with their respective target proteins to generate a polymerase chain reaction target sequence through proximity-dependent DNA polymerization. This sequence was measured using real-time polymerase chain reaction and normalized in two steps, producing a semi-quantitative Normalized Protein eXpression (NPX) unit. To evaluate analyte stability specimens were measured initially and then again after 24 hours at room temperature (23± 2° C).

Results

Protein expression (NPX) units for 36 of the 92 inflammatory markers had higher concentrations in saliva compared to serum. Serum (78/92) and saliva 78% (72/92) showed similar coverage for the number of markers in the panel where at least 75% of subjects had values exceeding the limit of detection (LoD). SMD preservation buffer improved the average stability of inflammatory protein biomarkers by 19.6% compared to neat saliva and 10.2% compared to serum over 24-hours at room temperature. However, the relative stability of individual between protein markers[DV1] [HM2] varied. For proteins in SMD buffer, initial concentrations of analytes were highly predictive[HM3] of values 24 hours later, having Pearson correlation coefficients > 0.7, for 23 of the analytes and greater than 0.5 for 47. The change in protein marker in serum over time was only moderately predictive of the protein's stability in saliva with or without preservative, having correlations of 0.33± 0.20 and 0.47 ± 0.19 (mean ± standard deviation), respectively.

Conclusion

This study demonstrates potential utility of saliva in proteomic applications utilizing the Olink platform. The SMD preservation buffer stabilized inflammatory biomarkers in saliva samples likely through protease inhibition and antimicrobial action. Preservation of self-collection salivary samples by subjects without cold storage enables noninvasive sampling and reliable measurements even when samples are collected by users at remote locations or under challenging conditions.

B-277

Analytical validation of whole blood suitability for MeMed BV, a host-protein test for differentiating between bacterial and viral infection

M. Hainrichson[1], E. Simon[1], R. Kalfon[1], N. Senderovich[1], N. Shamir[1], Y. Kaminer-Israeli[1], R. Navon[1], S. Halabi[2], A. Klein-Kremer[3], E. Eden[1]. *[1]MeMed, Haifa, Israel, [2]Carmel Medical Center, Haifa, Israel, [3]Hillel Yaffe Medical Center, Hadera, Israel*

Background:

MeMed BV® is an FDA-cleared test for differentiating bacterial and viral infections. It is based on computational integration of serum levels of TNF-related apoptosis-induced ligand (TRAIL), interferon gamma-induced protein-10 (IP-10), and C-reactive protein (CRP). The test result is a score between 0 and 100 that correlates with increasing likelihood of bacterial infection (or co-infection). Here, we assessed the analytical performance of MeMed BV using whole blood (WB).

Methods:

Precision for each BV analyte and the score were assessed using 3 clinical WB samples representing bacterial, viral and equivocal scores. Each specimen was analyzed in four runs on five MeMed Key® analyzers in accordance with CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures. For the matrix comparison study, paired samples of serum and WB were collected from patients with suspected acute bacterial/viral infection. The study was performed in accordance with CLSI EP35Ed1E Assessment of Equivalence or Suitability of Specimen Types. Limit of Quantitation was established based on CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.

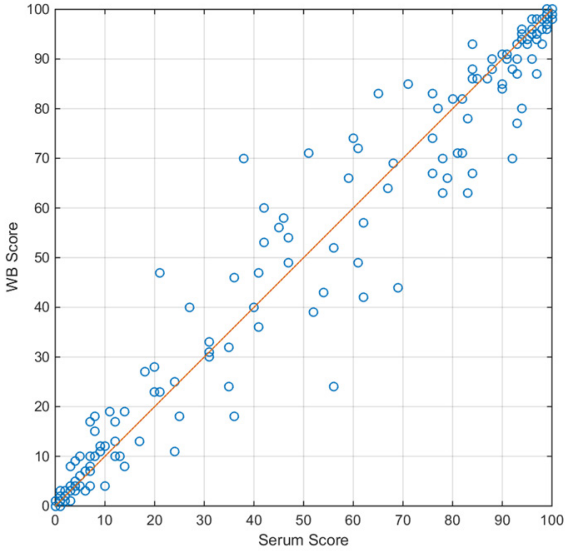
Results:

Precision results passed the pre-determined acceptance criteria for the analytes and score. The precision coefficient of variation range was 4.6-6.9%, 3.1-5.8%, and 4.0-12.0% for TRAIL, IP-10 and CRP, respectively. The precision standard deviation for the score was 0.0-3.0 score units.

For the matrix comparison study, 216 patients were recruited, aged 1-92 years (median 42.0) and 45.8% female. Passing-Bablok regression analysis of test scores from serum versus WB yielded a slope of 1.00 (95% confidence interval, CI 0.99-1.00) and intercept of 0.00 (95%CI 0.00-0.06) fulfilling pre-defined acceptance criteria (Fig. 1). Limit of Quantitation was established to be 1mg/L, 100 pg/mL, and 15pg/mL for CRP, IP-10 and TRAIL, respectively, the same values as for serum.

Conclusion:

Suitability of MeMed BV® for whole blood was demonstrated.



B-278

Pilot Clinical Study of the CarePoint Assay

J. S. Briggs[1], A. H. Brooks-Starks[1], T. J. Elenberger[1], R. Brenneman[1], K. R. Scarpato[2], S. S. Chang[2], J. C. Bassett[3], J. Linehan[4]. *[1]PhageTech, Inc, Irvine, CA, [2]Vanderbilt University Medical Center, Nashville, TN, [3]Hoag Hospital, Newport Beach, CA, [4]St. John's Cancer Institute, Santa Monica, CA*

Background: An estimated >710,000 individuals live with bladder cancer in the United States, with approximately 82,290 new cases expected this year (2023). Recurrence rates for bladder cancer are high: approximately 65% of patients with non-invasive or in situ tumors and 73% of patients with more advanced disease experience recurrence within five years. Consequently, patients treated for bladder cancer require prolonged surveillance to safeguard against tumor recurrence. The gold standard of surveillance, cystoscopy – an uncomfortable, expensive, and invasive procedure – is recommended every three to 12 months. Consequently, there is a need for an inexpensive, effective, noninvasive diagnostic tool as an alternative to cystoscopy.

The CarePoint Assay (CPA) is a 30-minute electrochemical immunoassay for the detection of disease-state biomarkers in human urine. The analytical performance of CPA has been evaluated for method comparison with a three-hour sandwich immunosorbent assay (ELISA) for bladder tumor antigen (BTA), a diagnostic biomarker for bladder cancer.

Method: Receiver operating characteristic (ROC) curves were calculated by simple logistic regression with Prism9 (GraphPad) using patient urine samples across multiple medical centers (n = 51), comparing BTA concentration (continuous variable) with high-grade tumor status (binary variable). Patient urine samples were evaluated by CPA using commercial potentiostats for comparison with a sandwich ELISA. The data was analyzed using simple linear regression.

Results: Patient urine evaluated by CPA was interpolated by simple linear regression using a three-point calibration with standard spiked into urine pooled from healthy donors. Data produced by sandwich ELISA was interpolated by five-parameter nonlinear regression using a 12-point standard curve. Both methods showed good correlation: (r)=0.93. Simple logistic regression comparing analyte concentration to high-grade tumor status produced a sensitivity and specificity of 52% and 88% with an AUROC of 0.71 for the CPA, and a sensitivity and specificity of 72% and 77% with an AUROC of 0.77 for the sandwich ELISA. Sensitivities and specificities were calculated using Youden's *J* statistic.

Conclusions: We established proof of concept for a rapid, electrochemical immunoassay for a diagnostic biomarker of bladder cancer. The CPA correlated well with sandwich ELISA, an established method for measuring biomarkers in bodily fluids. Future work will focus on refinement of the CPA architecture for point-of-care use and subsequent evaluation of analytical performance.

B-280

Sensitivity and Specificity of beta-2 Transferrin Gel Electrophoresis with Immunofixation for Evaluating Nasal Cerebrospinal Fluid Leaks

H. ZARI, A. JURAS, C. Chen, K. Veasey, J. Craig, J. L. Carey, B. C. Cook. *Henry Ford Health, Detroit, MI*

Background: Cerebrospinal fluid (CSF) rhinorrhea usually presents as unilateral clear thin nasal drainage, is caused by a direct communication from the subarachnoid space to the sinonasal cavities and can lead to intracranial infection, pneumocephalus, or death. Diagnosing CSF rhinorrhea can be challenging. While the gold standard for CSF leak confirmation is surgical exploration, testing nasal fluid for beta-2 transferrin (B2Tf) via agarose gel electrophoresis followed by immunofixation (AGEI) remains the initial non-invasive CSF confirmatory test of choice. However, AGEI requires subjective interpretation of immune-stained protein bands. Multiple studies have reported high sensitivity and specificity of B2Tf AGEI (90-100%), but other studies have shown wider ranges from 70-100%. Due to small sample sizes and methodologic differences between studies, comparing results between studies is problematic. The purpose of this study was to assess the sensitivity and specificity of B2Tf AGEI in confirming or excluding CSF rhinorrhea in patients with unilateral clear thin nasal drainage, and to determine the inter-reviewer variability of AGEI interpretation. **Methods:** This was a retrospective observational study of patients who had B2Tf AGEI performed on their unilateral clear nasal drainage from 2020 through 2022. A pathologist and four trained medical laboratory scientists conducted visual reviews of archived gels and their results were compared to the documented CSF rhinorrhea status. Results were also compared to the original test results documented in patients' charts. Sensitivity and specificity, and inter-reviewer agreement were assessed for B2Tf AGEI confirming or excluding CSF rhinorrhea. The gold standard for confirming CSF rhinorrhea was surgical exploration. The gold standard for excluding CSF rhinorrhea included either negative surgical exploration or rhinorrhea resolution after medical therapy aimed at treating rhinitis or rhinosinusitis. **Results:** Sensitivity and specificity varied for the five reviewers and documented test results, with sensitivities ranging from 65.0 to 86.0% (mean ± SD, 73.0 ± 5.7) and specificities from 61.4 to 88.6% (84.1 ± 11.0). The false positive rate was 8.7%, and false negative rate was 13.9%. Sensitivity and specificity for the documented test results ranging from 57.1 to 82.1% (mean ± SD, 72.1, 9.3) and specificities from 75.0 to 97.26% (91.6 ± 9.4). The abilities of the reviewers to reliability identify a CSF leak was moderate, with kappa statistics ranging from 0.313 to 0.646 (weighted average 62.2, 4.0). Two of the four reviewers demonstrated substantial to almost perfect agreement to the expert reviewer, with the other two demonstrating moderate agreement. **Conclusions:** B2Tf AGEI demonstrated lower sensitivity and specificity than some previous reports, however this study had a larger sample size and very well-defined clinical standards for CSF and non-CSF rhinorrhea. While false results were present in 9 to 14% of cases, the test is non-invasive, and is helpful in stratifying patients into those more or less likely to have CSF rhinorrhea. There was also substantial inter-reviewer variability, highlighting the challenges of subjective interpretation of this testing modality. Larger prospective studies would be helpful to more accurately determine the diagnostic accuracy of B2Tf AGEI, as well as explore practices to help limit inter-reviewer variability.

B-281

Analytical Validation of the Mindray High Sensitivity Cardiac Troponin I Assay

B. Fabre-Estremera[1], K. M. Schulz[2], A. Ladd[2], A. Buño Soto[1], F. S. Apple[3]. *[1]La Paz University Hospital, Madrid, Spain, [2]Hennepin Healthcare Research Institute, Minneapolis, MN, [3]Hennepin Healthcare/HCMC, Minneapolis, MN*

Background: According to recent global guidelines for patients presenting with ischemia, high sensitivity cardiac troponin I and T (hs-cTn) are the preferred biomarkers for the diagnosis and risk assessment of acute myocardial infarction (MI) and myocardial injury. The purpose of our study was to perform an analytical validation study of the Mindray hs-cTnI assay.

Methods: Analytical studies were designed according to Clinical and Laboratory Standards Institute protocols (CLSI). We used one reagent lot and one CL1200i chemiluminescence Immunoassay Analyzer (Mindray Bio Medical Electronics Co., Shenzhen, China). Following the CLSI EP17 A2 document, the limit of blank (LoB) and limit of detection (LoD) were assessed following analysis of a) Mindray controls without cTnI concentrations added for the LoB and b) 4 lithium heparin (LiHep) fresh samples with low cTnI concentrations, for 3 days for LoB and LoD, with 5 replicates per day (60 replicates per study). For the precision study, according to the CLSI EP15-A3 protocol, we used 12 LiHep fresh samples, measured for 10 days, with 2 runs per day (separated by a minimal time of 2 hours) and in replicates of 3 (n = 720). For the 12 samples for the precision study, 7 were chosen with hs-cTnI concentrations close to the manufacturer sex-specific 99th percentile upper reference limits (URL; 31 ng/L for males and 15 ng/L for females). The CLSI EP06 protocol was followed to study linearity, using 9 fresh LiHep samples analyzed the same day in replicates of 7 (n = 63). A blank sample between every sample was used to avoid carryover. Our analytical specification for linearity was an allowable deviation <10%. Analyses were performed with Analyze it for Excel (Method Validation edition, version 6.15).

Results: Using both parametrical and non-parametrical analyses, LoB was <0.1 ng/L, with a relative light unit (RLU) of 5,394. LoD was 0.1 ng/L according to the parametrical analysis. Regarding imprecision, for samples ranging from cTnI concentrations of 1 to 106 ng/L, repeatability had a coefficient of variation (%CV) from 1.15% to 3.83%, and within-laboratory imprecision ranging from 1.69% to 5.03%. Using samples with hs-cTnI concentrations close to the manufacture's sex-specific upper reference limits (9-36 ng/L), the repeatability and within-laboratory precision was 1.27-1.61% and 1.92-2.52%, respectively. The measuring interval for the linearity study ranged from 1.1 to 30,772 ng/L. All analytical parameters met CLSI limits of acceptable performance. No carry over was detected.

Conclusion: Our analytical observations of the Mindray hs-cTnI assay demonstrate excellent LoB, LoD, imprecision and linearity, that were in alignment of with the manufacturer's claims and published guidelines for hs-cTnI.

B-284

Evaluating the range of serum neurofiament light chain at different ages in Chinese

S. Guo[1], Z. Meng[2], M. Tang[2], Y. Ren[2], B. Ying[2]. *[1]West China Hospital, Sichuan University, Chengdu, China, [2]West China Hospital, Scihuan University, Chengdu, China*

Background: Neurofilament light chain (NFL) has recently been identified as a sensitive biomarker for diagnosis and prognosis in many neurological diseases. However, studies focused on NFL levels ranges in healthy individuals remain few, especially in the Chinese population. The clarification of the reference range among healthy individuals at different ages would be useful for further clinical utility. **Objectives:** To evaluate the serum neurofilament light chain range at different ages in healthy Chinese and analyze the potential contributing factors for NFL levels. **Methods:** Healthy individuals undergoing rigorous cognitive and neuroimaging detection were enrolled. Serum samples and plasma samples of different anticoagulants were collected for NFL levels detection by the ultra-sensitive Simoa HD-1 platform. The tendency of NFL levels to change with age and potential influence factors were analyzed. **Results:** We enrolled three hundred and ninety-nine healthy participants. The positive correlation between serum NFL levels and age was observed and the regression formula was further established. There were no statistical differences between the <20 years group and the 20-29 years group (p=0.2512), as well as the 60-69 years group and the 70–79 years group (p=0.0528) and differences,were statistically significant among the remaining pairwise groups. We further observed the positive correlation between serum tNFL levels and age was 0.76 in total(p<0.0001). The NFL levels between the serum and heparin plasma are statistically different and there was no statistical difference between the serum and plasma of other anticoagulants. **Conclusions:** The age-related change pattern was uncovered, providing important assistance for the clinical use of NFL to monitor the potential neurological alterations for people of different ages. **Keywords:** neurofilament light chain, serum, healthy people, age.

B-288

The Beginning of the End: AGO2 Protein Identified and Validated as a Novel Biomarker for Multi-Cancer Diagnosis through Bioinformatics Analysis and Immunoassay

A. HASHMI[1], G. Hutvagner[2], S. Sidhu[3]. *[1]University of Technology/NSW Health Pathology, Sydney, Australia, [2]University of Technology Sydney, Sydney, Australia, [3]Kollings Research Institute, Sydney, Australia*

Background: Despite significant progress in cancer research, the complexity and heterogeneity of the disease still pose major challenges to accurate diagnosis and effective treatment. Recent advancements in biomedical technologies have led to the identification of reliable potential cancer biomarkers. In this sense, the link between microRNAs (master regulators of gene expression) & cancer is well-established, but there has been little research on the microRNA machinery itself. We aim to investigate whether changes in the expression of genes & proteins involved in the miRNA pathway can be used for multi-cancer detection.

Method: We analyzed RNASeq data from the TCGA (The Cancer Genome Atlas) and GTEX (The Genotype-Tissue Expression) projects to compare the expression of core components in the miRNA biogenesis pathway, including AGO2, DGCR8, XPO5, RAN, DROSHA, DICER, and TARBP2, in normal and tumorous samples across 22 tissue types, including adrenocortical, AML, breast, colon, lung, liver, esophageal, prostate, pancreas, stomach, thyroid, rectum, uterus and ovarian cancer. We accessed and analyzed the TCGA and GTEx datasets using the UCSC Xena Browser. We also measured the protein concentrations of all these microRNA regulators in 15 normal adrenal cortex, 15 benign adenoma, and 15 adrenocortical cancer tissue homogenate samples using commercial ELISA kits. The Human Protein Argonaute-2 (EIF2C2) ELISA Kit-AE45910HU (Abebio-Co.Ltd) was used for the assay procedure, which was performed according to the manufacturer's instructions. Statistical analysis was performed using GraphPad Prism, Version 9 (GraphPad Software, CA, USA). A p-value of less than 0.05 was considered statistically significant.

Results: Our RNASeq analysis results showed that among all microRNA regulators, AGO2 is most highly significantly over-expressed in samples derived from cancer patients compared with normal controls (P<0.05). In addition, we analysed the protein expression of AGO2 in adrenocortical tissue homogenate samples. Our results demonstrated that the protein expression of AGO2 in adrenocortical carcinoma samples was significantly higher than in normal adrenal cortex and benign adrenocortical tumour samples (P<0.001).

Conclusion: Our study highlights the potential role of Argonaute 2 protein (AGO2) in cancer development and progression. AGO2 is a key component of the RNA-induced silencing complex, which regulates gene expression. Our findings show that AGO2 is overexpressed in cancer samples compared to normal controls across a range of cancer types. This suggest that AGO2 could be used as a promising marker for the development of more accurate and specific methods for cancer diagnosis, in combination with other markers. Furthermore, the potential for using AGO2 protein expression in serum samples as a non-invasive diagnostic tool is a promising area for future research. This could be achieved through automated immunoassay, making it a practical diagnostic tool for use in routine clinical practice.

B-289

Acute Kidney Injury urinary biomarker, Neutrophil gelatinase-associated lipocalin (NGAL) analytical performance evaluation on Roche cobas c502 chemistry platform

V. Samara[1], V. Buggs[2], L. Song[2]. *[1]University of Chicago, Chicago, IL, [2]University of California Los Angeles, Los Angeles, CA*

Background: Acute kidney injury (AKI) is a sudden and serious kidney damage condition that affects more than 1.2 million people every year. KDIGO guidelines suggest serum creatinine increase as a diagnostic marker for AKI but elevation of serum creatinine levels can take up to seven days. Neutrophil gelatinase-associated lipocalin (NGAL) has been identified as an early tubular injury biomarker for AKI that is detected within three hours of AKI patients' urine. In this study, we evaluated technical performance of BioPorto NGAL assay on Roche cobas c502 chemistry analyzer. **Methods:** BioPorto's (Hellerup, Denmark) particle enhanced turbidimetry immunoassay (PETIA) NGAL assay was installed on cobas c502 chemistry analyzer

(Roche, Indianapolis, U.S.A) following vendor-specific application parameters with few modifications. Linearity was determined with BioPorto's linearity material and verified by serial dilutions of a high NGAL patient urine sample. Limit of quantitation was determined in urine samples with values 20, 25 and 35 ng/mL, run in duplicate for 10 days. Precisions were determined by running urine samples containing 100ng/mL and 400 ng/ml NGAL 20 times for within-run precision and twice a dayX10 days for between-run precision, respectively. To determine the accuracy of the NGAL method on cobas 502, 40 sample results (provided by the vendor) were compared with those measured on Siemens Atellica using BioPorto's NGAL assay. Reference interval verification was carried out in 40 urine samples collected from healthy adult volunteer-donors. **Results:** The linearity was found to be excellent from 0-3000 ng/mL. Limit of quantification was found at 26 ng/mL. Precision results are in the table below.

	Within-run Precision		Between-run Precision	
	Mean	CV	Mean	CV
Level 1	121 ng/mL	4.3%	106 ng/mL	4.8%
Level 2	401 ng/mL	1.8%	399 ng/mL	2.6%

Method comparison showed a slope of 0.90 and intercept of 32.9. The reference interval was verified to be <50 ng/mL (in accordance with current NGAL reference cutoff for AKI diagnosis).

Conclusion: Performance of BioPorto's NGAL PETIA assay was validated in urine on cobas c502 chemistry analyzer.

B-290

Development of a LCMS/MS method for the quantitation of infliximab in serum

B. Zhong, J. V. Abonamah, D. Payto, A. McShane. *Department of Laboratory Medicine, Cleveland Clinic Foundation, Cleveland, OH*

Background

Quantitative bottom-up proteomics of biotherapeutics has been successfully introduced in the clinical laboratory for therapeutic drug monitoring in recent years. The general workflow includes multiple steps: sample extraction/purification, reduction, alkylation, enzymatic digestion, desalting, and LC-MS/MS analysis of signature peptides. In this study, we developed an infliximab drug assay with desired operational efficiencies needed in a clinical laboratory. Evaluations included reduction/alkylation removal, trypsin digestion length, trypsin quality, online/offline/no sample desalting, multiple/single signature peptides, and whole protein/peptide internal standardization. **Methods** For sample preparation, a general procedure was initially followed. Briefly, protein precipitation with ammonium sulfate, re-suspension with buffer, reduction, alkylation, overnight enzymatic digestion, desalting, and LC-MS/MS analysis. To simplify the workflow, the steps of reduction and/or alkylation were omitted. The trypsin digestion time was varied from 2h, 4h, and overnight. TPCK-modified trypsin from two different vendors (Thermo Scientific and Millipore Sigma) were also compared for digestion efficiency. SILu™MAb infliximab stable-isotope labeled monoclonal antibody (Millipore sigma) was compared with customer synthesized stable-isotope labeled signature peptides as the internal standard. Different trapping cartridges were evaluated for online sample desalting and different analytical columns were evaluated for target peptide analysis. A Transcend TurboFlow TLX UHPLC System coupled to a TSQ Altis triple-quadrupole mass spectrometer was used (Thermo Scientific) operating in multiple reaction monitoring mode (2 transitions for each peptide and internal standard). Two signature peptides YASE (Sequence: YASESMSGIPSR) and GLEW (Sequence: GLEWVAEIR) from different chains of infliximab were analyzed. **Results** For sample preparation, the omission of protein reduction/alkylation from the workflow did not significantly affect YASE quantitation. The peak area of GLEW decreased by about 30% without reduction/alkylation. The evaluation of three calibration curves corresponding to the digestion time of 2, 4, and overnight respectively showed that 4 h was optimal for all the levels of calibrators if both signature peptides were used for quantitation. TPCK-modified trypsin from two different vendors were equal in digestion efficiency. The calibration curve using SILu™MAb infliximab stable-isotope labeled monoclonal showed superior linearity (R^2 >0.98) and recovery for each calibrator versus using stable-isotope labeled signature peptides as internal standard. With the optimized conditions, both signature peptides can be used for the accurate quantitation of infliximab. However, for monitoring both peptides simultaneously, the chromatography separation needed 14.5 min. On the contrary, 9 min is needed for monitoring a single peptide YASE. **Conclusion** In summary, systematic optimization of sample preparation and LC-MS/MS analysis was accomplished in this study. The entire workflow was simplified by removing the steps of reduction and alkylation and using optimal digestion time, which increases the efficiency and significantly facilitate the implementation of this assay.

B-291

Proximity Labeling at Sites of Protein-Protein Interactions Using a Switchable DNA Catalyst

A. Ogorek, X. Zhou, J. Martell. *University of Wisconsin - Madison, Madison, WI*

Background: Extracellular protein-protein interactions (PPIs) play a crucial role in cell-cell adhesion, communication, and signaling. The dysregulation of PPIs in cancer caused by differential protein expression can drive oncogenic factors. Traditional methods to study PPIs disfavor weak or transient interacting protein partners, leaving them undiscovered. Proximity labeling (PL) is an emerging tool in which a catalyst is attached to a protein of interest (POI) and creates reactive species that tag nearby endogenous proteins. Tagged proteins are subsequently isolated and identified as potential interacting partners with the POI. If PL could be activated at a more specific subcellular location, such as the site of a PPI, more detailed information could be unveiled concerning the interactome of cancer-relevant PPIs. Recent advances in PL involve the use of a synthetic catalyst that can be localized to a POI via an antibody or ligand and avoids the need for PL enzyme-POI fusion proteins to be constructed, allowing for analysis of primary tissue samples. **Methods**: To create a PL catalyst with pseudo-allosteric regulation, we created a switchable DNA catalyst. The DNA catalyst is composed of synthetic photocatalyst and quencher moieties covalently linked to DNA oligomers such that when hybridized together the photocatalyst is quenched and inactive. The DNA scaffold affords conformational control that is utilized to change the distance between photocatalyst and quencher, thus regulating photocatalyst activity. Split triggers composed of DNA oligos that bind to catalyst-DNA and simultaneously disrupt quencher-DNA hybridization were used to create an AND logic gate where both split triggers need to be in proximity to activate the PL catalyst. The split triggers were localized to POIs via connection to aptamers, short pieces of synthetic DNA that bind targets. Using the c-Met homodimer as a model system, we first labeled c-Met expressing HEK293T cells with aptamer-split trigger DNA oligomers as well as the quenched photocatalyst. After the catalyst was activated at the site of the PPI, we performed proximity labeling using a biotin-phenol substrate in the presence of an oxidizing agent and 450 nm light. Fluorescent streptavidin was used to visualize sites of biotinylation and detected using fluorescence microscopy. **Results**: We observed extracellular biotin signal only of c-Met transfected cells as evidenced by the expression of a co-transfection plasmid encoding a fluorescent nuclear protein. When one of the split triggers had the aptamer motif removed, meaning it couldn't be localized to the cell surface, no labeling was observed which shows that both split probes must be localized to the cell surface to activate labeling. Additionally, controls including omission of light, photocatalyst, or biotin phenol showed no labeling. Using a free-floating version of the photocatalyst to label cells, we confirmed that lysates contained biotinylated proteins via western blot. **Conclusion**: We localized two split triggers to interacting proteins to activate a PL catalyst and performed proximity labeling at the site of the PPI. This technique demonstrates non-genetic split proximity labeling to enable downstream analysis of cancer cell proteomes in an ultra-specific manner and expands the toolkit for spatiotemporally controlled proximity labeling.

B-293

Non-renal sources of erythropoietin: complications for interpreting erythropoietin assays

R. Alshamali[1], J. P. Marrow[1], M. J. Platt[1], K. R. Brunt[2], J. A. Simpson[1]. *[1]University of Guelph, Guelph, ON, Canada, [2]Dalhousie University, Saint John, NB, Canada*

Background: Erythropoietin (EPO) is a glycoprotein that stimulates the production of red blood cells, in response to systemic hypoxia. Generally, the kidney is thought to be the sole producer of EPO. Emerging data shows *Epo* mRNA expression can occur in non-renal tissues, yet the significance of this remains uninvestigated. Clinically, EPO assays are primarily used to identify the underlying cause of anemia and polycythemia. Interestingly, EPO serum levels are elevated following a myocardial infarct (MI); the source is always assumed to be kidney with no further investigations into non-renal sources of EPO. Here we investigate the heart as a novel source of serum EPO following a MI. **We hypothesize** that the heart, not the kidney, produces EPO in response to organ-specific hypoxia (i.e., MI).

Methods: To assess which organs are producing EPO following a MI, we examined EPO mRNA expression. mRNA levels correlate strongly with EPO protein synthesis, as EPO is exclusively regulated at the transcription level. To induce a MI, CD1 mice were subjected to ligation of the lateral anterior descending artery. Left ventricle and

kidney tissues were collected at 24 hours, 4 weeks and 18 weeks following MI or sham surgery; tissue was snap frozen and stored at -80°C for qPCR analysis. Hematocrit was measured at 2-, 4-, and 18-weeks following MI or sham surgery.

Results: 24 hours following a MI, the left ventricle showed a ~100-fold increase in EPO mRNA expression in comparison to sham group (p=0.0012); the kidney showed no significant differences in EPO mRNA between MI and sham group. Hematocrit was measured from the carotid artery. At 2- and 4- weeks following surgery, hematocrit in MI group was significantly elevated in comparison to baseline hematocrit levels (Baseline: 40.8% ± 4, 2-week: 47.8% ± 3.1, and 4-week: 46% ± 2.6 (p=0.0003, p=0.0103, respectively). Whereas there were no significant differences in hematocrit between baseline and the sham group.

Conclusion: Here we show that following a MI, the heart, not the kidney, produces EPO. Subsequently, there is an increase in hematocrit as a result of cardiac-specific EPO production. Further, perturbations in EPO serum levels are not disease specific. In cases where EPO assays are conducted on MI patients, interpretation of EPO levels should be cautioned as it may not be exclusively diagnostic of the underlying cause of anemia or other bone marrow disorders. Therefore, an alternative diagnosis may be warranted for elevated EPO levels in this specific patient population.

B-294

Delineating the diversity of recombinant monoclonal antibodies isolated from Alpaca polyclonal mixture.

D. Narang[1], T. Le Bihan[2], T. N. De Villavicencio Diaz[2], B. Ma[2]. *[1]Rapid Novor Inc, Kitchener, ON, Canada, [2]Rapid Novor Inc., Kitchener, ON, Canada*

Introduction: Polyclonal antibodies are a mixture of antibodies produced by plasma B-cells and are often obtained by injecting a specific antigen into a host system. They are commonly used for research and diagnostic purposes and are inexpensive and relatively quick to produce. But they lack batch-to-batch reproducibility making them less reliable than recombinant monoclonal antibodies (mAbs). Our team designed a polyclonal sequencing method to sequence the mAbs directly from the immunoserum and then used recombinant technology to reliably express the mAbs. The diversity of the epitopes of these recombinant mAbs are then examined with Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS).

Methods: In this study, we used a novel mass-spectrometry-based approach to do *de novo* sequencing of the Alpaca IgGs directly from the immunoserum without analyzing the RNA-seq data. Using our established REpAb sequencing platform and a machine learning-based bioinformatic analysis, we successfully compiled complete amino acid sequences of individual alpaca IgG clones found in the original polyclonal mixture. Using this approach, we isolated ten unique sequences from the polyclonal mixture and expressed them recombinantly. In addition, we performed Hydrogen-deuterium exchange Mass spectrometry experiments on 4 of the ten mAbs to identify their binding sites. For HDX analysis, the antigen digestion was performed using Pepsin/protease XIII column, and the peptides were identified using Waters SELECT SERIES Cyclic IMS Mass spectrometer.

Preliminary data: The four mAbs which showed strong binding to the antigen were chosen to characterize further using HDX-MS experiments. The preliminary MS/MS experiments provided us with approximately 90% of the amino acid sequence coverage of the antigen. Interestingly, the HDX-MS results showed that different mAbs bind to different antigen regions, which is evident from the deuterium exchange. Overall, our results showed that the monoclonal antibodies isolated from the polyclonal mixture bind to different antigen regions and can represent the diversity in the polyclonal mixture.

Novel aspect: De novo sequencing functional mAbs from immunoserum and studying their epitope diversities with HDX-MS may help discover novel therapeutics antibodies.

Therapeutic Drug Monitoring and Toxicology

B-297

Effortless and streamlined workflow for absolute quantitation of therapeutic monoclonal antibodies using Promise Proteomics mAbXmise kits and TSQ Altis Plus mass spectrometer

Y. Song[1], D. Lebert[2], J. Guillaubez[2], S. Samra[1], E. Goucher[1], B. Hart[1]. *[1]Thermo Fisher Scientific, San Jose, CA, [2]Promise Proteomics, Grenoble, France*

Background: Laboratories continuously seek improved productivity and efficacy for clinical testing, ultimately impacting the turn-around time and sample throughput. Various efforts have been made including rapid and high-throughput testing, automation, and high-end instrumentation. This also applies to therapeutic drug monitoring (TDM) of therapeutic monoclonal antibodies (mAbs). In particular, mass spectrometry (MS) has gained significant popularity in clinical laboratories for TDM of mAbs due to its great versatility to measure such complex biological proteins qualitatively and quantitatively. Here we present the streamlined workflow for the absolute quantitation of therapeutic mAbs using the Promise Proteomics mAbXmise kits and the Thermo Scientific™ TSQ Altis™ Plus mass spectrometer. The mAbXmise kit is the first commercial ready-to-use kit that offers all the necessary reagents for the LC-MS/MS assay including the stable-isotope labeled (SIL) proteins of the target mAbs (SIL-mAbs). The TSQ Altis™ Plus mass spectrometer is the latest generation of the TSQ MS series, offering superior acquisition speeds, sensitivity, selectivity, and robustness for routine and high-volume targeted protein quantitation.

Methods: The experimental workflow followed the instructions provided by Promise Proteomics with minor modifications (refer to instructions manual - https://customer.mabxmise.com). The kits used in this study include pre-coated plates with a full-length SIL-mAb corresponding to each target mAb sequence as internal standards with purity > 95% and isotopic corporation > 98%. Thus, the ITDM1 kit contains 2 SIL-mAbs and the OTDM1 kit contains 7 SIL-mAbs pre-coated in each plate. The kits also provide buffer stocks, reagents, 6 calibration standards including zero, and 2 QC controls. Each kit provides 12 strips capable of 96 sample preparation. The calibrators and QC samples were added to the mAbXmise plate and subjected to immunocapture followed by trypsin digestion. LC-MS/MS was performed using a Thermo Scientific™ Vanquish™ Flex UHPLC system hyphenated to TSQ Altis™ Plus mass spectrometer.

Results: Highly comparable results were observed across six replicates for both OTDM1 and ITDM1 kits with R^2 values > 0.99 and % RSD of peak areas of peptides <15. After confirming the complete sample preparation of 96 samples, an evaluation of LC-MS analytical performance was performed. The results show great linearity with R^2 > 0.998 over measured calibration concentration points for all the OTDM1 and ITDM1 target peptides, respectively. Also, the data support the reproducible LC-MS measurement of the entire plate providing % RSD < 10. The accuracy of QC samples was determined to be between 94 and 103 % for OTDM1 peptides with a precision of < 7 % CV and between 95 and 107 % for ITDM peptides with a precision of < 9 % CV. The response of CAL1 (LLOQ) was 5 times higher than CAL0 for all the target peptides with accuracy between 96 and 112 % and precision of < 11 % CV. Additionally, extremely reproducible retention times were observed from all SIL-mAb peptides with RT difference ± 0.03 minutes during the evaluation.

Conclusion: The workflow tested in this report generated sensitive and robust data with high confidence, emphasizing the capability of MS-based TDM of mAbs as routine testing.

B-298

Retrospective analysis of fentanyl positivity at a large academic center reveals trends in illicit fentanyl use

M. Berry[1], L. Cohen[2], D. Manthei[1], C. Gherasim[1]. *[1]Michigan Medicine, Ann Arbor, MI, [2]College of Pharmacy, University of Michigan, Ann Arbor, MI*

Background: National drug-involved overdose deaths continue to rise with deaths involving synthetic opioids (primarily fentanyl) showing the most significant upward trend. Fentanyl is a synthetic opioid with short-acting analgesic activity and a 50-100 times higher potency compared to morphine. Pharmaceutical fentanyl is typically used to treat patients with severe or chronic pain. In contrast, "illegal" fentanyl has frequently been added to other drugs including heroin, cocaine, methamphetamine,

and MDMA to increase their potency and contributing to overdose deaths. In this study, we have investigated trends in fentanyl and fentanyl analogs positivity at a large academic medical center.

Methods: Retrospective analysis of results reported over seven years (2015-2021) for untargeted urine drug screens included testing performed for acute exposure and compliance. Presence of fentanyl, fentanyl analogs, and other prescribed or illicit used drugs was determined by gas chromatography - mass spectrometry (GCMS). Overall positivity was calculated as a percentage of total reported results.

Results: Fentanyl, and fentanyl analog positivity was determined for 40,782 urine drug screen results. Overall positivity for fentanyl and fentanyl analogs increased 3.8-fold over the past seven year from 1.53% (2015) to 5.78% (2021). Co-positivity with other illicit drugs such as cocaine and heroin also increased from 7% and 8% respectively, in 2015, to 42% and 13%, in 2021. Presence of fentanyl analogs such as fluorofentanyl, despropionylfentanyl, and acetylfentayl increased in the recent years as well.

Conclusion: Our data reveals increased positivity of fentanyl and fentanyl analogs present with cocaine, heroin, or their metabolites suggesting increased use of illicit fentanyl. The high prevalence supports the inclusion of fentanyl on routine urine drug screen immunoassays, or at a minimum as a reflex with a positive opiate screen.

B-299

Liquid chromatography-mass spectrometry measurement of drugs of abuse and alcohol biomarker phosphatidylethanol (peth 16:0/18:1) using volumetric dried blood spot device

G. Göksu Gürsu, M. E. Maviş, H. Yılmaz. *SEM Laboratuar Cihazlari, Istanbul, Turkey*

Background: Reliable biomarkers have great significance in the investigation of alcohol consumption patterns and in the treatment of alcohol-induced diseases. Direct biomarkers to detect alcohol intake are non-oxidative products of ethanol metabolism [1,2]. Phosphatidylethanol (PEth), blood-based direct alcohol biomarker, represents a group of unnatural phospholipids formed on leukocytes and predominantly erythrocyte membranes. The presence of PEth in blood samples is an indisputable indicator of ethanol consumption [3,4]. Moreover, detecting drugs of abuse (DOA) including opiates, amphetamines, cocaine metabolites, benzodiazepines, THC-metabolites, MDMA, methadone, fentanyl etc. is important to establish patterns of illicit drug usage. In recent years, DBS has been gaining interest in plenty of research areas owing to ease of specimen collection and storage [5,6].The main objective of this study was to determine the concentration of PEth-16:0 18:1 in DBS samples collected using a volumetric dried blood spot (DBS) device (*Hemaxis™ DB*) and to identify/quantify drugs of abuse (including 119 parameters-*Jasem Clinical Toxicology mixture*) using another blood spot punch of the same DBS sample.

Methods: According to *Jasem* method, DBS samples were prepared by sampling from spiked 20 µL of blood drop located onto paraffin film. PEth-16:0 18:1 and DOA were extracted from entire blood spot of DBS specimens implementing two different extraction reagents at room temperature for 10 min then subjected to HPLC system equipped with Agilent 6470 TQ. The total run times from injection to injection for PEth and DOA were 4.0 min and 12.0 min respectively.

Results: The linearity and accuracy of the methods were evaluated using 5 DBS calibrators levels for PEth and 6 DBS calibrators levels for DOA. Linearity was confirmed in the range 10 to 1000 ng/mL ($r^2 > 0.995$) for PEth 16:0/18:1 and in the range 2 to 100 ng/mL for DOA ($r^2 > 0.99$). For two methods linearity, RSD% (inter-intraday) and accuracy were within the analytical acceptable ranges.

Conclusion: The measurement of blood PEth levels provides a precious tool to determine the chronological profile based on retrospective alcohol intake in a reliable way. Besides, simultaneous analysis of DOA containing 119 parameters ensures comprehensive analytical solution and clinically relevant information. Both of *Jasem* LC-MS/MS approaches are centred on simple sample preparation following non-invasive sample collection method. Prior to rapid extraction, applying volumetric DBS device assures to collect fixed volume of blood regardless of blood haematocrit level making the measurements more consistent.

References [1] Cabarcos P., Álvarez I., Tabernero M. J., Bermejo A. M., *Anal. and Bioanal. Chem.*, 2015, 407, 4907-4925. [2] Ulwelling W. and Smith K., *J. Forensic Sci.*, 2018, 63(6):1634-1640. [3] Laksoa H-A., Wuolikainen A., Sundkvist A., Johanssond I., Marklunda S. L., *Clin. Mass Spectrom.,* 2019*,* 11, 37-41. [4] Berg T., Eliassen E., Jørgenrud B., Kabashi S., Alexey Petukhov A., Bogstrand S. T., *J. Clin. Lab. Anal*, 2018. [5] Beck O., Modén N. K., Seferaj S., Lenk G., Helander A., *Clin. Chim. Acta*, 2018, 479, 38-42. [6] Bakhireva L. N., Shrestha S., Gutierrez H. L., Berry M., Schmitt C., Sarangarm D., *Alcohol and Alcoholism*, 2016, 51(3), 275-280.

B-300

Importance of clinical chemistry and metabolism parameters for the management of patient with chronic nitrous oxide intoxication.

G. GRZYCH[1], E. GERNEZ[1], D. Marchand[1], S. DEHEUL[1], F. Zerimech[1], V. MASSO[1], A. BENNIS[2], R. Diesnis[2], M. Joncquel[1], I. KIM[1], J. NIGUET[3], J. Guichard[1], M. GIROT[1], C. TARD[1]. *[1]CHU Lille, Lille, France, [2]CH Roubaix, Roubaix, France, [3]GHICL, Lille, France*

Background: Recreational use of nitrous oxide (N2O) has increased exponentially recently and leads to severe neurological disorders. A recent report from European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) showed that the recreational use of N2O is a growing and became a major health issue in Europe. For biological monitoring, serum or urine N2O assays are not routinely performed because they do not allow to ensure a real exposure due to the very short half-life of this gas in the body. Functional disorders of vitamin B12 metabolism, characteristic of N2O intoxication, could be explored by two markers: homocysteine (Hcys) and methylmalonic acid (MMA). We experimented here, management of N2O abused patient thanks to functional markers as Hcys and MMA.

Methods: We collected clinical and biological data from patients with declared chronic N2O consumption. The patients treated in Lille University Hospital between september 2020 and december 2022 were included in the study. The clinical evaluation included the Peripheral Neuropathy Disability (PND) score which was evaluated by a neurologist.

Results: Our study included 202 patients with N2O abused, Globally, plasma functional markers concentration are increased in case on consumption (Table). We noticed that clinical sign of patient evaluated by PND score correlated with plasma MMA (p-value < 0.01) but not with plasma hcys (p-value >0.05).

Conclusion: The use of functional markers as Hcys and MMA makes it possible to follow the metabolic disturbances of nitrous oxide abused patients and evaluated clinical outcomes. However, in view of the thrombotic risk related to high Hcys and the need to quickly adapt the nutritional treatment, biological and in particular Hcys, current reporting time is not satisfactory using mass spectrometry method. Hence, our medical laboratory aim to improve our method evaluating immunoassay techniques in order to reduce the turnaround time while maintaining analytical reliability.

Clinical gravity according to PND score	0	1	2	3
Numbers	84	51	47	20
Ratio Men/Women	9.5	2.92	1.61	1.37
Homocysteine HCY	37	66.85	94.39	82.25
Méthylmalonic Acid MMA	0.51	3	4.66	5.54
Numbers (2020-2021-2022)	5-13-66	3-5-37	1-21-24	0-2-17

Table. Characteristics of patients according to clinical severity. Groups are based on the severity of their clinical symptoms based on Peripheral Neuropathy Disability (PND) score: level 0: no symptoms, level 1: patients with distal sensory disorders without gait disorders, level 2: patients with walking disorders, but they can walk without help, and level 3: patients with walking disorders who need help or bedridden patients. Values of HCY and MMA are expressed in µmol Data are expressed as mean.

B-301

In-vivo continuous therapeutic drug monitoring with electrochemical aptamer-based sensors

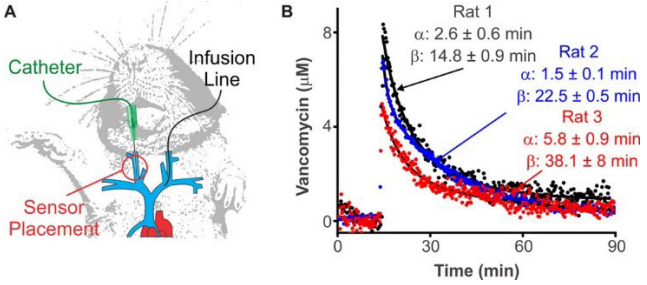
V. Goncales[1], A. A. Chamazketi[1], M. Harrison[1], K. Leung[1], J. Gerson[1], R. H. Batchelor[1], A. Farnkopf[1], A. Hodges[1], K. Plaxco[2]. *[1]Nutromics, Melbourne, Australia, [2]University of California Santa Barbara, Santa Barbara, CA*

Therapeutic drug monitoring (TDM) is a clinical practice involving quantification of plasma drug concentrations to adjust treatment efficacy and minimize toxicity in patient care. TDM is relevant for drugs with a narrow therapeutic index, defined as the plasma concentration range over which a molecule is therapeutically effective without causing significant adverse effects. For narrow therapeutic index drugs, indirect predictors of a patient's metabolism, such as age, body mass, or genotype, are insufficient to ensure effective dosing, and metabolic variability across different individuals needs to be considered. The importance of TDM is illustrated by the difficulty in treating a patient with vancomycin, an essential antibiotic used to combat gram-positive bacterial infections. The practice typically involves determining trough concentration,

peak concentration, or exposure to the vancomycin area under the curve, necessitating blood sampling. After sampling, the current practices require patient blood to be sent to a laboratory, which can only provide an answer from hours after sample collection.

Figure 1 - Real-time continuous monitoring of subject-specific vancomycin pharmacokinetics with electrochemical aptamer-based sensors. Reprinted with permission from. Copyright American Chemical Society

Electrochemical aptamer-based (E-AB) sensors provide significant opportunities to make TDM a practice as frequent and convenient as the measurement of blood sugar has become for people with diabetes. This is because E-AB sensing is a molecular approach to continuously measuring concentrations of specific molecules. We present the selection of an aptamer against vancomycin and its adaptation into an E-AB sensor. Using this sensor, we then demonstrate the opportunities for rapid (seconds) measurement of plasma vancomycin and the real-time measurement of subject-specific vancomycin pharmacokinetics on rats (Figure 1) [3]. Such capability provides a route toward improving TDM and the personalized delivery of pharmacological interventions.



B-302

Increased False-Positives vs Increased Interference Flags: Using the AU 5800 to Compare Two Ethyl Glucuronide Assays

A. N. Jackson[1], S. P. Wyness[1], S. La'ulu[1], J. W. Rudolf[2], K. L. Johnson-Davis[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]University of Utah Health, Department of Pathology, Salt Lake City, UT*

Background

Ethyl glucuronide (EtG) is a metabolite of ethanol that can be detected in urine for up to three to five days following consumption of alcohol. Urine specimens are routinely screened for EtG to assess ethanol exposure for compliance and/or abuse with presumptive positive samples being confirmed using quantitative Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). LC-MS/MS is costly and labor intensive. Therefore, providing a sensitive and specific screening option for EtG in urine is beneficial, with it being more cost-effective, efficient, and widely available. Evaluation of the performance characteristics of different EtG assays can aid in identifying an assay that most closely agrees with LC-MS/MS.

Methods

To evaluate agreement, 100 residual urine specimens (approximately 50 positive/50 negatives identified using ARK Ethyl Glucuronide Assay) were thawed, mixed, and analyzed. Specimen were analyzed using ARK and Immunalysis Ethyl Glucuronide Urine HEIA on the Beckman Coulter AU 5800. Discrepant results were investigated using LC-MS/MS. Additionally, 15 false-positive samples (identified positive by ARK but confirmed negative by LC-MS/MS) were evaluated on both ARK and Immunalysis assays. Lastly, 7 samples were analyzed on ARK and Immunalysis because of the inability to quantitate by LC-MS/MS due to interfering substance. Results were evaluated using EP Evaluator and Microsoft Excel.

Results

Of the 100 samples analyzed, 8 samples were identified as discrepant between the two immunoassays. These 8 discrepant samples tested positive on ARK and negative on Immunalysis, resulting in an 83.3% positive agreement and a 100.0% negative agreement between ARK and Immunalysis. For these 8 discrepant samples, LC-MS/MS confirmed 7 samples negative and 1 sample positive suggesting that using ARK would result in 14.6% of presumptive positives confirming negative. Of the initial 100 samples, 7 samples resulted with interference flags by Immunalysis, indicating the presence of an unknown interfering substance within the sample. Of these samples with interference flags, 3 were among the 8 discrepant samples. Evaluation of identified false-positive samples (n=15) by ARK resulted in 90.9% negative agreement for Immunalysis compared to 0.0% negative agreement by ARK. Of these 15 false-positive samples tested, 3 had interference flags identified using Immunalysis. Between agreement samples (n=100) and false-positive samples (n=15), a total of 10 interference flags were identified by Immunalysis, 6 of which were observed to have discrepant results between ARK and Immunalysis. ARK identified no interference flags. Interference was confirmed for 7 samples using LC-MS/MS, with Immunalysis detecting interference in 57% of those samples, and ARK reported no interference flags. In total, LC-MS/MS results were obtained for 37 samples, of which, only 2 false-positive and 1 false-negative samples were observed using Immunalysis.

Conclusion

While the assays offered by ARK and Immunalysis are similar in that they screen for EtG, we observed the accuracy between the two assays to be quite different, when compared to LC-MS/MS. Using ARK reagents would result in greater number of presumptive positives. Of note, the instrument flags accompanying Immunalysis results appear to be indicative of an interfering substance that ARK is not detecting and then subsequently reports as (falsely) positive.

B-303

Comparison of Tacrolimus Concentrations Obtained by the Newly Approved Alinity i Tacrolimus Assay with a Reference Liquid-Chromatography combined with Tandem Mass spectrometry Assay

K. Woodard, T. Kisler, A. Dasgupta. *University of Kansas Health System, Kansas City, KS*

Background: Tacrolimus belongs to the calcineurin-inhibitor class of medications and used for preventing and maintenance of organ rejection in transplant recipients alone or in combination with other medications. Due to narrow therapeutic window and significant nephrotoxicity, this drug is routinely monitored with a suggested therapeutic range of 5-15 ng/mL. Recently, Abbott Laboratories (Abbott Park, IL) received FDA approval for using tacrolimus immunoassay on the Alinity i platform. We compared tacrolimus values in 101 transplant patients using Alinity i analyzer and a reference method based on liquid chromatography combined with mass tandem spectrometry (LC-MS/MS). **Methods:** Tacrolimus immunoassay on the Alinity i analyzer is a chemiluminescent microparticle immunoassay (CMIA) requiring pretreatment of whole blood specimen prior to analysis. The analytical measurement range of the assay is 2-30 ng/mL. The left-over blood specimens were sent to Eurofins-Viracor reference laboratory in Lenexa, KS for analysis using LC-MS/MS method. After liquid-liquid extraction, specimens were analyzed using ascomycin as the internal standard for the presence of tacrolimus (assay is capable of analyzing other immunosuppressants simultaneously). UPLC C-18 column (waters) was used for separation using two mobile phases (A: 2.0mM ammonium acetate in 0.1% formic caid in water and B: 2.0 mM ammonium acetate in 0.1% formic acid in methanol). Mass spectrometer was operated in positive ionization electrospray mode (tacrolimus m/z 821.6 >768.6, ascomycin m/z 809.756.6. **Results:** The total precision of the low control was 3.6% (mean: 4.48 ng/mL; CV 0.16 ng/mL, n=20) while the total precision of the high control was 5.1% (mean: 25.4 ng/mL, SD: 1.29 ng/mL, n =20). The linearity of the assay (2.0-30.0 ng/mL) was verified by running various calibrators as unknown. Comparing tacrolimus values in specimens from 101 transplant patients (49 kidney, 19 bone marrow transplant, 16 liver and 15 heart transplant) using the reference method (LC-MS/MS) as the x-axis and the corresponding values obtained by the tacrolimus immunoassay using Alinity I analyzer, we observed the following regression equation: y = 0.97 x + 1.005 (n =101, r = 0.95). For kidney transplant recipients, the regression equation was y = 0.96 + 1.20 (n =49, r = 0.94). **Discussion:** Excellent correlation between tacrolimus values obtained by the newly FDA approved tacrolimus immunoassay with the reference LC-MS/MS based assay indicates further improvement in the performance of immunoassays for therapeutic drug monitoring of immunosuppressants. This may be due to reduced cross-reactivity of metabolites as indicated in the package insert. **Conclusions:** The newly approved tacrolimus immunoassay for the application on the Alinity I analyzer can be used for routine therapeutic drug monitoring of tacrolimus in clinical laboratories.

B-304

Performance of Alternative Proficiency Testing Program for Bayesian Forecasting Tool of Infliximab Exposure in Clinical Pharmacokinetic Laboratory

S. Caonguyen, O. McLachlan, D. Pham, P. Hughes, T. Dervieux. *Prometheus Laboratories, San Diego, CA*

Background: We have developed a Bayesian (PK) forecasting and dosing tool for Infliximab (IFX), a monoclonal antibody targeting Tumor necrosis factor α. This novel

dosing tool employs machine learning and proficiency of the algorithm must be established. Our objective was to implement an alternative proficiency testing program (APT) in the routine clinical (PK) laboratory.

Methods: The test (PredictrPK® IFX) is validated and measures serum IFX and antibodies to IFX (ATI) concentrations using a homogenous mobility shift assay by size exclusion HPLC and albumin (ALB) by nephelometry. Determinations are inputted with weight, dose, and interdose interval provided on test requisition in the Bayesian forecasting PK tool which uses non-linear mixed effect to calculate the individual parameter estimates, Clearance and predicted trough concentrations. APT is conducted using five previously tested patient samples, blinded to testing personnel and retested in the same manner as the routine method of analysis every six months. APT consists of two components, a component evaluating standalone IFX, ATI, and ALB analytes, and a second component evaluating the Bayesian forecasting PK tool in estimating Clearance and trough concentrations. Criteria for acceptable performance is set at target value ± 20% for standalone analytes and forecasted PK estimates. Acceptable ATP score corresponds to 4/5 samples with passing grade.

Results: The results from two consecutive APT results with six months intervals is presented in Figure 1. Comparing the results to the original assayed data at the time of collection, the APT results showed IFX concentration, ATI and ALB within 20% of target value. Clearance and estimated trough concentrations calculated using the Bayesian forecasting tool also met acceptability criteria (100% passing grade).

Conclusion: We have implemented an APT for Bayesian forecasting tool in the clinical PK laboratory.

Figure 1. Proficiency Testing Results for IFX, ATI, Estimated IFX Clearance and Estimated IFX Drug Concentration at Trough

	Clinical Data			IFX (µg/mL)			ATI (U/mL)		
APT 1 Samples	Weight (kg)	Dose Total (mg)	Dosing Interval (weeks)	Measured Value	Target Value	Target Percent (%)	Measured Value	Target Value	Target Percent (%)
Sample1	52.2	600	8	12	13	7	< 3.13	< 3.13	N/A
Sample2	68.2	400	4	2	3	12	11	12	6
Sample3	59.6	600	8	26	26	2	< 3.13	< 3.13	N/A
Sample4	88	500	8	8	7	20	< 3.13	< 3.13	N/A
Sample5	60.3	300	6	10	9	9	< 3.13	< 3.13	N/A

	Albumin (g/dL)			Estimated IFX Clearance (L/day)			Estimated IFX conc. at Trough (µg/mL)		
APT 1 Samples	Measured Value	Target Value	Target Percent (%)	Computed Value	Target Value	Target Percent (%)	Computed Value	Target Value	Target Percent (%)
Sample1	4	4	17	0	0	2	11	11	0
Sample2	4	4	11	1	1	1	3	3	0
Sample3	4	4	5	0	0	0	35	34	3
Sample4	5	5	8	0	0	4	11	9	22
Sample5	3	3	3	0	0	4	5	5	0

	Clinical Data			IFX (µg/mL)			ATI (U/mL)		
APT 2 Samples	Weight (kg)	Dose Total (mg)	Dosing Interval (weeks)	Measured Value	Target Value	Target Percent (%)	Measured Value	Target Value	Target Percent (%)
Sample1	39.1	700	4	29	28	4	< 3.13	< 3.13	N/A
Sample2	64.4	700	4	49	49	1	< 3.13	< 3.13	N/A
Sample3	54.8	274	8	22	22	0	< 3.13	< 3.13	N/A
Sample4	43.6	436	6	14	12	17	< 3.13	< 3.13	N/A
Sample5	95	800	8	2	2	0	< 3.13	< 3.13	N/A

	Albumin (g/dL)			Estimated IFX Clearance (L/day)			Estimated IFX conc. at Trough (µg/mL)		
APT 2 Samples	Measured Value	Target Value	Target Percent (%)	Computed Value	Target Value	Target Percent (%)	Computed Value	Target Value	Target Percent (%)
Sample1	4	4	0	0	0	7	34	33	3
Sample2	5	4	0	0	0	2	54	55	2
Sample3	5	4	0	0	0	1	23	22	5
Sample4	5	5	0	0	0	6	18	15	20
Sample5	3	3	0	0	0	0	3	3	0

B-305

A Novel Dilute-n-shoot Method for Quantitating Isotonitazene and Desmetonitazene in Human Urine by LC/MS

P. J. Simms. *Silver State Diagnostics, LAS VEGAS, NV*

Background: Isonitazene is a benzimidazole derived opioid analgesic drug related to etonitazene, which has been sold as a designer drug. According to doctors isotonitazene has the potency of 20-200 times of fentanyl. The side effects of isotonitazene are like fentanyl and has been linked to many fatalities in Europe and the US. It has led to a number of overdoses through secondhand contact, by smelling or coming in contact with skin. It is slowly replacing fentanyl as the deadliest street drug in the US. We developed a dilute-n-shoot method that would test for isotonitazene and its metabolite metodesnitazene in human urine. This method used filtration to remove unwanted particulates and protein. It reduces the number of steps needed to prepare samples for liquid chromatography/mass spectrometry analysis compared to methods that require centrifugation. This assay allows for easier training of staff. In addition, it is easily adaptable to increase sample load. **Method:** Isotonitazene and metodesnitazene urine samples were prepared for LC/MS analysis by diluting them 1:20 in 0.1% formic acid in water. These samples were spiked with internal standard and filtered through a 0.2 µm Captiva 96-well filter plate. Isotonitazene and metodesnitazene concentrations were measured using LC/MS in the positive ion mode. The separation was obtained using a phenylhexyl UPLC column, 50 x 2.1mm, 1.8µm, along with a 50:50 acetonitrile/methanol/water formic acid gradient mobile phase. **Results:** The separation was completed in a proximately 4.5 minutes with good resolution between isotonitazene and metodesnitazene. Validation studies demonstrated that this method was linear from 2 to 100 ng/mL, with $r^2 \geq 0.990$ for both isotonitazene and metodesnitazene. Interday and intraday precision for both analytes were acceptable, with CV values < 5.0%. Accuracy measurements at 5, 25 and 80 ng/mL gave results where the percent difference was ≤ 20% for the 4 ng/mL concentration and ≤ 15% for the 25 and 80 ng/mL. A study of interfering compounds showed that there were no interferences that would affect the accuracy of isotonitazene and metodesnitazene. Recovery studies showed that > 90% of each analyte was recovered from the filtration step. The LOQ was determined to be 2 ng/mL and the LOD was determined to be 0.5 ng/mL. The reportable range was determined to be 2 to 200 ng/mL. The results of this validation will be discussed in detail in this poster. **Conclusion:** The method was linear and precise for both analytes over a range of 2 to 100 ng/mL. The method was reproducible and easy to use. Isotonitazene and metodesnitazene could easily be quantitated in human urine using this method.

B-306

Performance Evaluation of an Automated Assay for Measurement of Tacrolimus* on the ADVIA Centaur XP System

T. Chuang[1], R. Swan[2], O. Sankiewicz[2], N. Casson[2], S. Baxter[2]. *[1]Siemens Healthcare Diagnostics Inc., Tarrytown, NY, [2]Randox Laboratories Limited, Antrim, United Kingdom*

Background:

Tacrolimus (FK506) (PROGRAF) is a macrolide antibiotic of fungal origin (Streptomyces tsukubaensis). Tacrolimus is an inhibitor of calcineurin, a phosphatase that activates T-cell proliferation. At the cellular level, tacrolimus binds a family of binding proteins termed FK506-binding proteins (FKBPs). The correlation between steady-state trough concentration and area under the curve (AUC) provides reliable therapeutic monitoring for tacrolimus exposure at the trough level. The objective of this study was to determine the performance characteristics of the Tacrolimus assay on the ADVIA Centaur® XP System.

Methods:

The ADVIA Centaur Tacrolimus assay is a competitive immunoassay using direct chemiluminescent technology. Free tacrolimus in the patient sample competes with the bound tacrolimus in the solid phase for binding with the acridinium ester-labeled anti-tacrolimus antibody. The amount of tacrolimus present in the patient sample has an inverse relationship to the amount of relative light units detected by the system.

Results:

The ADVIA Centaur Tacrolimus assay demonstrated a mean %CV of 3.1% for intra-assay and 6.0% for total precision (across a sample range of 3.10 to 26.15 ng/mL). Close correlation to LC-MS/MS was demonstrated, with r, slope, and intercept of 0.957, 0.973, and 0.475, respectively. LoD and LoQ were 0.7 ng/mL and 1.9 ng/mL, respectively. Likewise, comparison to the Atellica IM assay resulted in r, slope, and intercept of 0.966, 1.09, and -0.508, respectively. Linearity was demonstrated across a range from LoQ to 30.0 ng/mL. Tacrolimus metabolite percent cross-reactivity ranged from 1.7 to 34.2%. A panel of potential interferants including the following were tested and found not to interfere (≤10%): cephalosporin to 100 µg/mL, erythromycin to 13.8 mg/dL, phenobarbital to 10 mg/dL, rapamycin to 5 µg/mL, sulfamethoxazole to 150 µg/mL, tobramycin to 3.3 mg/dL, trimethoprim to 4.2 mg/dL, bilirubin (conjugated) to 60 mg/dL, bilirubin (direct) to 60 mg/dL, and rheumatoid factor to 500 IU/mL.

Conclusion:

The resulting data demonstrates that the ADVIA Centaur Tacrolimus assay is an accurate and precise method of quantifying tacrolimus concentration in EDTA whole-blood samples. *Assay under development by Randox Laboratories Ltd. for Siemens Healthineers. Not available for sale. Future availability cannot be guaranteed.

B-307

Direct Quantitation of 77 Therapeutic and Clinical Toxicology Drugs in Dried Blood Spots using the Fully Automated Transcend DSX-1 System

J. Guo, C. Patterson, R. Gibson, K. van Natta, S. Samra. *Thermo Fisher Scientific, San Jose, CA*

Background: The dried blood spot (DBS) sampling technique is advantageous over the traditional liquid blood collection due to its minimal invasiveness, smaller sample volume, improved analyte stability, and ease of storage and transportation, resulting in its increasing usage in therapeutic drug monitoring and clinical toxicology. Here, we describe a fully automated workflow to rapidly extract and quantify a wide range of therapeutic drugs and drugs of abuse in DBS using the Thermo Scientific™ Transcend™ DSX-1 system, fulfilling the routine drug monitoring needs in the clinical laboratories.

Methods: Calibrators in blood are spotted on the DBS cards, analytes are extracted with the innovative flow-through desorption technology, and their corresponding internal standards (IS) are introduced via an automated IS addition module in the dried spot autosampler. The 2-dimensional TurboFlow technology allows the removal of interferences from the extracted samples before analytical separation. An integrated software controls every step of the sample desorption and separation. Analyte quantitation is performed on the Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer, and the data is analyzed in TraceFinder™ software.

Results: A total of 77 therapeutic and clinical toxicology drugs from eleven classes, including anticonvulsants, antidepressants, antihistamines, antipsychotics, benzodiazepines, cocaine, dissociatives, opioids, and stimulants, are quantified in a single injection from DBS cards using a rapid automated method on a Transcend DSX-1 system. DSX-1 combines a dried spot module for direct analyte extraction with Transcend™ UHPLC for online sample separation using the TurboFlow technology. The method only takes 4.3 minutes from analyte extraction to MS detection. Good calibration curves with $R^2 > 0.98$ are achieved using a weighting factor of 1/x, and the limit of quantification (LOQ) values are established with % RSD and % CV < 15, |% Diff| < 20, and relative ion ratio < %20. The LOQ values are all in the low ng/mL levels, which largely meet the screening and confirmation sensitivity needs of analytical methodologies in routine clinical laboratories.

Conclusion: A fully automated LC-MS-based workflow is developed to rapidly and reliably quantitate 77 compounds therapeutic and clinical toxicology drugs in dried blood spots.

B-308

Focal Adhesion Kinase Activation is Involved in Tension Development During Contractile Stimulation of Mouse Detrusor Smooth Muscle to Modulate Bladder Dysfunction-Related Lower Urinary Tract Symptoms

S. Maher[1], H. Kalil[2], M. Bayachou[2], G. Liu[3]. *[1]Cleveland Clinic, Cleveland, OH, [2]Cleveland State University, Cleveland, OH, [3]Case Western Reserve University, Cleveland, OH*

Background: The function of the bladder is to store and expel urine. Bladder dysfunction-related lower urinary tract symptoms (LUTS) is a common urological problem and negatively affects health-related quality of life. Investigation of detrusor muscle contraction mechanisms is necessary to understand its underlying pathophysiology. Voiding occurs through bladder muscle contraction, which is mediated primarily by the increased intracellular Ca^{2+} concentration-induced myosin regulatory light chain (MLC) phosphorylation. Recent studies showed that focal adhesion kinase (FAK)-regulated focal adhesion assembly and actin polymerization are involved in vascular and airway smooth muscle contraction. However, the role of FAK activation in visceral smooth muscle contraction is not well documented. This study aims to determine if FAK activation is involved in mouse detrusor smooth muscle contraction.

Methods: Normal adult male and female mouse bladders were harvested. The trigone and dome areas were cut, and the bladder body was used. The mucosa was carefully removed. Detrusor muscle strips (1.5-2 × 8-10 mm) were prepared and then suspended in a 20 ml tissue bath filled with physiological Krebs solution and bubbled with 95% O_2 and 5% CO_2 to obtain a pH of 7.4 at 37°C. The isometric contraction was recorded. After establishing a stable baseline, the strips were stimulated with 120 mM potassium chloride (KCl), which was used to normalize the contractility induced by subsequent stimuli. One of the following specific inhibitors or the same volume of vehicle (DMSO) was applied and incubated for 30 min: PF-573228 (2 μM, a FAK inhibitor) or latrunculin B (1 μM, an inhibitor of actin polymerization). Then, the contractile responses to KCl (90 mM), electrical field stimulation (EFS, 2-32 Hz), or carbachol (CCh, $10^{-7.5}$-$10^{-4.5}$ M) were measured. In a separate experiment, the detrusor strips were stimulated with CCh (10 μM) after incubation with PF-573228 (2 μM) or vehicle (DMSO) for 30 min. Once the peak contraction was reached, the strips were snap-frozen for immunoblotting analysis of the phosphorylated FAK (p-FAK) and MLC (p-MLC). Statistical analysis was performed (GraphPad Prism 6). The contractile responses to KCl and EFS between vehicle and inhibitor groups were compared using a paired t-test. Nonlinear regression was used to compare CCh-induced dose-response curves. For the expression of phosphorylated proteins, one-way ANOVA, followed by Tukey's posthoc test, was used for analysis.

Results: KCl-induced contractile responses decreased significantly after incubation with PF-573228 or latrunculin B compared to the corresponding vehicle group ($p<0.0001$). The contractile responses induced by EFS were significantly inhibited by preincubation with PF-573228 at 8, 16, and 32 Hz ($p<0.05$) or latrunculin B at 16 and 32 Hz ($p<0.01$). Following the application of PF-573228 or latrunculin B, CCh-induced dose-response contractions were significantly lower than those in the corresponding vehicle-treated strips ($p=0.0021$ and 0.0003, respectively). Immunoblotting analysis demonstrated that CCh stimulation caused increased expression of p-FAK and p-MLC, whereas preincubation with PF-573228 led to decreased expression of p-FAK but not p-MLC

Conclusion: **:** FAK activation is involved in tension development induced by contractile stimulation in mouse detrusor muscle, probably through enhancing actin polymerization, but not MLC phosphorylation.

B-309

Development and Validation of a LC-MS/MS Method for Highly Concentrated Tacrolimus and Cyclosporine Specimens Prepared from Clinical Pharmaceutical Products to Assess Adsorption by Feeding Tubes

Y. Xiao, E. Leung. *Children's Hospital Los Angeles, Los Angeles, CA*

Background: Tacrolimus and cyclosporine are common immunosuppressive drugs used in the prevention and treatment of solid-organ transplant rejection (e.g., kidney, liver, heart). Other indications include several autoimmune diseases such as Crohn's disease, autoimmune hepatitis, and rheumatoid arthritis. To prevent toxicity while achieving therapeutic efficacy, tacrolimus and cyclosporine in whole blood specimens are commonly measured in medical centers so that their dosages can be precisely adjusted. Different from adult patients, pediatric patients often require smaller doses and the use of feeding tubes for drug administration. In order to assess nonspecific adsorption by feeding tubes, we modified our in-house clinical method to measure highly concentrated tacrolimus and cyclosporine specimens prepared from clinical pharmaceutical products.

Methods: Highly concentrated tacrolimus and cyclosporine specimens were serially diluted with dimethyl sulfoxide (2,000 times for tacrolimus and 20,000 times for cyclosporine) before measurements by our clinical method using liquid chromatography mass spectrometry. The method for highly concentrated tacrolimus and cyclosporine was validated, with analytical measurement range determined with in-house prepared concentrated specimens at varying concentrations, precision (repeatability and reproducibility) assessed by multiple measurements of concentrated specimens at three different concentrations, accuracy assessed by recovery studies, and matrix effect assessed by post-column infusion and by matrix dilution with dimethyl sulfoxide.

Results: The method was linear from 10 ug/mL to 80 ug/mL for tacrolimus, and 1 mg/mL to 8 mg/mL for cyclosporine with $r^2 > 0.99$. Repeatability and reproducibility were <10% CV. Minimal matrix effect was observed. Recovery studies using commercial calibrators to measure specimens prepared from reference materials and dimethyl sulfoxide identified no significant bias for tacrolimus but an average positive bias of 20% for cyclosporine, and a correction factor was used to finalize cyclosporine results for specimens prepared from clinical pharmaceutical products. The positive bias was eliminated when calibrators were prepared in dimethyl sulfoxide.

Conclusion: We have developed and validated a LC-MS/MS method for the measurement of highly concentrated tacrolimus and cyclosporine in specimens prepared from clinical pharmaceutical products. We hypothesize that one or more components in the commercial calibrators have contributed to the positive bias of cyclosporine in those specimens. Our method can be used to support studies that assess nonspecific adsorption by feeding tubes.

B-310

High Through-put Online Solid Phase Extraction of Immunosuppressive Drugs from Whole Blood on Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) in a Tertiary Hospital Laboratory

J. Wong, C. J. Gea, G. J. Goh, C. P. Yeo. *Singapore General Hospital, Department of Clinical Pathology, Singapore, Singapore*

Background:

Cyclosporine A and Tacrolimus are immunosuppressive drugs that are commonly used in solid organ transplant and are characterized by a relatively narrow therapeutic index, potential drug interactions and significant intra- and inter-individual variability. Therapeutic drug monitoring is therefore beneficial for optimizing dosage regimens, preventing rejection of the graft and reducing toxicities in transplant recipients receiving immunosuppressive drug therapy. A commercial assay (RECIPE ClinMass LC-MS/MS Advanced kit) for simultaneous measurement of immunosuppressants in whole blood using LC-MS/MS methodology has been adapted for assay performance evaluation in our laboratory on the Agilent 1290 Infinity II liquid chromatograph coupled to an Agilent 6470 triple quadrupole mass spectrometer. Our study evaluated the analytical performance of Cyclosporine A and Tacrolimus on the RECIPE assay and compared it against our existing in-house LC-MS/MS assay.

Methods:

The RECIPE ClinMass® LC-MS/MS Advanced Immunosuppressants assay kit consist of mobile phase solvents, precipitation reagents, stable isotopic labeled internal standards, solid phase extraction and analytical columns, lyohilized whole blood calibrators and quality controls. Drug analytes were extracted from whole blood using single-step protein precipitation followed by on-line solid phase extraction on the LC, eluted on isocratic chromatography and detected on positive ESI MS ionisation mode. The RECIPE assay run time is under 3 minutes. Performance validation parameters included within/total assay imprecision, linearity, recovery, limits of detection (LOD)/quantification (LOQ) and carry-over. Method correlation studies using fresh patient whole blood specimens (n = 212 Cyclosporine A; n = 120 Tacrolimus) were performed on existing in-house assay on Agilent 6460 LC-MS/MS and the RECIPE assay on Agilent 6470 LC-MS/MS systems.

Results:

Within run and total imprecision for Cyclosporine A and Tacrolimus on the RECIPE assay were determined to be ≤4.4% and ≤7.5% on the Agilent 6470 LC-MS/MS. The assay demonstrated linearity across the analytical measurement range up to 1500 ug/L for Cyclosporine A and up to 55 ug/L for Tacrolimus. Analytical recoveries obtained for both drugs ranged between 93-100%. The LODs were assessed to be 13.0 ug/L and 0.70 ug/L for Cyclosporine A and Tacrolimus respectively. LOQs were assessed to be 22.7 ug/L and 1.30 ug/L for Cyclosporine A and Tacrolimus respectively. Results of carry-over studies were insignificant. Participation in the CAP Immunosuppressive Drugs External Quality Assessment Survey showed good agreement with LC-MS/MS peers. Method correlation with in-house assay demonstrated good agreement: (1) Cyclosporine A: Passing Bablok regression RECIPE = 0.94 (In-house) + 17.2, Bland Altman mean bias of +2.0%, Spearman correlation coefficient of 0.985; (2) Tacrolimus: Passing Bablok regression of RECIPE = 1.03 (In-house) - 0.2, Bland Altman mean bias of -0.2%, Spearman correlation coefficient of 0.988

Conclusion:

The RECIPE ClinMass® LC-MS/MS Immunosuppressants assay performed within manufacturer's specifications except for LOD/LOQs which were determined at higher concentrations. The assay is adaptable to contemporary LC-MS/MS systems and confer benefits from rapid turnaround time (< 3 minutes), simplified specimen preparation procedures and ready prepared reagents. The advantages allow enhancements in workflow efficiency and ease of staff training in our laboratory and the assay is suitable for routine clinical use in high workload clinical laboratories.

B-311

Increasing Xylazine Findings in Clinical Samples

A. Reinhart. *NMS Labs, Horsham, PA*

Background: Xylazine is a veterinary tranquilizer first appearing in illicit fentanyl preparations in 2019. It is believed to extend a fentanyl high and is now more commonly used as its own drug of abuse. Clinically, xylazine is of concern as it does not respond to naloxone or other antidote during overdoses. There are many forensic findings of increasing xylazine use since its original appearance, but little in the literature concerning clinical xylazine findings, possibly because xylazine is rarely screened in routine drug screening. We sought to find clinical cases with positive xylazine results from 2019-2022. Determined data included demographics (where possible to discern), sex distribution, age ranges, and frequency.

Methods: We queried our laboratory information management system (LIMS) for reported xylazine findings under expanded clinical drug screen utilizing liquid chromatography time of flight mass spectrometry (LC-TOF) for screening and liquid chromatography tandem mass spectrometry (LC-MS/MS) for confirmation, and specific xylazine testing utilizing LC-MS/MS.

Results: In 1928 clinical cases reported between January 2019 and January 2023, the xylazine positivity rate increased from 1.28% (2021; n = 13 positive xylazine results) to 5.05% positivity (2022; n = 29 positive xylazine results) despite a significant decrease in panels and tests with xylazine ordered (n = 1008 in 2021, n = 574 in 2022). The ages of the patients ranged 0-57 years; eleven cases concerned pediatric patients. Of cases that indicated the sex of the patient, 69% were male (n = 29). These cases were found in ten different states (AZ, CT, DE, IN, MA, NJ, OH, PA, UT, VA), with 18 incidental findings. While other drugs were present in incidental finding cases, all cases with an incidental positive xylazine result were also positive for fentanyl and norfentanyl, and several cases were also positive for fentanyl analogues, most commonly 4-ANPP, which occurred in 50% of these cases.

Conclusion: Xylazine use continues to be on the rise and should be of concern for emergency medicine doctors, poison control centers, and clinical laboratories.

B-313

Aberrant lithium results after hdl assay on roche cobas c503 instrument

A. Aravamudhan, C. Mills, D. Li, P. Muluhngwi. *University of Minnesota Medical Center Fairview, Minneapolis, MN*

Background: Lithium is prescribed clinically to manage mania in patients with bipolar disorder. However, blood levels higher than 1.5 mmol/L (12 hours post administration) indicate toxicity accompanied by severe nausea and vomiting, severe hand tremors, confusion, and vision changes. If neglected, lithium toxicity can lead to coma, brain damage, or even death. Diligent monitoring of blood lithium levels periodically is critical to preventing lithium toxicity. *Case Presentation*: A 37 y/o female with schizophrenia and bipolar disorder on lithium since 2019 visited the outpatient clinic for her routing biannual lithium check. The patient's plasma lithium levels were detected at 3.9 mmol/L (previously 0.4mmol/L; Therapeutic range: 0.60 - 1.20 mmol/L; Toxic: >2.00 mmol/L). The cause for elevated lithium level was unclear; EKG and vital sign were unremarkable and patient was asymptomatic for toxicity. Nevertheless, patient was then managed with IV infusions and admitted to emergency room for follow up. A redrawn lithium sample ~22 hours later was 0.3 mmol/L, suggesting no toxicity. We then investigated the discrepancy in lithium levels reported by the laboratory. Retesting of the original sample detected lithium at 0.5 mmol/L, a value significantly less than the previously reported 3.9 mmol/L. Our laboratory had recently introduced the Roche Cobas® pro integrated solutions into the lab and chemistry testing (including Lithium) are performed on the Roche Cobas c503 analyzers (an arm of the Roche pro integrated solutions). Our goal was to investigate the observed phenomenon and find potential solutions, especially as it had significant implications to patient care. **Method:** We reviewed all high lithium levels recently tested our new instrument within a week of this incident. Seven samples (lithium values >1.2 mmol/L) were identified and lithium measurements repeated. We also investigated and ruled out the possibility of carry over contamination by rerunning a low lithium sample before and after a high lithium sample. **Results**: Retesting all seven samples resulted in lower lithium level with an average % change of 365%. Further investigations and discussions with manufacturers alluded to interference after HDL testing on the same instrument. To investigate this hypothesis, we reran two previously reported low samples (0.3 and 0.6 mmol/L) immediately after running the HDL cholesterol assay on the Roche Cobas c503 analyzer. Retesting resulted in lithium levels of 7.32 and 1.36 mmol/L (2313.3 % and 126.7 % change) respectively. **Conclusion**: Our investigation suggests that running the HDL cholesterol assay prior to the lithium assay on the Roche Cobas c503 analyzer results in positive interference. As oppose to other Roche chemistry analyzers (such as the Roche c501), the Roche Cobas c503 analyzer has no wash step between the HDL cholesterol and the lithium assays. Detergents in buffers and reactive byproducts (like peroxide) of the HDL cholesterol assay, may leave behind salts in the instrument probe that can interfere with the lithium assay. We are currently working on adding a wash step between the HDL cholesterol and lithium assays. In the interim, we are repeating lithium testing on high lithium samples.

B-314

Evaluation of Everolimus QMS Immunoassay on Roche Cobas c502 and Indiko Plus Analyzers

V. Buggs, L. Song, I. Metushi. *Department of Pathology and Laboratory Medicine. University of California Los Angeles, Los Angeles, CA*

Background: Everolimus has a narrow therapeutic window and significant intra-individual variability. Rapid turn-around-time and precise results are critical for therapeutic drug monitoring and adequate dose adjustment. The Thermo Scientific Quantitiative Microsphere System (QMS®) everolimus immunoassay is the only FDA-approved immunoassay for measuring everolimus in human blood. The analytical performance of the Thermo Scientific QMS® everolimus immunoassay on Indiko Plus and Roche c502 platforms were evaluated and compared to LC-MS/MS. **Methods**: Whole blood EDTA-anticoagulated patient samples were pretreated with methanol and precipitation reagent (immunoassay) or zinc sulfate/methanol (LC-MS/MS) to remove proteins before analysis. Imprecision was determined by running quality control and patient samples 20 times. Fifty transplant samples were used for method comparison. Deming regression, correlation coefficient (R^2), and % bias plot analysis were performed using EP Evaluator® software. **Results**: Imprecision (%CV) for QMS everolimus assay on Roche c502 was 16.7% (mean = 4.4 ng/mL) and 9.7% (mean = 10.3 ng/mL) for patient pools, and 16.5% (mean = 4.7 ng/mL), 10.3% (mean=7.8 ng/mL), 10.7% (mean = 14.5 ng/mL) for quality control. QMS everolimus assay imprecision on Indiko Plus platform had CV of 3.1% (mean = 4.3 ng/mL), 3.3% (mean = 9.3 ng/mL), 2.8% (mean 14.3 ng/mL) for liver transplant patients and 3.3% (mean = 4.2 ng/mL), 3.3% (mean 8.3 ng/mL), 3.1 % (mean 15.6 ng/mL) for quality control. Patient comparison studies between Roche c502 vs. LC-MS/MS revealed a R^2 of 0.82, slope of 1.3 and %bias of -2.7%. QMS on Indiko Plus vs. LC-MS/MS revealed a R^2 of 0.84, slope of 0.96 and %bias of -21.9%. **Conclusions**: The Indiko Plus platform had a constant negative bias vs. LC-MS/MS while the Roche's platform had lower average bias but it resulted in greater scatter in the therapeutic range (3-8 ng/mL) vs. LC-MS/MS. Overall, we observed increased imprecision on the c502 analyzer.

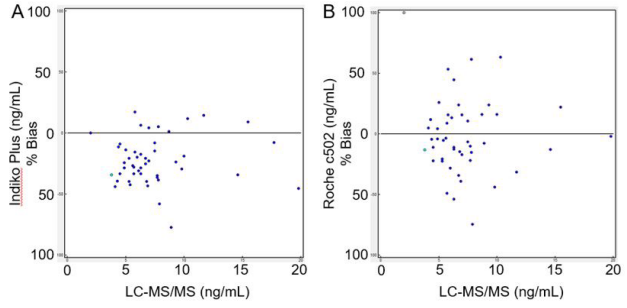


Figure 1: Percent bias plots for Everolimus QMS, A) Indiko Plus, and B) Roche Cobas c502

B-315

Investigation Into Extraction Procedure Substitution In The Abbott Architect i2000 Tacrolimus, Sirolimus, and Cyclosporine Assays

A. Chandras, D. R. Mendu, D. C. Kirchhoff. *The Mount Sinai Hospital, New York, NY*

Background: Tacrolimus, cyclosporine, and sirolimus are commonly used immunosuppressive drugs with narrow therapeutic windows requiring consistent monitoring to avoid drug toxicity. The Abbott Architect i2000 tacrolimus, sirolimus, and cyclosporine assays each require a pre-analytical whole blood lysing extraction step prior to analysis on the i2000. This extraction is largely manual, requiring the combination of an extraction reagent with the patient sample, followed by an incubation and centrifugation/pelleting steps, after which, the resulting supernatant is assayed for drug concentration. Each assay has its own unique extraction reagent and incubation steps, albeit all three procedures are quite similar. A clinician-requested investigation into a questionably elevated tacrolimus level led us to investigate the effect of substituting extraction reagents and procedures between the three assays. In this study, we investigated the effect of substituting extraction procedures and reagents on assayed immunosuppressive drug values between the Abbott tacrolimus, sirolimus, and cyclosporine immunoassays.

Methods: Whole blood was obtained from three patients who had previously had either their tacrolimus, sirolimus, or cyclosporine level assayed within the normal therapeutic range. For each patient, the cyclosporine, tacrolimus, or sirolimus level was re-assayed in triplicate, with the substitution of the other two assay's extraction procedures performed prior to analysis. Such as: tacrolimus extraction was performed, then assayed for sirolimus or cyclosporine. This procedure was repeated for all three drugs and extraction procedures, affording 9 trials in total. The procedure was repeated with three samples obtained from patients with no measurable immunosuppressive drug levels. Additionally, cross-reactivity in the assay extraction reagents was assessed by measuring, in triplicate, immunosuppressive drug concentration on each extraction reagent without the addition of patient whole blood.

Results: Analysis in patients undergoing immunosuppressive drug therapy afforded the following changes in assayed drug concentration: Tacrolimus substituting sirolimus, cyclosporine extractions, 5.60 to 8.13, 8.47 ng/mL; sirolimus substituting cyclosporine, tacrolimus extractions 13.63 to 4.60, 8.07 ng/mL; cyclosporine substituting sirolimus, tacrolimus extractions 274.6 to 391.3, 757.30 ng/mL. Analysis in patients with no measurable drug content resulted in no change in assayed drug concentration with exception of tacrolimus, in which tacrolimus substituting sirolimus, cyclosporine extractions afforded <1.50 to 4.40, 4.00 ng/mL. Cross-reactivity trials yielded the following drug concentrations: tacrolimus precipitant: tacrolimus: >30.00, cyclosporine: >1500, sirolimus: 5.97 ng/mL; cyclosporine precipitant: cyclosporine: >1500, tacrolimus: 7.13, sirolimus 1.53 ng/mL; sirolimus precipitant: sirolimus: 5.00, tacrolimus: 8.67, cyclosporine: <25.00 ng/mL.

Conclusions: The use of incorrect extraction reagents and procedures resulted in both increased and decreased assayed immunosuppressive drug concentrations. These changes in drug concentration were observed in both samples containing, and not containing, immunosuppressive drugs. Significant cross-reactivity was observed in all three assay extraction reagents resulting inappropriately elevated assayed drug concentrations in all three assays. This highlights the need for comprehensive technologist training and effective pre-analytical workflows to prevent errors, such as reagent substitution, from occurring. This study has clinical implications into investigations of spurious immunosuppressive drug values, and substitution of assay extraction reagents and procedures should be investigated when performing root cause analysis into incorrectly reported immunosuppressive drug concentrations.

B-316

Retrospective Data Analysis Reveals Unusual Metabolism Pattern of Ethanol in Pediatrics as Compared to Adult and Geriatric Populations

K. Baryeh[1], R. Zhou[2], L. M. Zuromski[3], G. A. McMillin[1], M. D. Krasowski[2], K. L. Johnson-Davis[1]. *[1]University of Utah Health, Salt Lake City, UT, [2]University of Iowa, Iowa City, IA, [3]ARUP Laboratories, Salt Lake City, UT*

Background: Ethanol is utilized in many pharmaceutical formulations and in the treatment of methanol poisoning. It is also consumed recreationally and is the most abused drug globally. About 95% of consumed ethanol is metabolized by alcohol dehydrogenase into acetaldehyde, followed by conversion of the acetaldehyde into acetic acid by acetaldehyde dehydrogenase. Less than one percent is metabolized via nonoxidative pathways, which may include: glucuronidation, sulfation, and the formation of fatty acid esters of ethanol. In neonates the glucuronidation pathway has been reported to be underdeveloped and matures with age. In this work, the patterns and concentrations of phase II nonoxidative metabolites of ethanol, ethyl glucuronide (EtG) and ethyl sulfate (EtS), were assessed in random urine collections of pediatric, adult, and geriatric patients.

Methods: Test results (n= 63498) from urine samples tested for EtG and EtS by quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) at our facility were utilized for this study. For both analytes, the lower limit of positivity was 100 ng/mL. The age range under consideration was 0 - 100 years. Data was partitioned into age groups: 0 - 17 years, 18 - 80 years and 81 - 100 years. The EtG and EtS concentrations across the partitions were compared.

Results: Across all ages, 60 - 65% of patients had both EtG and EtS present in urine. Between 5 - 10% had EtG present without EtS, and 25 - 35% of patients had neither of the ethanol metabolites present. In all age groups there was a low positivity rate of EtS without the presence of EtG (0-1%). All age partitions showed a similar pattern with the highest percentages of patients showing the presence of both metabolites, and the lowest percentage of patients showing the presence of EtS only. Markedly, there were no pediatric patient samples that had the sulfated metabolite present in the absence of the glucuronidated metabolite; this was statistically significantly different than this proportion in adults (Fisher's Exact test, p=.025). This finding suggests greater glucuronidation activity in the pediatric population

Conclusion: From the data presented in this work, EtG is more prevalent relative to EtS in urine samples of patients assessed for ethanol exposure. This work also shows

that glucuronidation may be the predominant pathway for ethanol metabolism in pediatric populations. Although the level and frequency of ethanol exposure, as well as the time between exposure and sampling were unknown for the patients studied, the data offers insights into the relative distribution of EtG and EtS in random urine samples for pediatric and adult populations.

B-317

Poor Standardization of Specimen Validity Tests Leads to Significant Variation of Urine Adulteration Positivity Rates

E. M. Fatica[1], L. R. Ksiazek[2], C. D. Koch[2]. *[1]University of Cincinnati, Cincinnati, OH, [2]Sanford Health, Sioux Falls, SD*

Background: Drugs of abuse testing is performed in multiple settings including pain management, pre-transplant evaluations, and employment screening. It is important to be able to accurately assess the integrity of urine specimens to detect efforts to mask the presence of drugs through dilution, substitution, or other means of adulteration (e.g., adding bleach or other chemicals). These detection methods, however, are not standardized and therefore adulteration detection performance may vary widely. The purpose of this study was to compare several specimen validity testing (SVT) methods.

Methods: Results for SVT testing performed in January 2023 were retrospectively reviewed for urine specimens submitted for occupational drug testing (ODT) or for which comprehensive drug screening (CDS) was ordered. Specimens screening positive or near the cut-offs for adulteration/dilution by UrineCheck®7 (UC7) test strips (creatinine, specific gravity (SG), pH, bleach, pyridinium chlorochromate (PC), nitrites, glutaraldehyde) or the Thermo DRI® (SG, pH, oxidants) and Beckman creatinine assays were included in the study. Additional testing by the Roche Cobas Pro SVT assays (creatinine, SG, pH, oxidants, nitrites, chromate), pH meter (Fisher accumet AB315) and SG refractometer (Reichert® Goldberg TS Meter) was performed (n=7 ODT, 22 CDS). Nitrite-positive specimens submitted for routine urine analysis (UA) (n=6) were also included to assess each assay's interference potential (i.e., false positive) from nitrites produced from urinary tract infections.

Results: Of the 244 ODT results, 5 were "dilute" and 7 were positive for bleach, PC and/or nitrites by UC7 test strips. There were 603 CDS results, of which 23 had a urine creatinine <20mg/dL but only 6 of these also had a low SG (<1.003) by DRI assays. Only one specimen was flagged for low pH by all methods except the DRI assay.

Of the 5 ODT specimens that were positive for bleach and PC by UC7, none exceeded the cutoffs when tested on the DRI oxidant (100µg/mL), Roche oxidants (200mg/L), or Roche nitrites (500mg/L) assays, however these specimens did have higher values than the other specimens in the ODT group for all assays. The UC7 assay was the most sensitive for oxidant detection although this study was not designed to differentiate true from false positive results. The Roche assay was the most sensitive to urinary tract infection-related nitrites although values were still well below the positive cutoff (Mean±SD=46.7±11.1mg/L). Surprisingly, pH had poor agreement among methods but good overall concordance. For the assessment of specimen dilution, creatinine values correlated well but specific gravity generally did not. Additionally, overall concordance was poor (14/35 specimens) when manufacturer-specific cutoffs were applied. Notably, cutoffs varied greatly between assays which likely contributed to the poor concordance more so than the variation in measured values.

Conclusion: These findings suggest that the choice of urine SVTs can have a significant impact on positivity rate due to the lack of standardization. While we have determined that sensitivity and specificity varies significantly between assays, further studies are needed to evaluate method accuracy and overall adulteration detection performance.

B-318

Development of Non-competitive Immunoassay System for Cyclosporine A Using Alpaca Naïve Library-derived VHH

S. Fukuya[1], M. Tanaka[1], Y. Hotta[1], Y. Kitamori[1], N. Kiyose[2], N. Miyazaki[2], N. Ise[1], K. Aoyagi[1]. *[1]Fujirebio inc., Tokyo, Japan, [2]ARK Resource Co., Ltd., Kumamoto, Japan*

Background: Cyclosporine A is a potent immunosuppressive drug. It has a narrow therapeutic index, and therefore the measurement of cyclosporine's blood concentration is essential to obtain optimal therapy. Cyclosporine A, a small cyclic polypeptide, is generally measured by competitive immunoassay, which is theoretically inferior to noncompetitive sandwich immunoassay in terms of sensitivity and specificity. In this study, we report a novel sandwich immunoassay for cyclosporine A using an alpaca naïve library-derived variable domain of heavy chain of heavy chain antibody (VHH) developed against an immunocomplex of cyclosporine A and anti-cyclosporine A mouse monoclonal antibody. **Methods:** We generated a VHH against an anti-cyclosporine A mouse monoclonal antibody complexed with cyclosporine A by alpaca naïve VHH phage display library. A bivalent tandem VHH was generated using the established VHH, and a new sandwich immunoassay for measuring cyclosporine A was constructed using the tandem VHH conjugated with alkaline phosphatase. A prototype fully automated sandwich immunoassay was developed on a fully automated chemiluminescence analyzer using magnetic particles immobilized with the mouse monoclonal antibody and the tandem VHH conjugated with alkaline phosphatase. The prototype assay was further developed by implementing an automated whole blood pretreatment process with strong denaturing agents. The performances of the prototype immunoassays were investigated. **Results:** The established VHH showed a highly specific reaction to the complex of cyclosporine A and the anti-cyclosporine A mouse monoclonal antibody, and a sandwich immunoassay for cyclosporine A was successfully developed using the VHH and the mouse monoclonal antibody. The tandem bivalent VHH exhibited higher reactivity to the complex than the monomeric VHH. The fully automated reagent prototype achieved a measurement range of 30 to 1,500 ng/mL. Measurement of cyclosporine A in whole blood samples by the prototype reagent showed a correlation coefficient of 0.96 with mass spectrometry, and a correlation coefficient of 0.95 with a commercial IVD reagent. **Conclusion:** We successfully developed a sandwich reagent prototype for cyclosporine A using VHH established by alpaca naïve VHH phage display library. This is the first report of a fully automated reagent for cyclosporine A measurement including an automated whole blood pretreatment process.

B-319

Drugs of Abuse: Correlation of Immunoassay Absorbance with Concentration by LC-MS/MS and False Negative Rates on the Roche Analyzer

L. Militello, P. P. Gqamana, V. Zhang. *University of Rochester, ROCHESTER, NY*

A common practice for drug abuse testing is to first screen using immunoassay (IA) then confirm using tandem mass spectrometry (LC-MS/MS) if the screening result is indeed positive. Immunoassays are qualitative and a positive result is based on an absorbance reading over a cutoff value specified by the manufacturer. Despite that, one method of investigating unexpected results is to check for an absorbance close to the cutoff value, as that may indicate an analyte concentration close to the limit of detection and the need for further investigation. Here we investigate the validity of this approach regarding potential false positives/false negatives by using quantitative LC-MS/MS assays. These explicitly model instrument response as a function of concentration and are expected to be more reliable than absorbance. This abstract aims to answer two questions: Is there a relationship between absorbance by IA and concentration of analyte as measured by LC-MS/MS that can be used in clinical interpretations? What is the false negative rate (FNR) by IA screening on the Roche automated analyzer?

We collected discarded patient urine samples that were screened for amphetamines (AMP), cocaine (COC) and THC to get a total of 10 negative and 10 positive samples of each analyte with a range of absorbance values, as well as preliminary data for benzodiazepines, opiates, and oxycodone. All samples were tested again 5 times in one day using a Roche Cobas c501 chemistry analyzer. The cutoff concentrations for the immunoassays are 500 ng/mL for AMP, 300 ng/mL for COC and 50 ng/mL for THC. In-house LC-MS/MS assays gave quantitative measurements with a limit of quantitation of 30 ng/mL for AMP and COC and 10 ng/mL for THC. The results obtained from LC-MS/MS were considered as reference values to determine the FNR of the IAs. The linear regression between absorbance and concentration was calculated in Excel using the average absorbance of each sample.

The amphetamine IA results showed a concordance with LC-MS/MS of 80%, a FN rate of 20%, and an R^2 of 0.80 between absorbance and concentration. The cocaine IA results showed a concordance with LC-MS/MS of 65%, a FN rate of 41%, and an R^2 of 0.24. The THC IA results showed a concordance with LC-MS/MS of 95%, a FN rate of 9%, and an R^2 of 0.94.

The amphetamine IA shows moderate agreement with LC-MS/MS and accurately detected 80% of the positive specimens. The absorbance and concentration show good fit to the regression model. The cocaine IA shows fair agreement with LC-MS/MS and has an LOD too high to detect the presence of drug in most specimens that were found positive by LC-MS/MS. The lack of fit to the regression model is due to specimens with very high concentrations of COC that do not cause a proportional increase in absorbance. The THC IA shows excellent agreement with LC-MS/MS and excellent fit to the regression model. These findings indicate the use of absorbance may be able to assist in test interpretation for specimens near the cutoff value.

B-320

Development of Two-Step Sandwich CLEIA Kit for Tacrolimus with Automated Sample-Pretreatment Process, LUMIPULSE Presto® iTACT® Tacrolimus.

A. Yamaguchi, S. Funakoshi, C. Iwashita, K. Baba, S. Sekiya, Y. Hirano, Y. Hotta, N. Ise, K. Moriyama, K. Umeda, A. Kaneko, K. Aoyagi. *FUJIREBIO Inc., Hachioji City, Tokyo, Japan*

Background:

Tacrolimus (The other name: FK506) is an immunosuppressive drug used for preventing rejection of allotransplantation and treating atopic dermatitis or rheumatoid arthritis. The complex consisting of Tacrolimus and FK506 binding protein binds to calcineurin and suppresses expression of Interleukin-2 (IL-2) and IL-2 induced immune response. Although tacrolimus is a useful immunosuppressive drug, this medicine causes serious side effects, such as renal dysfunction, opportunistic infections due to the excessive immunosuppression, and so on. Therefore, the measurement of the tacrolimus concentration in blood is required to obtain the maximum tacrolimus therapeutic effect without side effects. It is known that 95 percent of tacrolimus in blood localizes in the inside of erythrocyte, and this localization imposes cumbersome pretreatment process of whole blood. Almost all diagnostic reagents for tacrolimus need the manual whole blood pretreatment process that defaces the reliability of measurement values. And the existing diagnostic reagents often show the high cross-reactivity against the metabolite products of tacrolimus. To solve these inconveniences, we developed a highly-specific chemiluminescence enzyme immunoassay (CLEIA) for tacrolimus using a new automated sample-pretreatment process on a fully automated LUMIPULSE® L2400 analyzer. In this study, we report the basic performance of this new tacrolimus assay named as LUMIPULSE Presto® iTACT® Tacrolimus.

Methods:

LUMIPULSE Presto® iTACT® Tacrolimus is the two-step sandwich CLEIA with the on-board sample-pretreatment process. All evaluations were executed on LUMIPULSE® L2400 analyzer (FUJIREBIO INC.). The on-board sample-pretreatment process is performed simultaneously with the first immune-reaction in sandwich CLEIA, and the total reaction time is 20 minutes. Limit of detection (LoD) and Limit of quantification (LoQ), within-run precision, day-to-day reproducibility, linearity, endogenous interferences, anticoagulative interferences, tacrolimus metabolites interferences and the correlation with current tacrolimus assay were evaluated in LUMIPULSE Presto® iTACT® Tacrolimus.

Results:

Both of LoD and LoQ of LUMIPULSE Presto® iTACT® Tacrolimus were 0.12 ng/mL. Within-run precisions (% CVs) using three control samples were 0.4 - 2.5 %. Day-to-day reproducibility in five days were 0.4 - 1.9 %. We determined the linearity ranged from 0.29 to 48.80 ng/mL. Up to 19.7 mg/dL of conjugated bilirubin, 18.8 mg/dL of free bilirubin, 1620 FTU of chyle, 2000 mg/dL of triglycerides, 1000 IU/mL of RF, and 1010 ng/mL of HAMA, and 4 - 12 g/dL of proteins including albumin did not affect tacrolimus measurements. In addition, there was no influence by EDTA 2K and EDTA 2Na up to 10 mg/mL. The cross-reactivities of four tacrolimus metabolites (M-I, M-II, M-III, and M-IV) were respectively 0.4 - 1.0 %, 0.0 - 0.8 %, -0.7 - 0.2 %, and 0.7 - 1.6 %. Comparing to the existing chemiluminescence immunoassay (CLIA) with 128 specimens, the correlation coefficient was 1.0, and the slope calculated by Passing-Bablok method is 1.04. Comparing to the existing electrochemiluminescence immunoassay (ECLIA) with 137 specimens, the correlation coefficient was 1.0, and the slope calculated by Passing-Bablok method is 1.07.

Conclusion:

The fundamental performance of LUMIPULSE Presto® iTACT® Tacrolimus is satisfactory and the on-board sample-pretreatment process of this assay is expected to contribute to reducing the burden and inconvenience of laboratory personnel.

B-321

Development of Chemiluminescent Enzyme Immunoassay (CLEIA) for Detecting Methotrexate in Blood

E. Inagaki[1], K. Baba[2], y. Imai[2], A. Kaneko[2], K. Aoyagi[2]. *[1]FUJIREBIO Inc., Hachioji, Tokyo, Japan, [2]Fujirebio Inc., Hachioji, Tokyo, Japan*

Background: Methotrexate (MTX) is a folate metabolism antagonist used in the treatment of rheumatoid arthritis, psoriasis, and antineoplastic agents. Therapeutic drug monitoring (TDM) is required to avoid serious adverse reactions of high-dose MTX/leucovorin rescue therapy or in patients with possible delayed excretion due to renal dysfunction or other factors. Although there are many reagents for MTX measurement based on the immunoassay method, they have a narrow measurement range to 2 µmol/L. Therefore, high concentration samples often make users conduct dilution and retest. In addition, most reagents have a cross-reactivity rate of over 40% with 2,4-diamino-N10-methylpteroic acid (DAMPA), one of the metabolites of MTX, so users must take note in the interpretation of measurement results. We have established an antibody with high specificity against MTX, and have developed a fully-automated, chemiluminescence immunoassay for LUMIPULSE® L2400 system for detecting MTX in blood, Lumipulse Presto® Methotrexate, which can significantly improve the issues described above. **Methods**: MTX assay is a one-step competitive immunoassay on the LUMIPULSE® L2400 System (FUJIREBIO INC.), which requires 10 µL of sample. The resulting reaction signals are derived within 30 minutes / sample, and are proportional to the amount of MTX in the sample allowing quantitative determination of MTX in plasma or serum. **Results**: The detection limit of the assay was 0.019 µmol/L, and the limit of quantitation was 0.030 µmol/L. A 20-day precision study was performed during a 31-day period using two controls and three panel specimens, and within-laboratory precision showed CV ≤ 3.1 %. Linearity was demonstrated over the range 0.017 - 28.821 µmol/L. No interference was observed with unconjugated (≤20.1 mg/dL) or conjugated bilirubin (≤20.2 mg/dL), chyle (≤1770 FTU), hemoglobin (≤450 mg/dL), and RF (rheumatoid factor, ≤5522 IU/mL). The correlation coefficient and the regression slope of this assay and LC-MS/MS were 1.00 and 1.0, respectively (N=171). Compared to existing immunoassay reagents, the assay showed much lower cross-reactivity to DAMPA. **Conclusion**: The performance of our novel MTX assay using LUMIPULSE® L2400 system was satisfactory, suggesting it is useful for routine analysis. In particular, the measurement range is wide, 0.030 to 25.000 µmol/L, which greatly reduces the frequency of dilution and retest of high concentration samples. Used with the auto dilution system in LUMIPULSE® L2400, it is possible to measure almost all clinical specimen automatically. Because of the low cross-reactivity with DAMPA, it is expected to be utilized in patient receiving glucarpidase. **Acknowledgement:** Some of the samples were provided in accordance with “Guidelines for clinical measurement and diagnosis technology improvement project”. The guidelines are promoted by Tsukuba Medical Laboratory of Education and Research Center, and University of Tsukuba Hospital, Japan.

B-322

Assessment of MassTrak™ Immunosuppressant Calibrator and Control Sets: Confidence in your Calibrators

G. Hammond, S. Balloch, L. Calton. *Waters Corporation, Wilmslow, United Kingdom*

Background:

The analytical performance of the Waters MassTrak Immunosuppressant Calibrator and Quality Control Sets (IVD) were evaluated using a laboratory developed LC-MS/MS method, to analyze all four immunosuppressants in a single run.

Methods:

The MassTrak Immunosuppressant Calibrator and Quality Control Sets were used to quantify cyclosporine, everolimus, sirolimus and tacrolimus in human whole blood. Samples (50µL) were treated with zinc sulfate and acetonitrile containing internal standards. A water/methanol/ammonium acetate gradient was used with a Waters C18 HSS SB Column on a Waters ACQUITY™ UPL™ I-Class FL and Xevo TQ-S micro Mass Spectrometer with an injection-to-injection time of less than 2 minutes.

Results:

No system carryover was observed from high concentration samples. Precise quantification (≤20% CV, ≤15% bias) at concentrations equal to or lower than the lowest concentration calibrator was demonstrated. Total precision and repeatability (3 pools, 5 replicates, 5 days; n=25) were determined to be ≤7.1% CV. The method was linear over the measuring interval of 19.3-1500 ng/mL (cyclosporine) and 0.77-39 ng/mL (everolimus, sirolimus and tacrolimus). Addition of high concentrations of several endogenous materials did not affect quantification. External quality assurance samples for all drugs met the scheme acceptance criteria (cyclosporine: n=39, range 0-2658 ng/mL, mean % bias +1.0%; everolimus: n=35, range 0-21.9 ng/mL, mean % bias +0.9%; sirolimus: n=34, range 0-74.2 ng/mL, mean % bias -7.5%; tacrolimus: n=40, range 0-23.0 ng/mL, mean % bias -0.5%).

Conclusion:

Performance of the Waters MassTrak Immunosuppressant Calibrator and Quality Control Sets, with the developed method described provide good method performance. The accuracy data demonstrates confidence in the MassTrak Immunosuppressant Calibrator Set, providing harmonization across laboratories.

This method is an example of an application using the instrumentation, software and consumables described in this document. This method has not been cleared by any

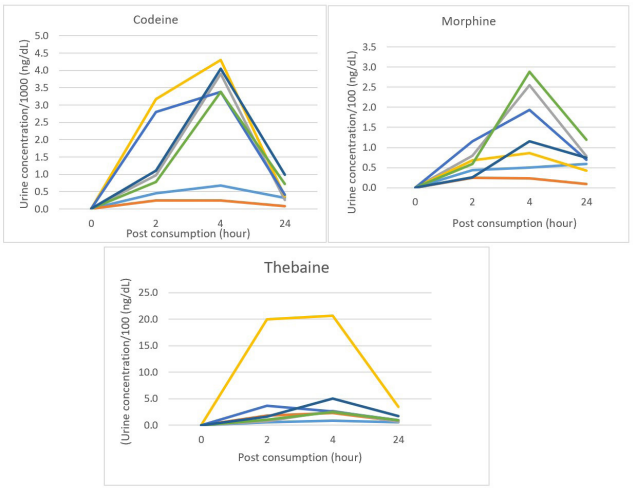
regulatory entity for diagnostic purposes. The end user is responsible for completion of the method development and validation. MassTrak Immunosuppressant Calibrator and Quality Control Sets are not available for sale in all countries.

B-323

Urinary Thebaine Improves Opioid Interpretation In Pain Management

N. Pourfarrokh[1], N. Nguyen[2]. *[1]Baylor Scott and White Temple, Temple, TX, [2]Baylor Scott & White Health, Temple, TX*

Background Poppy seed from Papaver somniferum plant is used in bakery results in positive urine test for Morphine and Codeine up to 48 hours. This leads to prescription compliance failure in the setting of clinical pain management. To mitigate misinterpretation, Thebaine testing was introduced to confirmatory opioid panel. Thebaine is naturally found in poppy seeds but not in prescription opiate formularies. A positive Thebaine result helps identify poppy seed consumption rather than from opioid prescription abuse. **Method** Each of the seven healthy adult volunteers (age 20-50) consumed one poppy seed filled kolache. Urine samples were then collected from each person at pre and post-consumed time points at 2, 4, and 24 hours. All samples were screened on Abbott Architect c16000 immunoassay (Abbott Park, IL) for opioids at 300 ng/mL cutoff. The samples were quantitated using ThermoFisher Vanquish Quantis LC-MS/MS (Waltham, MA) for Codeine, Morphine, and Thebaine at AMR 10-500 ng/mL, 10-500 ng/mL, and 5-250 ng/mL, respectively. Data acquisition/analyses were performed on TraceFinder software. The deuterated internal standards were purchased from Cerilliant Corporation (Round Rock, TX). All samples met calibrators and quality controls quality criteria. **Results** All post-consumption immunoassay results were positive for opioid. All LC-MS/MS positive results showed various levels of Codeine, Morphine, and Thebaine at 230-4300 ng/mL, 9-290 ng/mL, and 5-210 ng/mL, respectively (Figure 1). **Conclusion** Poppy seed consumption results in positive opioid class by immunoassay and positives Codeine, Morphine by LC-MS/MS. Detection of Thebaine in these samples would enable accurate clinical interpretation for poppy seed dieters and abusers of Codeine and Morphine prescription.



B-324

Comparison of Two Urine Fentanyl Assays on the Abbott Alinity c Analyzer

E. T. Laryea, S. Manning, J. H. Nichols. *Vanderbilt University Medical Center, Nashville, TN*

Background: Mass spectrometry (MS) remains the gold standard, however immunoassays can provide a preliminary test result while the patient is still in the emergency room or clinic.

Methods: The ARK Fentanyl II assay (ARK Diagnostics Inc, Fremont, CA) was compared to the Immunalysis SEFRIA Fentanyl (Immunalysis Corporation, Pomona, CA) assay on the Abbott Alinity c analyzer (Abbott Park, IL) using leftover deidentified urine samples. MS analysis was performed at ARUP Laboratories (Salt Lake City, UT) to quantitate the amount of fentanyl or norfentanyl metabolite in each sample. Medications prescribed at the time of sampling were reviewed to assess for possible cross reactivity with the assays.

Results: A total of 142 urine samples were analyzed with 70 MS positive and 72 MS negative samples. Positive and negative assay specific quality control precision (N=10) was 3.0% and 9.6% CV for the ARK Fentanyl II assay and 10.2% and 15.9% CV for the Immunalysis SEFRIA assays respectively. ARK Fentanyl II Sensitivity = 95.7%, Specificity = 94.4%, PPV = 94.4%, NPV = 95.8% and Efficiency = 95.1% compared to the Immunalysis SEFRIA Fentanyl Sensitivity = 95.7%, Specificity = 81.9%, PPV = 83.8%, NPV = 95.2% and Efficiency = 88.7%. Upon repeat analysis, 4 of 4 ARK and 6 of 13 Immunalysis false positives reported as negative, indicating some variability around the cutoff concentration. Possible carryover from high samples has also been reported (ARK Technical Note 20-001-August 2020) and high samples were analyzed prior to some of the false positive samples. Six MS positive samples were reported as negative (false negatives), 3 samples by each of the assays. However, 4/6 of those samples had combined fentanyl and norfentanyl concentrations < 3 ng/mL by MS.

Conclusion: The ARK Fentanyl II and Immunalysis SEFRIA assays demonstrated acceptable performance in the detection of fentanyl in our patient population.

Performance of Two Urine Fentanyl Assays

	ARK Positive	ARK Negative
MS Positive (N=70)	67	3
MS Negative (N=72)	4	68

	Immunalysis Positive	Immunalysis Negative
MS Positive (N=70)	67	3
MS Negative (N=72)	13	59

B-325

Retrospective Study Comparing Immunoassay Screen and Mass Spectrometry Confirmation Results in an Opioid Dependant Population

M. J. Bennett, J. Luong, D. W. Kinniburgh. *Alberta Centre for Toxicology, University of Calgary, Calgary, AB, Canada*

Background: Retrospective analysis of clinical laboratory data is employed to monitor the performance of instruments, evaluate test utilization and support operational changes. Urine drug testing in the context of opioid use disorder is used to assist in the management of patients. This testing is typically performed by an immunoassay screen followed by mass spectrometry confirmation. Immunoassay screens identify drug class and can cross-react with off-target substances to produce false-positive or false-negative results. Mass spectrometry confirmations provide more sensitive and specific results. The objective of this study was to utilize historical data to determine the concordance between immunoassay and mass spectrometry results from patients in an opioid dependency treatment program.

Methods: Urine drug screening results spanning a 3-year period (2020-2023) were mined to identify samples that screened positive for amphetamines, benzodiazepines, opiates, fentanyl/carfentanil, oxycodone/oxymorphone, and cocaine. These results were compared to the mass spectrometry confirmation results. The immunoassay results were collected on an Olympus AU480 instrument and the mass spectrometry results were collected using an in-house developed dynamic multiple reaction monitoring method on both Agilent 6470 and 6460 triple quadrupole instruments.

Results: Of the 15,850 urine drug records reviewed, 28.8% screened positive for amphetamines, 23.5% screened positive for benzodiazepines, 14.2% screened positive for opiates, 23.8% screened positive for fentanyl/carfentanil, 4.2% screened positive for oxycodone/oxymorphone, and 11.2% screened positive for cocaine. Of the amphetamine class samples that screened positive, 95.3% confirmed positive and 4.7% confirmed negative. Of the benzodiazepine class samples that screened positive, 50.8% confirmed positive and 49.2% confirmed negative. Of the opiate class samples that screened positive, 79.8% confirmed positive and 20.2% confirmed negative. Of the fentanyl/carfentanil samples that screened positive, 97.2% confirmed positive and 2.8% confirmed negative. Of the oxycodone/oxymorphone samples that screened positive, 58.5% were confirmed positive and 41.5% confirmed negative. Of the cocaine samples that screened positive, 99.8% confirmed positive and 0.2% confirmed negative.

Conclusion: Our findings determined there were discrepancies among all classes when comparing immunoassay versus mass spectrometry results. There was good agreement between immunoassay and mass spectrometry results in the detection of fentanyl/carfentanil, cocaine and amphetamines. The differences between methods were most significant in the benzodiazepine, opiate and semi-synthetic opioid classes.

This may be due to different cut-off levels between methods, specificity limitations of immunoassays and/or changing drug use patterns resulting in mass spectrometry multiple reaction monitoring false negative results.

B-326

Home collected oral fluid specimens are a viable option for telemedicine based toxicology testing.

J. P. Canner, F. H. Moonschi. *Gravity Diagnostics LLC, Covington, KY*

Background: A standard treatment for Substance Use Disorder (SUD) is Medication Assisted Therapy (MAT) consisting of a buprenorphine-naloxone combination medication. Typically, a urine specimen is collected during the in-office visit for the patient to receive MAT. Urine drug testing (UDT) ensures compliance and adherence with the treatment protocol and is often mandated for MAT. However, access to in-office visits and the ability to provide a specimen for UDT can be problematic for individuals undergoing MAT for SUD due to factors such as limited access to reliable transportation or distance to facilities. The COVID-19 pandemic has eased restrictions around telemedicine, including for the administration of MAT. Telemedicine can be a valuable tool to provide greater access to MAT for individuals by removing transportation barriers to treatment. Gravity Diagnostics partnered with various facilities to provide a telemedicine observed oral fluid toxicology (OFT) collection process. To understand if telemedicine OFT could provide the same information as in-office collected specimens, we compared telemedicine OFT to in-office OFT specimens and for one facility telemedicine-OFT to in-office UDT.

Methods: Gravity Diagnostics provides OFT through LC-MS/MS quantitative testing for over 72 analytes. Using a deidentified data set from our LIS, we identified OFT specimens that were collected either in-office or through telemedicine during a 6 month period. The ability to determine the effectiveness of telemedicine OFT was measured by examining the unexpected positivity rate of 72 analytes when compared to in-office OFT. We used 2-way ANOVA to determine if the detection rate of unexpected analytes between two collection methods were significantly different. Additionally, we identified a facility that conducted in-office UDT and telemedicine OFT on the same patient population. These data were compared in a similar manner with the unexpected positivity rate for 47 analytes subjected to 2-way ANOVA.
Results: When the unexpected detection rates between the in-office and telemedicine OFT were compared with a 2-way ANOVA, a p-value of 0.554 was found between the collection methods. This result indicated that the detection rates of unexpected analytes between the in-office and telemedicine collections were not significantly different. Similarly, when the unexpected detection rates between the UDT and the telemedicine-OFT were compared, a p-value of 0.391 was found between the collection methods. This result indicated that the detection rates between in office UDT and telemedicine OFT were not significantly different when compared within the same patient population.

Conclusion: Our data showed that telemedicine-OFT are comparable to in-office OFT for the detection of unexpected positive analytes. In addition, when the same patient population was tested by either telemedicine-OFT or in-office UDT, no significant difference in detection rates of unexpected positives is observed. Therefore, we are confident that we can utilize telemedicine-OFT to provide greater access to health care for patients undergoing MAT for SUD.

B-327

Evidence of Missed Novel Psychoactive Substances (NPS) in Unexpected Fentanyl Positives

G. Campbell, J. Schrecker, A. Holt, M. Hardison, R. Heltsley. *Aegis Sciences Corporation, Nashville, TN*

Background:

Recent data published by the CDC indicated that overdose deaths in the United States began to increase significantly in 2020 and continued throughout 2021. Synthetic opioids, such as illicitly-manufactured fentanyl (IMF), have been a primary driver in overdose deaths. There has been a concomitant increase in both the prevalence and diversification of novel psychoactive substances (NPS) and IMF. We performed a blinded secondary analysis of remnant samples to further characterize the incidence in which individuals are encountering NPS when using non-prescribed fentanyl. Various classes of NPS compounds (e.g., designer opioids, designer benzodiazepines, xylazine) were evaluated in samples that were originally positive for fentanyl but did not include a request from the provider for NPS testing to be performed.

Methods:

This study was IRB approved. A sampling of 200 urine or oral fluid specimens collected from individuals as part of their routine medical treatment, wherein definitive testing for fentanyl and norfentanyl was performed and the drug and metabolite were both identified above the upper limit of quantitation of the assay, underwent additional analysis for NPS. Samples were excluded from blinded secondary analysis if any NPS testing, regardless of drug class, was originally ordered and performed. Additional testing that was performed included the following classes of drugs: designer opioids, designer benzodiazepines, synthetic cannabinoids, synthetic stimulants, hallucinogens/dissociatives, xylazine, tianeptine, and phenibut.

Results:

Specimens within the sample set (N: 200) that were originally positive for fentanyl without a previous request for NPS testing originated from 30 different states within the US. These samples were originally collected between 9/19/2022-1/12/2023. Of those that underwent additional analysis after initial reporting of positive fentanyl results, 117 (58.5%) had at least one analyte within an NPS class identified. Designer opioids (N: 93, 47%), and xylazine (N: 76, 38%), followed by designer benzodiazepines (N: 16, 8%), synthetic stimulants (N: 4, 2%), and hallucinogens/dissociatives (N:3, 2%) were the most frequent compounds detected on a per sample basis. No synthetic cannabinoids were detected within this sample set.

Conclusion:

Although much of the national focus around fatal and non-fatal overdoses centers on intentional and unintentional exposures to IMF, healthcare providers and the general public must be made aware of the prevalence of NPS. These compounds, which are often intentionally added to drugs sold as fentanyl to enhance the drug potency, can increase risk for an adverse event. Of paramount importance is that the effects of substances such as xylazine and designer benzodiazepines, which contribute to central nervous system depression, are not effectively reversed by administration of naloxone, a mainstay of harm reduction protocols. Knowledge of the prevalence of these compounds should impact clinical decision-making and individual risk mitigation plans. From a broader perspective, improving understanding of the incidence in which these drugs are found within communities could improve overall surveillance efforts on current drug use trends.

B-328

Analytical Performance of a Screening Test for Lead using Dried Blood Spots and ICP-MS

C. M. Orahoske[1], W. Cieslak[2], D. M. Payto[2], E. Chegwidden[2], J. Colón-Franco[2]. *[1]Cleveland State University, Cleveland, OH, [2]Cleveland Clinic Foundation, Cleveland, OH*

Introduction: There is no safe blood lead levels (BLL) in children and exposure can cause irreversible neurological harm. In 2021, the CDC decreased the blood lead reference value (BLRV) to 3.5 ug/dL from 5.0 ug/dL, based on the 97.5th percentile of BLL among US children under 5 years of age. Despite this, lead poisoning disproportionately affects Black children, those below poverty levels, and living in houses built before 1978. The American Academy of Pediatrics recommends targeted screening for BLL in children aged 12-24 months living in high prevalence areas. Children enrolled in Medicaid also require screening by 24 months of age. Only one-third of children who require screening receive it and lead screening declined by >50% during the COVD-19 pandemic. Quality improvement strategies have included electronic health record alerts and point of care testing. To improve screening rates in our pediatric population, we developed a screening method utilizing dried blood spot (DBS) cards. This study aimed to validate the accuracy and precision of this screening method with emphasis on the performance around the BLRV cut-offs, which would be used to determine the need to follow up with a confirmatory venous lead test.
Methods: The method was calibrated using certified reference material traceable to NIST SRM 3128 (VHG labs, Manchester, NH, USA) in whole blood. The calibrators, controls and patient samples were spotted onto Whatman 903 cards. A 6mm filter paper disc in extraction buffer was vortexed, allowed to sit at room temperature for 30 min and centrifuged. The samples were analyzed on the Thermo Fisher iCAP RQ and TQ (Whaltman, MA) in kinetic energy discrimination mode. The assay has a linear range of 1-100 µg/dL, and the recovery ranged from 92-102%. We evaluated this method for reproducibility, and accuracy across the measurement range. In this study, we focused on the reproducibility and accuracy of DBS at lead concentrations *around the BLRV cut-offs.* We used in 2 samples spiked with NIST CRM 3128 (Inorganic Ventures, Christiansburg, VA, USA) analyzed 10 times over 3 days and 13 leftover patient samples run over at least 5 days. The total allowable error was 2 µg/dL or 10%. **Results:** In two samples spiked to BLL of 3.5 and 3.9 µg/dL, interday precision (n=10) ranged from 3.4% to 17.4% (SD 0.1-0.7) in samples run for 3 days. Removal of an apparent outlier decreased the %CV ranged observed to <8.5%. The average bias for each day ranged from 0.1 to 0.6 µg/dL. In 13 patient samples with concentra-

tions around the BLRV ranging from 1.4 to 5.8 µg/dL, the coefficient of variation (CV) ranged from 0.2% to 32% with the largest SD at 0.8 µg/dL. The average bias for this sample set was -0.3 (11.7%). **Conclusion:** The assay's analytical performance is sufficient for screening purposes, but the clinical performance and the impact of introducing this test to enhance screening rates during 1 and 2 years well visits need to be evaluated further.

B-329

Comparison between Trough and AUC Monitoring of MPA in Patients with and without Organ Transplantation. Influence of the Co-administered Immunosuppressant

D. Svinarov. *Alexander Hospital, Faculty of Medicine, Medical University of Sofia, Sofia, Bulgaria*

Background: Monitoring of mycophenolic acid (MPA) could be performed by measurement of trough concentrations with target therapeutic range of 1.3 to 3.5 mg/L, or by calculation of area under the drug concentration versus time curve (AUC) utilizing 3 or 4 samples within a dosing interval, with a target range of 30 to 60 mg*h/L. AUC is considered more reliable compared to trough levels, and MPA kinetics are dependent on the type of the co-administered calcineurine inhibitor. This study compares trough versus AUC MPA monitoring received alone or in co-administration with Cyclosporine (CsA) or with Tacrolimus (TAC).

Methods: 77 transplanted patients receiving MPA, 25 of them on CsA, 49 on TAC, and 20 non-transplanted patients on MPA alone, were evaluated more than once, yielding 260 monitoring consults included for assessment: 45 for MPA alone, 108 for MPA combined with CsA, and 117 consults for the MPA combined with TAC. MPA, CsA, and TAC levels were measured by validated LC/MS/MS methods. Validated pharmacokinetic programs for the calculation of MPA AUC were used, adapted to the type of calcineurine inhibitor. Assessment was made by comparison of the distribution and coincidence between trough levels and AUC with respect to the above therapeutic ranges.

Results: Transplanted patents: when combined with CsA, MPA AUC was in the target range in 61% of cases, under it - in 31%, and in 8% - over it, while trough levels were 52% under, 42.5% within, and 5.5% over the therapeutic range; agreement between trough MPA and MPA AUC was poor - there was coincidence in 37.5% of sub-therapeutic, 63% of therapeutic and 0% of supra-therapeutic ranges, 3 cases were identified with sub-therapeutic trough and supra-therapeutic AUC; when combined with TAC, MPA AUC was in the target range in 63% of cases, under it - in 13%, and in 24% - over it; trough levels being 23% under, 60% within and 17% over the therapeutic range; agreement between trough MPA and MPA AUC was much better, compared to the combination with CsA: there was coincidence in 56% of sub-therapeutic, 84% of therapeutic and 80% of supra-therapeutic ranges. Nevertheless, 1 case was found with sub-therapeutic trough and supra-therapeutic AUC. In non-transplanted patients MPA AUC was in the target range in 47% of cases, under it - in 13%, and in 40% - over it; trough levels being 18% under, 53% within and 29% over the therapeutic range; agreement between trough MPA and MPA AUC showed coincidence in 75% of sub-therapeutic, 75% of therapeutic and 92% of supra-therapeutic ranges.

Conclusion: This study confirms that MPA AUC is more relevant than trough monitoring for dose individualization. It also shows that MPA trough levels are much more concordant with MPA AUC in non-transplanted patients and in transplanted patients on TAC, while in combination with CsA, discordance remains significant.

B-330

Homogeneous Enzyme Immunoassay for Hydrocodone

K. Chung, M. Pham, T. Houts, R. O'Malley. *ARK Diagnostics, Fremont, CA*

Background: Hydrocodone is a semi-synthetic derivative of codeine and produces opioid-like effects similar to morphine. Hydrocodone (Trade names: Vicodin, Lortab, Hycodan, Vicoprofen) is one of the most frequently prescribed opioids in the U.S. as an antitussive (cough suppressant) and narcotic analgesic agent for the treatment of moderate to severe pain. Its ease of prescription and opioid-like effects has led to widespread drug diversion and abuse. The two commercially available homogeneous immunoassays for the detection of hydrocodone in urine at a cutoff of 300 ng/mL suffer undesirable cross-reactivity to oxycodone and morphine at concentrations below 25,000 ng/mL. ARK Diagnostics has developed the ARK Hydrocodone Assay to detect hydrocodone at a cutoff of 300 ng/mL with no cross-reactivity to oxycodone, morphine, and codeine at concentrations below 100,000 ng/mL.

Methods: The ARK™ Hydrocodone Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, with a cutoff of 300 ng/mL and semi-quantitative range up to 800 ng/mL. The performance of this assay was evaluated on the Beckman Coulter AU680 Automated Clinical Chemistry Analyzer. Precision, spiked recovery, specificity, Histogram Overlap Analysis of ±25% controls and the cutoff, and method comparison with LC-MS/MS were evaluated.

Results: In semi-quantitative mode, total precision ranged from 3.9% to 9.9%CV. Spiked recovery of hydrocodone ranged from 86.2% (720 ng/mL) to 102.9% (240 ng/mL). The active metabolite, hydromorphone, showed an approximate equivalence to the 300 ng/mL hydrocodone cutoff at 299 ng/mL (100.3% cross-reactivity). Histogram overlap analysis showed no overlap between cutoff and control levels. Method correlation with LC-MS/MS using authentic urine samples showed an excellent agreement with a specificity of 100% and sensitivity of 96.9% (98 positives and 128 negatives).

Conclusion: The ARKTM Hydrocodone Assay measures hydrocodone and its metabolite hydromorphone in human urine with acceptable performance. The assay is sensitive, rapid, and applicable to a wide range of clinical chemistry analyzers.

B-331

Homogeneous Enzyme Immunoassay for the Quantitative Determination of Methotrexate

J. Nguyen-Choi, S. Mun, D. Wolfson, B. Moon, T. Houts, R. O'Malley. *ARK Diagnostics, Fremont, CA*

Background: Methotrexate (MTX), a classical antifolate, can be safely administered over a wide dose range as maintenance chemotherapy for acute lymphoblastic leukemia and treatment of nononcologic diseases including rheumatoid arthritis or psoriasis. When combined with leucovorin (LV) rescue, high-dose MTX (HDMTX; doses of 1,000-33,000 mg/m2) is usually administered as a prolonged i.v. infusion for a variety of cancers, including acute lymphoblastic leukemia, lymphoma, osteosarcoma, breast cancer, and head and neck cancer. HDMTX can be safely administered to patients with normal renal function by vigorously hydrating and alkalinizing the patient to enhance the solubility of MTX in urine. Serum levels may reach 1000 µmol/L or more. Pharmacokinetically guided LV rescue by monitoring MTX serum levels is required to prevent potentially lethal MTX toxicity. Ability to measure MTX accurately at 0.050 µmol/L enables clinical determination of non-toxic status

Methods: The ARK™ Methotrexate II Assay is a homogeneous enzyme immunoassay for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The assay was evaluated on the Beckman AU680 analyzer. The assay consists of two reagents, six-level calibrators, and six-level (0.070, 0.400, 0.800, 5.0, 50.0, 500.0 µmol/L) quality controls. Performance of the assay was determined by assessing precision, limit of quantitation, linearity, endogenous substances interference, high sample dilution (1:10 serial dilutions), on-board auto dilution (at 1:10 and 1:50), cross-reactivity, and method comparison.

Results: Within-lab precision (%CV) for control levels were 3.00% (0.070 µmol/L), 1.40% (0.400 µmol/L), 2.05% (0.800 µmol/L), 1.58% (5.0 µmol/L), 2.30% (50.0 µmol/L), and 1.62% (500.0 µmol/L). Limit of Detection and Quantitation: LoD was 0.004 µmol/L and LoQ was 0.030 µmol/L (4.87% CV, 113.6% analytical recovery). The ARK Methotrexate II Assay showed less than 8% deviation from linearity over the range 0.030 to 1.300 µmol/L. Endogenous substances did not interfere with measurement of MTX at the levels tested. High sample manual dilution (2.0, 20.0, 200.0, 1200.0, 5.0, 50.0, and 500.0 µmol/L) and on-board auto dilution (1:10 and 1:50) results were within 10% of nominal values. Cross-reactivity to compounds (potentially co-administered drugs, folate derivatives, and compounds of similar structure) tested was <10% interference. The major metabolite, 7-hydroxy MTX, at 50 µmol/L produced less than 10% interference for MTX levels of 0.050 and 0.500 µmol/L. Passing Bablok analysis of the results for 90 patient specimens of ARK MTX II Assay compared to liquid chromatography with tandem mass spectrometry (LC-MS/MS) was 1.03 + 0.00 (r2 = 0.98).

Conclusion: The ARK™ Methotrexate Assay II quantitated methotrexate accurately and precisely in serum and plasma. Performance on the Beckman AU680 automated clinical chemistry analyzer demonstrated excellent precision, recovery, and clinical accuracy versus LC-MS/MS.

B-332

Evaluation of the Analytical Performance of Seven Therapeutic Drug Monitoring Assays on the Atellica® CI 1900 Analyzer

A. Bogard, T. Coward, K. Kolewe, P. Paudel, C. Tyler, G. Arrode-Bruses, J. Rhea-McManus, J. Snyder. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput integrated chemistry and immunoassay analyzer employing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica CH Vancomycin (Vanc), Carbamazepine (Carb), Gentamicin (Gent), Phenytoin (Phny), Tobramycin (Tob), Valproic Acid (VPA), and Theophylline (Theo) assays* on the Atellica CI 1900 Analyzer.

Methods: The Atellica CI 1900 Vanc, Carb, Gent, Phny, Tob, VPA, and Theo assays use the same reagents and calibrators as the Atellica chemistry assays. Precision and method comparison (MC) were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 using native and contrived human serum samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. MC studies were performed according to CLSI EP09-A3. Individual native and contrived human serum samples were analyzed using the Atellica chemistry assays on both the Atellica CH and Atellica CI 1900 Analyzers. Precision and MC were evaluated with three reagent lots on one system.

Results: Representative precision and MC results observed from one reagent lot across indicated sample ranges are listed in the table below for each assay. Over the seven assays tested, repeatability and within-lab % CVs were <6.5% and <11.0%, respectively. Slopes determined by the Deming linear regression model were approximately equal to 1.

Conclusion: Evaluation of the Atellica CH Vanc, Carb, Gent, Phny, Tob, VPA, and Theo assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on the Atellica CH 930 Analyzer.

	Precision				Method Comparison	
Analyte (Assay)	Unit	Sample Range	Repeatability %CV range	Within Laboratory %CV range	Sample Range	Regression Equation for Comparative Assay
Vancomycin (Vanc)	µg/mL	6.1–46.1	0.8–2.3	1.5–2.8	4.1–45.9	y = 0.97x + 0.3 µg/mL
Carbamazepine (Carb)	µg/mL	2.7–17.1	0.7–1.0	1.2–4.4	0.6–19.1	y = 0.98x + 0.0 µg/mL
Gentamicin (Gent)	µg/mL	1.5–7.1	1.8–4.0	2.3–6.7	0.6–11.6	y = 0.99x – 0.1 µg/mL
Phenytoin (Phny)	µg/mL	7.6–32.8	2.0–3.8	2.4–5.7	2.7–37.1	y = 1.03x + 0.2 µg/mL
Tobramycin (Tob)	µg/mL	1.1–10.4	1.3–6.4	2.4–10.9	0.4–11.2	y = 0.98x + 0.0 µg/mL
Valproic Acid (VPA)	µg/mL	23.6–127.7	0.8–1.5	2.4–3.4	9.9–137.4	Y = 0.95x – 0.4 µg/mL
Theophylline (Theo)	µg/mL	7.2–29.3	1.3–2.5	2.3–3.2	2.4–38.8	y = 1.05x + 0.4 µg/mL

*The products/features mentioned here are not commercially available in all countries. Their future availability cannot be guaranteed.

B-334

How to Prevent the Next Wave in Overdose Crisis? - Detect Designer Benzodiazepines Using CEDIA and DRI Assays

J. Wu[1], A. Ouyang[2], P. Chua[3], L. Ye[2], R. Patel[2]. *[1]Thermo Fisher Scientific, FREMONT, CA, [2]Thermo Fisher Scientific, Fremont, CA, [3]Thermo Fisher Scientific, South San Francisco, CA*

Background: Benzodiazepines are a class of drugs used for relieving symptoms of anxiety, insomnia, agitation, muscle spasms, and alcohol withdrawal. Benzodiazepines are CNS depressants enhancing the effect of GABA neurotransmitter at GABA receptors and they are well-known for drug abuse potential and drug dependence. Designer benzodiazepines are new psychoactive substances emerging in the past two decades. Designer benzodiazepines are often taken by individuals who use hallucinogenic and stimulant drugs. The drug abuse can lead to respiratory depression, coma, or death. There exists a need to develop a new homogeneous enzyme immunoassay. Existing commercial immunoassay antibodies may also cross-react with designer benzodiazepines which have chemical structure closely related to classic therapeutic benzodiazepines. Herein, we evaluated designer benzodiazepines cross-reactivity using DRI Benzodiazepines and CEDIA Benzodiazepines assays and demonstrated the potential of detecting designer benzodiazepines in urines.

Methods: DRI assay is based on competition between drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) and free drug in the urine sample for a fixed amount of antibody binding sites. The enzyme activity is determined spectrophotometrically at 340 nm by measuring its ability to convert NAD to NADH. CEDIA technology is based on the bacterial enzyme β-galactosidase which has been genetically engineered into two inactive fragments, Enzyme Acceptor (EA) and Enzyme Donor (ED). The enzyme activity is then determined spectrophotometrically at 570 nm. Designer Benzodiazepines tested in this study are 3-Hydroxyphenazepam, Adinazolam, Bromazolam, Clonazolam, Cloniprazepam, Deschloroetizolam, Diclazepam, Etizolam, Flubromazepam, Flubromazolam, Flunitrazolam, Meclonazepam, N-Desmethylflunitrazepam, Nifoxipam, Nimetazepam, Nitrazolam, and Pyrazolam. Compounds were spiked into drug free urine and cross-reactivity was evaluated using DRI® Benzodiazepine Assay and CEDIA® Benzodiazepine Assays on the Beckman Coulter AU680.

Results: 14 out of 17 (82%) tested designer benzodiazepines showed high cross-reactivity (> 100%) in DRI benzodiazepine assay. 12 out of 17 (71%) tested designer benzodiazepines showed high cross-reactivity (> 100%) in CEDIA assays with 200 ng/mL cutoff and 300 ng/mL cutoff. Compared to NPS-benzodiazepines trend data, this study demonstrated that DRI and CEDIA benzodiazepine assays can detect more than 50% (10 out of 19) designer benzodiazepines over 2020 Q1 to 2022 Q4 in the United States.

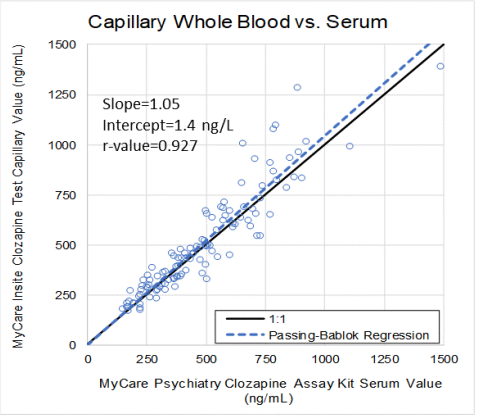
Conclusion: DRI and CEDIA Benzodiazepine Assays showed high cross reactivity of most seventeen designer benzodiazepines. The results provide potential tools for detect emerging designer benzodiazepines. **Note: This was preliminary data that has not been reviewed by any regulatory authorities/cleared by FDA.**

B-335

Rapid Quantitative Clozapine Measurements at the Point-of-Care using Capillary Blood

R. H. Christenson[1], M. R. Hilaire[2], S. J. Salamone[2], D. L. Kelly[1]. *[1]University of Maryland School of Medicine, Baltimore, MD, [2]Saladax Biomedical, Inc., Bethlehem, PA*

Background: Clozapine is the most effective medication for treatment-resistant schizophrenia, and is endorsed in professional guidelines. Therapeutic drug monitoring (TDM) is recommended for Clozapine due to high pharmacokinetic variability and risk of adverse drug reactions. The effectiveness of TDM for managing clozapine patients is limited by the lack of immediate results. **Methods:** A novel immunoassay, the MyCare Insite Clozapine Test (MCI), was developed that uses 20 µL of capillary blood (CP) and delivers 7-minute quantitative results for a portable point-of-care (POC) device. To validate the MCI system, matching CP and serum samples were collected from 113 patients at two sites. CP was tested immediately with MCI and compared to serum immunoassay results on a Beckman-Coulter AU480 analyzer. Precision was evaluated using three whole blood spiked samples and two assay controls over five days with five replicates per day. **Results:** The figure shows a correlation plot evaluated by Passing-Bablok regression of the POC MCI (y-axis) and the Beckman analyzer (x-axis). The regression line was y=1.05*x + 1.4 ng/L, r=0.927, indicating good agreement. Precision studies demonstrated that repeatability was ≤11% and within-lab precision was ≤13%. **Conclusion:** Quantitative Clozapine results are available in 7 minutes using CP in a portable POC device. A rapid POC alternative can achieve timely patient results in many testing environments. Rapid Clozapine testing has the potential for expanding utilization of this important drug by providing actionable POC results.



B-336

Comparison of the LZI Fentanyl Enzyme Immunoassay with ARKII and SEFRIA Fentanyl Assays on Beckman AU Analyzer

S. Manar[1], B. George[1], R. Huang[2]. *[1]Harris Health Ben Taub Hospital, Houston, TX, [2]Baylor College of Medicine, Harris Health Ben Taub Hospital, Houston, TX*

Background:

Fentanyl is a fast-acting synthetic opioid that can be prescribed for severe pain or found in illicit drugs. Routine clinical screening of fentanyl use is often performed on automated immunoassay analyzers, by measuring fentanyl and/or its primary metabolite, norfentanyl, in urine. Limited data has been published on performance of the relatively new LZI fentanyl enzyme immunoassay, which detects both fentanyl and norfentanyl but calibrated against norfentanyl. In this study, we evaluated the performance of LZI fentanyl assay on Beckman AU analyzer, in comparison to ARKII and SEFRIA assays calibrated against fentanyl.

Methods:

The LZI and ARKII fentanyl assays were implemented on Beckman AU 5800 analyzer according to manufacturer's instructions in our clinical laboratory. The LZI assay uses a cutoff value of 5 ng/mL for norfentanyl, while the ARKII assay uses a cutoff value of 1 ng/mL for fentanyl. The SEFRIA assay was performed on Beckman AU analyzer in a reference laboratory, with a cutoff value of 2 ng/mL for fentanyl. A qualitative comparison study was performed on a total of 42 deidentified urine samples, which were consecutively collected from clinical fentanyl screening test orders. Fentanyl screening results from the LZI and SEFRIA assays were available for all 42 samples, with ARKII results available for 28 out of the 42 samples. LC-MS confirmation test (cutoff value of 1 ng/mL for either fentanyl or norfentanyl) was also performed in a reference laboratory for samples with discrepant LZI results compared to either SEFRIA or ARKII results.

Results:

A within day precision of <10% (4.8-9.7%) and between day precision of <15% (7.0-13.9%) were observed for the LZI fentanyl assay on Beckman AU analyzers, using calibrator or quality control samples at cutoff level, 25% lower and 25% higher than cutoff level. LZI and ARKII results agreed on 27 out of 28 samples (10 positive and 17 negative). Only one sample tested positive by ARKII but negative by LZI assay, which was also negative by SEFRIA and LC-MS. When compared to SEFRIA assay, results from LZI assay agreed on 35 out of 42 samples (10 positive and 25 negative). The seven discrepant results (negative on SEFRIA but positive on LZI) were all confirmed positive by LC-MS. The false negative results observed on SEFRIA were likely due to a cutoff value of 2 ng/mL instead of 1 ng/mL. There was one sample tested positive by LC-MS only (<1.0 ng/ml for fentanyl and 3.3 ng/ml for norfentanyl), with inconclusive LZI results but counted as negative (positive on one instrument and negative on another). Overall, there was no false positive result reported by LZI assay in this study and 17 out of 18 positive samples were correctly identified, with a calculated sensitivity of 94%, specificity 100%, positive predictive value 100% and negative predictive value 96%.

Conclusion:

The Beckman AU LZI fentanyl assay with a cutoff value of 5 ng/mL calibrated against norfentanyl has shown comparable performance as existing fentanyl assays and can be implemented for routine clinical screening of fentanyl.

B-337

Case Report: Fatal Opioid Overdose Induced by Over-the-Counter Cold Medication Diphenhydramine

A. Whitt, S. Jortani. *University of Louisville, Louisville, KY*

Background: Accidental drug-related deaths frequently arise following ingestion of multiple co-intoxicants, including opioid analgesics. Hydrocodone, the most prescribed opioid for pain management, is metabolized by hepatic enzymes CYP2D6 and CYP3A4 to the pharmacologically active metabolite hydromorphone and the inactive metabolite norhydrocodone, respectively. As a result, inhibition of CYP2D6 by other pharmacologic agents can impair the analgesic properties of hydrocodone and prolong its half-life in circulation. One such example is diphenhydramine, which is a potent inhibitor of CYP2D6 activity. This case highlights a drug-drug interaction likely resulting in an accidental fatal opioid overdose. Case Presentation: A 58-year-old female had longstanding prescriptions for hydrocodone/acetaminophen (Norco® 10/325), alprazolam, carisoprodol, and diltiazem. The decedent had recently taken an over-the-counter cold medication, diphenhydramine (Benadryl), shortly preceding death. A toxicology report (NMS labs, Horsham, PA) from a post-mortem femoral blood sample detected the presence of the following therapeutic compounds by High Performance Liquid Chromatography/Time of Flight-Mass Spectrometry (LC/TOF-MS) analysis: acetaminophen (14 µg/mL), hydrocodone (410 ng/mL), dihydrocodeine (hydrocodol, 24 ng/mL), alprazolam (42 ng/mL), carisoprodol (88 ng/mL), diltiazem (110 ng/mL), and diphenhydramine (150 ng/mL). Hydromorphone, the active metabolite of hydrocodone, was not detected (< 2 ng/mL). With the exception of hydrocodone, which was present at fatal concentrations, all other compounds were within therapeutic range. Together, these observations are strongly suggestive of a drug-drug interaction leading to impaired hydrocodone metabolism. Conclusions: This case report highlights a fatal opioid overdose as a result of CYP2D6 inhibition by diphenhydramine, a readily available over-the-counter medication. Post-mortem toxicological analysis revealed hydrocodone was present in lethal concentrations, whereas its pharmacologically active metabolite, hydromorphone, was undetectable at time of autopsy. Further, the patient was prescribed a formulation of hydrocodone in combination with acetaminophen, which was present at therapeutic concentrations despite the two drugs having similar elimination half-lives. These toxicological findings suggest administration of diphenhydramine precipitated a deadly accumulation of hydrocodone in the deceased. Overall, this case report underscores the importance of understanding and preventing harmful drug-drug interactions in patients taking opioid medications for pain management.

B-338

A Clinical Research LC-MS/MS Method for the Analysis of Immunosuppressant Drugs in Whole Blood using Capitainer B Devices

S. Balloch, L. Calton, G. Hammond. *Waters, Wilmslow, United Kingdom*

Background:

Traditional laboratory analysis of the immunosuppressant drugs cyclosporine, everolimus, sirolimus and tacrolimus is well-established in clinical research. However there remains a need for individuals to undergo an invasive, time-consuming and disruptive process under the supervision of trained staff in order to collect a sufficient volume of whole blood for laboratory analysis. A reliable, remote sampling method may find utility in a clinical research setting. Here we describe the use of Capitainer® B qDBS devices to obtain analytically sensitive, precise and accurate data for cyclosporine, everolimus, sirolimus and tacrolimus analysis using small sample volumes. The Waters ACQUITY™ UPLC I-Class with Xevo™ TQ Absolute mass spectrometer was used to analyze these samples.

Methods:

An in-house laboratory developed LC-MS/MS method to analyze all four immunosuppressants in a single ruin was developed. Waters MassTrak Immunosuppressant Calibrator and Control Sets (IVD) and whole blood External Quality Assurance samples (LGC, Bury, UK) were used in conjunction with Capitainer® B qDBS devices to assess the performance of the method. Samples were collected using Capitainer® B qDBS devices, and the sample (10 µL) extracted using solvent containing internal standards. A water/methanol/ammonium acetate gradient was used with a Waters C18 HSS SB column on a Waters ACQUITY™ UPLC™ I-Class and Xevo TQ Absolute Mass Spectrometer operating in positive electrospray ionization mode with run time of less than 2 minutes.

Results:

Analytical sensitivity of the lowest calibrator at 1 ng/mL for everolimus, sirolimus and tacrolimus and 25 ng/mL for cyclosporine was demonstrated with S/N (PtP) > 10 across five analytical runs. We successfully demonstrated linearity of cyclosporine from 25-1500 ng/mL and everolimus, sirolimus and tacrolimus from 1-30 ng/mL, with r^2>0.995 over five analytical runs. Total precision and repeatability across the four immunosuppressants (2, 8 and 22 ng/mL for everolimus, tacrolimus and tacrolimus; 150, 400 and 900 ng/mL for cyclosporine) with five replicates over five analytical runs (n = 25) was ≤15% CV. External quality assurance samples for all drugs met the scheme acceptance criteria, with mean bias ≤15% CV.

Conclusion:

Using Capitainer® B qDBS devices and sample small volumes (10 µL) of whole blood, an in-house laboratory method was used to meet validation goals for analytical sensitivity, linearity, precision and accuracy for cyclosporine, everolimus, sirolimus and tacrolimus. Furthermore, the advantages conferred by volumetric absorptive microsampling, notably removing the requirement for travel and a venous blood draw and facilitating home sampling, render this technique applicable to clinical research. For Research Use Only. Not for Use in Diagnostic Procedures.

B-339

A Clinical Research LC-MS/MS Method for the Analysis of Antidepressants in Plasma

S. Balloch, L. Calton, G. Hammond. *Waters, Wilmslow, United Kingdom*

Background: Depression is common globally, with an estimated 3.8% of the population affected. The condition can impact individuals' ability to function in work, social and family settings. Many antidepressant drugs are currently prescribed, encompassing selective serotonin reuptake inhibitors (SSRIs), serotonin-nonresponsive reuptake inhibitors (SNRIs) and tetracyclic antidepressants (TeCAs). However pharmacokinetic and drug interactions are known, therefore a reliable quantitative clinical research method may play a role in researching the effects of their administration. Waters has developed a clinical research method for the following antidepressants in plasma; citalopram, desmethylfluoxetine, duloxetine, fluoxetine, fluvoxamine, O-desmethylvenlafaxine, sertraline and venlafaxine (10-1000 ng/mL); mirtazapine (5-500 ng/mL) and trazadone (30-3000 ng/mL).

Methods: Matrix matched calibrators and QCs were prepared using in-house stocks and pooled plasma. Samples (50 µL) were treated with internal standard in acetonitrile. A water/methanol/ammonium acetate gradient was used with a Waters™ XSelect™ Premier HSS T3 column on a Waters ACQUITY™ UPLC™ I-Class followed by detection on a Xevo® TQD mass spectrometer in a 5 minute run.

Results: No system carryover was observed following analysis of plasma samples containing the highest concentration calibrators. Analytical sensitivity investigations indicated precise quantification (≤20% CV, ≤17.6% bias) at concentrations equal to or lower than the lowest concentration calibrator. Total precision and repeatability were assessed (3 pools, 5 replicates, 5 days; n=25) and determined to be ≤10.0% RSD. Linearity experiments determined the method provided first or second order fits over the ranges analyzed; additionally, each run met acceptance criteria (coefficient of correlation ≥ 0.995, determined concentrations of calibrators ±15% of nominal, ±20% in the case of the lowest calibrator). Post-column infusion experiments demonstrated analytes eluted in regions free of major ion suppression or enhancement. Evaluation of matrix effects at low and high concentrations indicated compensation by the internal standard. Addition of high concentrations of several endogenous and exogenous materials did not affect quantification.

Conclusion: This quantitative method for clinical research demonstrates very good precision with minimal matrix effects and allows for the multiplexing of a panel of antidepressants in plasma in a short run time.

For Research Use Only. Not for Use in Diagnostic Procedures.

B-340

Performance of a Quality System for Targeted Monoclonal Antibodies in a Reference Clinical Pharmacokinetic Laboratory

J. McFarland, O. McLachlan, J. Tsang, M. Gowhari, J. Ho, P. Hughes, T. Dervieux. *Prometheus Laboratories, San Diego, CA*

Background: The homogenous mobility shift assay (HMSA) laboratory developed test (LDT) quantifies monoclonal antibody drug and anti-drug antibody levels and is useful for the management of inflammatory bowel diseases (IBD). We report the implementation and performance of a quality system for these LDTs, as well as longitudinal trends of exposure and immunization prevalence from a reference clinical pharmacokinetic (PK) laboratory (Prometheus Laboratories, Inc.).

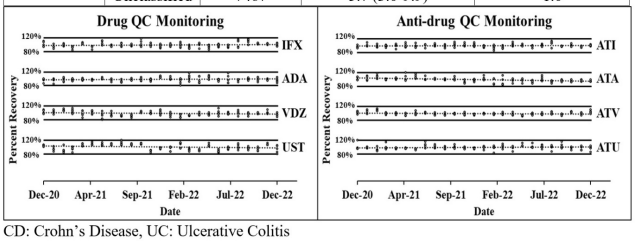
Methods: Quality control (QC) HMSA data was evaluated to verify conformance to specifications for infliximab (IFX), adalimumab (ADA), vedolizumab (VDZ), ustekinumab (UST) and their respective anti-drug antibodies (antibody to IFX [ATI], ADA [ATA], VDZ [ATV], and UST [ATU]). A QC panel consisting of five serum specimens spanning the reportable range per each drug or anti-drug antibody LDT was established, stored at subzero temperature, and tested monthly over a period of two years. Acceptable performance consisted of 80-120% accuracy from the target value. All specimens were blinded to operator and processed with the clinical PK load of testing. Exposure and immunization prevalence over three years of testing was evaluated. All patient data were de-identified before analysis. Median drug level (interquartile ranges [IQR]), and anti-drug percent positivity by international classification of disease was also estimated (ICD-10 based).

Results: The QC results establish that the drug (IFX, ADA, VDZ, UST) and anti-drug (ATI, ATA, ATV, ATU) LDTs report acceptable performance with 80-120% accuracy over the two-year evaluation period. Median drug concentrations with IQR were assessed for the clinical sample set differentiated by disease classification as depicted in **Figure-1**. Anti-drug positivity levels were higher for ATI (mean: 11.5%) and ATA (mean: 13.6%) than those from ATV (mean 2.0%) and ATU (mean: 0.6%).

Conclusion: The QC data presented supports the performance of HMSA for therapeutic drug monitoring (TDM) of IBD and other immune mediated inflammatory diseases.

Figure-1: Drug Median with IQR, Anti-drug Positivity, and QC Monitoring

Test	Disease	N	Drug Median (IQR) (µg/mL)	Anti-drug Positivity (%)
IFX	CD	40,191	10.5 (4.8-18.7)	10.8
	UC	8437	10.1 (3.7-19.3)	14.4
	Unclassified	29,950	11.4 (4.9-20.7)	11.6
ADA	CD	22,661	9.8 (5.4-14.6)	12.1
	UC	4178	8.6 (3.8-13.9)	17.8
	Unclassified	12,330	9.6 (4.8-14.9)	14.9
VDZ	CD	11,232	14.3 (7.9-23.2)	1.5
	UC	5893	14.6 (8.3-23.5)	2.3
	Unclassified	14,991	15.0 (8.6-23.9)	2.4
UST	CD	12,558	5.1 (2.7-8.7)	0.4
	UC	1428	5.2 (2.7-9.3)	0.4
	Unclassified	7467	5.7 (3.0-9.9)	1.0



CD: Crohn's Disease, UC: Ulcerative Colitis

B-341

Analytical Performances of Homogeneous Mobility Shift Assay for Infliximab and Biosimilars and Resulting Exposure Assessments in Large Cohort of Patients with Inflammatory Bowel Diseases

J. McFarland, J. Tsang, J. Ho, O. McLachlan, M. Gowhari, P. Hughes, T. Dervieux. *Prometheus Laboratories, San Diego, CA*

Background: Biosimilar for infliximab (IFX) are currently available for the treatment of various immune mediated inflammatory diseases including inflammatory bowel disease (IBD). We have validated the homogenous mobility shift assay (HMSA) for IFX biosimilars and describe exposure from a large population of patients with IBD treated with IFX and biosimilars.

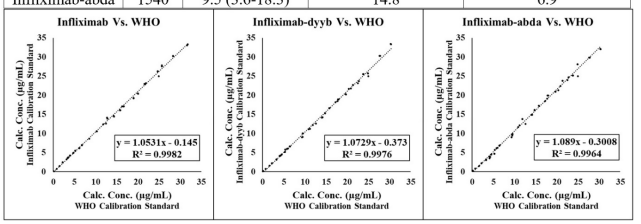
Methods: Validation of the biosimilars, infliximab-dyyb (Inflectra®) and infliximab-abda (Renflexis®), was conducted in a reference clinical laboratory (Prometheus Laboratories, Inc.) for performance equivalence to the 1st international World Health Organization (WHO) IFX standard (16/170) by HMSA. The evaluated performance characteristics consisted of accuracy, precision, sensitivity, specificity, range, and linearity using spiked human serum. Following validation and implementation in the clinical laboratory, the drug median with interquartile range (IQR) and antibody to drug (ATI) prevalence and percentage relative to low-titer antibody cut-point of 10 U/mL (Am J Gastroenterol 2021;00:1-12), using de-identified patients, was evaluated by comparing treatment with IFX or the two validated biosimilars.

Results: The reportable range for the biosimilars was 0.98 µg/mL (precision: 5.5% CV; accuracy: 110.7% recovery) to 34 µg/mL (precision: 6.3% CV; accuracy: 105.4% recovery). Biosimilar intra-day, inter-day precision (CV) and accuracy (% recovery) across the reportable range were 3.8%, 3.6% and 105.1%, respectively. Furthermore, HMSA calibrated with IFX originator or biosimilar showed congruent results when compared to calibration with the WHO standard for the determination of drug concentrations using 20 clinical samples spanning the reportable range (**Figure-1**). Median drug concentrations with IQR were 10.9 µg/mL (4.8-19.7 µg/mL) for infliximab originator, 9.8 µg/mL (3.9-18.1) for infliximab-dyyb, and 9.5 µg/mL (3.6-18.3) for infliximab-abda over a three-year period of testing, which also demonstrated similar ATI positivity rates.

Conclusion: HMSA is validated to be used as a tool for therapeutic drug monitoring (TDM) of patients treated with IFX originator and biosimilars.

Figure-1: Drug Median with IQR and Anti-drug Positivity with Percentage >10 U/mL

Drug	N	Drug Median (IQR) (µg/mL)	Anti-drug Positivity (%)	Anti-drug >10 U/mL (%)
Infliximab	70,212	10.9 (4.8-19.7)	11.1	5.1
Infliximab-dyyb	5800	9.8 (3.9-18.1)	13.9	6.5
Infliximab-abda	1540	9.5 (3.6-18.3)	14.8	6.9



B-342

Fentanyl urine drug screen comparison of the ARK II and Lin Zhi immunoassays

K. Lam[1], M. Menlyadiev[2], R. Suhandynata[2], R. Fitzgerald[2], L. Song[1], I. Metushi[1]. *[1]University of California Los Angeles, Los Angeles, CA, [2]University of California San Diego, San Diego, CA*

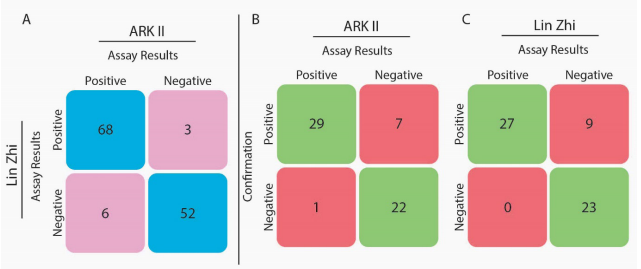


Figure 1. Conparison of A) Lin Zhi and ARK II immunoassays for the detection of fentanyl/norfentanyl (n = 129). B) ARK II vs. comfirmed results (n = 59); (C) Lin Zhi vs. comfirmed results (n = 59).

Background. Given the opioid epidemic, screening for fentanyl in urine has become increasingly important. Routine opiate immunoassays cannot identify fentanyl, given its structural variance from the more common opiates such as morphine and codeine. Therefore, the detection of fentanyl by immunoassay urine drug screen depends on antibody specificity for fentanyl. The ARK II is a widely used immunoassay that will detect fentanyl and norfentanyl. Recently, a novel fentanyl assay by Lin-Zhi has become available on the Roche platform; here, we evaluate its performance. **Methods.** One hundred twenty nine urine samples were analyzed for fentanyl across the Lin-Zhi and ARK II assay on the Cobas c502 platform. Samples were either analyzed immediately upon request for drug of abuse screening or frozen for subsequent analysis. Liquid chromatography coupled tandem mass spectrometry (LC-MS/MS) and/or prescription was used as confirmation for the presence of fentanyl. Our of the total 129 tested samples, so far 59 have been confirmed and additional studies are ongoing. The mass spectrometry assay has a limit of detection of 1 ng/mL for fentanyl/norfentanyl. The ARK II immunoassay has a 1 ng/mL cut-off for fentanyl and 7% cross-reactivity for norfentanyl; the Lin Zhi has a 5 ng/mL cut-off for norfentanyl and 131.58% cross reactivity for fentanyl. **Results.** There were 9/129 discrepant samples between ARK II and Lin Zhi immunoassays as shown in Figure 1. From the 59 confirmed samples Ark II resulted in one false positive sample and seven false negative samples (Figure 1B) while the Lin Zhi assay resulted in 9 false negatives. **Conclusion.** Given the current data set, there appear to be some discrepant results between the ARK II and Lin Zhi immunoassays. Most of the discrepant results observed have concentrations of fentanyl and norfentanyl that are close to the lower limit of immunoassay detection.

Tumor Markers and Cancer Diagnostics

B-343

Validation of glucose test in pancreatic cyst fluid

K. Lam, I. Metushi, L. Song. *University of California Los Angeles, Los Angeles, CA*

Background: Pancreatic cysts may be detected in 40 to 50 percent of patients who undergo abdominal magnetic resonance imaging for unrelated reasons. Mucinous cysts such as intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN) are known precursor lesions of pancreatic ductal adenocarcinoma. Therefore, it is imperative to identify mucinous cysts from nonmucinous cysts. In addition to cytology analysis, biomarker analysis in cyst fluid obtained by endoscopic ultrasound-guided fine needle aspiration can also provide information for the differential diagnosis of a mucinous cyst. Carcinoembryonic antigen (CEA) has been used as the biomarker to differentiate mucinous and nonmucinous pancreatic cysts with a sensitivity of around 63% and specificity of around 88%. Recently, glucose in pancreatic cyst fluid has been shown to have a better sensitivity (92%) and specificity (87%) in the differentiation of mucinous cysts using a threshold of <50 mg/dL. Therefore, it is consequential for clinical laboratories to validate glucose tests in pancreatic cyst fluid samples. In this study, we report the validation of the glucose test in pancreatic cyst fluid samples using the glucose assay on an automated chemistry analyzer.

Methods: The glucose assay, GLUC3, on Roche Cobas c702 analyzer was used to measure glucose in pancreatic cyst fluid samples. For linearity and recovery studies, cyst fluid samples were spiked with stock glucose solutions of known concentrations in a way that the spiked fluid samples always contained no less than 90% of the cyst fluid. Linearity was determined by assaying nine samples with glucose concentrations from 0-718 mg/dL. For the recovery study, various amounts of glucose were added to a cyst fluid sample containing undetectable glucose. The expected glucose concentrations in these samples are 23.2, 46.4, 92.7, and 159 mg/dL, respectively. Intra-run and inter-run precisions were determined by measuring glucose ten times in a run and in 10 separated runs, respectively, in 9 cyst fluid samples containing various amounts of glucose ranging from 0 - 718 mg/dL. The effect of hemoglobin was tested by spiking one cyst fluid sample with a hemolysate. Stability was determined by testing one sample at 0 and 24 hours at room temperature, respectively.

Results: The assay was linear from 0 - 718 mg/dL of glucose with a slope of 1.05 and an intercept of 3.5. The recovery ranged from 103% to 111% in samples containing 23.2, 46.4, 92.7, and 159 mg/dL glucose, respectively. Both the intra-run and inter-run precisions for the nine samples with different glucose concentrations had a coefficient of variation of <5%. The samples were stable at room temperature for the study period of 24 hours. No interference was observed at hemoglobin of 477 mg/dL.

Conclusion: Our study indicates that the glucose assay, GLUC3, on Roche Cobas c702 analyzer can be used to measure glucose in pancreatic cyst fluid samples. Our results show that glucose measurement in pancreatic cyst fluid had a good recovery and reproducible results with precision being <5%. This test may aid in the differential diagnosis of pancreatic mucinous cysts.

B-347

Performance Validation of an In-House Assay for Soluble Mesothelin Related Peptides

J. A. Erickson[1], K. Doyle[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]University of Utah School of Medicine, Department of Pathology, Salt Lake City, UT*

Background: Mesothelin is a glycosylated phosphatidylinositol glycoprotein located on the surface of mesothelial cells. Mesothelin is released from the cell in a form denoted as Soluble Mesothelin Related Peptides (SMRP), that can be measured in the blood. Although mesothelin is produced by normal cells, studies have shown an overexpression in individuals suffering malignant mesothelioma, with an increase in serum SMRP concentration associated with the disease.

The in-house ARUP MESOMARK® ELISA is a 96-well microtiter plate formatted sandwich immunoassay for the quantitative measurement of SMRP in human serum. The ELISA incorporates two separate monoclonal antibodies specific for SMRP (including megakaryocyte potentiating factor); one (4H3) bound to the microtiter well for capturing SMRP and the second (OV569), enzyme labeled with horseradish peroxidase (HRP), for bound SMRP detection. After several incubation and wash steps to remove unbound materials, color is produced by HRP turnover of a chromogenic

substrate during a final incubation step. A direct relationship between the color intensity and the SMRP concentration of the specimen is generated, with results expressed as nmol/L SMRP.

Methods: Deidentified residual serum specimens sent to ARUP Laboratories for routine testing, as well as serum specimens from healthy volunteers, were collected under University of Utah's Institutional Review Board approved protocols. Commercial kit (Fujirebio Diagnostics, Inc., Malvern, PA) testing was completed according to manufacturer's protocol. ARUP ELISA measurements were conducted according to an established in-house protocol. ARUP ELISA performance characteristics evaluated included a method comparison against the commercial assay, linearity, recovery, precision, analytical sensitivity, and dilution studies. A reference limit was also verified.

Results: A method comparison study against the commercial SMRP ELISA generated a slope of 0.989, intercept of 0.0422, r^2 of 0.998 and bias of -1.2% (n = 45, Deming regression, Bland-Altman analysis). Clinical agreement near the 1.5 nmol/L cutoff was 100%. The limit of detection was 0.3 nmol/L (20 determinations each of a blank and low-level serum pool). Linearity was established by combining serum specimens with high and low SMRP concentrations at different ratios, creating 10 specimens of varying SMRP concentrations. Each specimen was tested in triplicate. Regression analysis (measured vs. expected concentration) produced a slope of 1.002, intercept of -0.739 and r^2 of 0.998, with percent recoveries ranging 86.1 to 100.0%. Precision was determined from two serum pools of differing SMRP concentrations tested over 5 days, four replicates per pool per day. Repeatability and within-laboratory CVs were 3.0 and 5.0% at 1.6 nmol/L, and 1.7 and 1.7% at 17.3 nmol/L, respectively. Back-calculated results from a 10-fold dilution study of four serum specimens were within -9.8 to -8.1% of their corresponding neat values. The previously established reference limit of 1.5 nmol/L was verified for the ARUP ELISA, with 95% of results below the cutoff value (n = 20, sera from healthy volunteers).

Conclusions: The in-house ARUP MESOMARK ELISA demonstrates acceptable performance for quantifying SMRP in human serum and compares favorably to a well-established commercial ELISA. A reference limit of 1.5 nmol/L was also verified. Overall, the ARUP MESOMARK ELISA is acceptable for serum SMRP testing.

B-355

Measurable residual disease detection in mantle cell lymphoma (MCL) subjects using NGS B-Cell MRD assay

P. Shah[1], Y. Huang[1], A. Jacobsen[1], N. Hill[1], A. Zlotnicki[1], O. Diaz II[1], A. Medina[2]. *[1]Invivoscribe, San Diego, CA, [2]Hospital Universitario de Salamanca, SALAMANCA, Spain*

Background: Mantle Cell Lymphoma (MCL) is an aggressive, rare form of non-Hodgkin B-cell lymphoma. Measurable Residual Disease (MRD) assessment can provide useful information when assessing and tracking response to therapy, refining treatment and predicting clinical outcome for subjects with B-cell lymphoproliferative diseases. One method of MRD detection is to identify and track specific clonal immunoglobulin heavy chain (*IGH*) gene rearrangement sequences on a next-generation sequencing (NGS) platform. Here, we present the NGS-based B-cell MRD Assay with 10^{-6} sensitivity for detecting MRD in malignancies in mantle cell lymphoma (MCL).

Methods: Residual DNA samples from different specimen types (44 BM and 77 PB) at different post-treatment time points were obtained from anonymized 47 MCL subjects that had been enrolled in a study approved by the Spanish Group of Lymphoma and Autologous Stem Cell Transplantation (clinical trial: NCT02682641, publication reference: 10.1200/JCO.21.02321). 121 of these follow up samples were tested by the B-cell MRD Assay by tracking the clonal sequences detected in corresponding baseline samples. Among them, 90 follow up samples were paired PB and BM samples. The B-cell MRD Assay workflow consists of DNA extraction and PCR based library preparation with proprietary multiplex master mixes (MMx) targeting either *IGH* Framework 1 (FR1) or 3 (FR3) regions, which both track the patient- and tumor-specific CDR3 sequence. Purified libraries are then equimolar pooled and sequenced on Illumina's NextSeq Dx platform. The FASTQ output files from sequencing are analyzed using Invivoscribe's B-Cell MRD Software-NextSeqDx. Results from Invivoscribe were compared to the results from Salamanca lab that were generated by qPCR.

Results: Based on the results from B-cell MRD Assay, 115/121 (95%) samples were valid, 42/115 (37%) valid samples had, "MRD Detected", and 73/115 (63%) samples had, "MRD Not Detected", with an "MRD Detection" rate of 28% in PB and 50% in BM specimen types. When comparing MRD detectable status between paired PB and BM samples from 36 MCL subjects, 30/44 (68%) paired samples had same MRD call and were concordant, whereas 14/44 (32%) calls were discordant. More specifically, 12/14 (86%) discordant samples had PB specimen type with a "MRD Not detected" call, whereas the paired BM specimen showed a "MRD Detected" call. Overall, in comparison with the MRD results (n=84) provided by Salamanca lab using qPCR method, the B-cell MRD Assay demonstrated concordance of 80%, PPA of 79%, and NPA of 80% for MRD detection in MCL samples.

Conclusion: Good concordance was observed between the qPCR used at Salamanca and Invivoscribe's B-cell MRD assay. It was important to note that the BM specimen type was determined to be more sensitive in detecting MRD when compared to PB in assessment of paired PB and BM samples. These results demonstrate that the B-cell MRD Assay can be utilized for MRD detection in follow-up sample types for MCL subjects.

B-356

Advancements in Commercial Viability of a Blood Test for the Early Detection of Breast Cancer

C. Kehelpanalla[1], D. Pascovici[2], D. Li[1], K. Heffernan[1], M. David[1], K. Huynh[3], G. Lamoury[4], K. Ekroos[5], P. J. Meikle[3], A. M. Batarseh[1]. *[1]BCAL Diagnostics Limited, Sydney, Australia, [2]Insight Stats, Croydon Park, Australia, [3]Baker Heart and Diabetes Institute, Melbourne, Australia, [4]Royal North Shore Hospital, St Leonards, Australia, [5]Lipidomics Consulting Ltd., Esbo, Finland*

Background: Mammography is the current diagnostic standard for breast cancer screening and monitoring. However, accessibility challenges, accuracy issues and patient discomfort all contribute to reduced patient compliance and utilization, resulting in a need for more effective diagnostic tools. A blood test to detect early-stage breast cancer is sought to increase the screening detection rate, provide more accurate monitoring tools, and improve outcomes for patients. We have previously reported a series of lipidomic studies and derived a lipid signature from plasma enriched extracellular vesicles (EVs) that effectively distinguished people with localized breast cancer from cancer-free controls. Here we report on a significant refinement to the test methodology allowing the assessment of the lipid signature directly from plasma samples and its performance, with the aim of advancing the commercial viability of the test as we move towards clinical application.

Methods: Lipids in EVs from fasted breast cancer and control blood samples (4 separate cohorts (n=793)) were extracted and analysed by liquid chromatography-high resolution mass spectrometry (LC-HRAM-MS). Over 400 manually curated lipids were quantified. From these, an independent review and retrospective analysis of the data established a lipid signature. The lipid signature was modelled on each of the cohorts using leave-one-out internal cross-validation. Following variable selection, a lipid signature capable of distinguishing breast cancer samples from controls was derived. Furthermore, we investigated if the lipid signature can be assessed from plasma instead of EVs with the aim of further streamlining the process. We analysed the lipids in cancer and control plasma samples (n=256) previously used for EV preparations, corresponding to patients from Cohorts 3 and 4, and applied the signature derived using EVs on plasma lipidomic data. NIST Standard Reference Material (SRM) 1950-Metabolites in Frozen Human Plasma was used for inter-laboratory quality control.

Results: Breast cancer subjects were differentiated from controls by the lipid signature with an area under the curve (AUC) of 0.77-0.89 across four cohorts. Assessing the signature directly from plasma, the test achieved a comparable AUC of 0.84. This improvement would make the test more clinically viable and easier to perform.

Conclusion: We derived a lipid signature, which shows high potential for distinguishing breast cancer samples from controls directly from plasma instead of EVs, reducing the test complexity. Ongoing studies will optimize the plasma lipidomic signature and prospectively compare the test against mammographic and pathological diagnoses.

B-358

Application of Surface-Enhanced Raman Spectroscopy to Improve Sensitivity of Clinical Assays

D. Sheik[1], C. Ummadisetti[1], K. Byers[1], V. Suresh[2], K. Pate[3], M. Matthews[3], V. J. Davisson[2]. *[1]Amplified Sciences, West Lafayette, IN, [2]Purdue University, West Lafayette, IN, [3]Wasatch Photonics, Morrisville, NC*

Background: Current efforts to expand the pool of available markers that can be analyzed in clinical settings is hampered by limited volumes of biomaterial-rich fluids and low concentrations of certain analytes, coupled with poor sensitivity of existing detection techniques. Although progress has been made using advanced sample preparation or assay techniques such as miRNeasy or ELISA for genomic and protein assays, respectively, little research has focused on adapting new optical methods as detection modes in the clinic. Amplified Sciences has developed an enzymatic activity

assay platform using SERS detection to improve diagnostic sensitivities in multiple disease indications. Along with the experts at Wasatch Photonics, research focused on adaptation of this assay platform has enabled investigation of multiple markers of disease that had previously been undervalued or overlooked, as well as stacking of multiple markers in precious, low-volume samples. **Methods**: Building upon previously published data using gastricsin as a marker to identify benign, non-mucinous pancreatic cysts, Amplified Sciences has added several SERS laser wavelengths, and different sample preparation methods to the existing platform. To examine the sensitivities of laser and dye combinations, solutions representative of assay samples containing rhodamine-dimer dye-labeled peptide were tested using Wasatch Photonics Raman spectrometers at 532, 638, 785, and 830 nm laser wavelengths. Samples at different pH were analyzed after addition of colloidal Au or Ag nanoparticles to determine the impacts of sample preparation on SERS spectral features. After drying in a desiccator overnight, the samples were reanalyzed to assess the stability of the SERS signals. As a pertinent application of this technology, an activity assay was developed for the known COVID protease 3ClPro. Release of rhodamine dimer dye as a result of 3ClPro cleavage of the substrate was quantified using SERS. The data were analyzed using Bruker OPUS Software package, and plotted using Graphpad Prism 9. **Results**: Each laser, buffer, and metal used for SERS provided a unique spectral signature, suggesting that different transitions are available and potentially tunable depending on the application under investigation. Sensitivity was, as expected, best with the 532 nm SERS system purportedly due to enhancement factors, and the resonance impacts of rhodamine dyes at 532 nm. It was found that the Limit of Detection of Rhodamine-dimer dye was 100-fold lower with SERS compared to fluorescence, even after a 3-fold dilution with colloidal Ag. However, the remaining lasers could be more finely tuned, and, thus, provide alternate spectral properties, and different sensitivities which has implications when new dye classes are introduced into the platform. Furthermore, when SERS was applied to the 3ClPro assay, it was determined that activity could be detected below the dimerization concentration of 3ClPro, described in the literature as ~25 nM. Also, a monomeric version of 3ClPro that is expected to have no reactivity with the substrate was found to display activity above background at concentrations as low as 1 µM. **Conclusion**: SERS is an appropriate method for measuring activity in clinically relevant markers of disease, with tunable characteristics based upon the dye composition and the spectrometer in use.

B-359

Bap1 overexpression enhances epithelial-mesenchymal transition in cholangiocarcinoma

H. You[1], W. Liao[2], W. Huang[1]. *[1]Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, [2]Kaohsiung Medical University, Kaohsiung, Taiwan*

Background: BRCA1-associated protein 1 (BAP1) is a nuclear protein that considered as a tumor suppressor by its involvement in DNA double-strand-break repair. However, BAP1 overexpression was observed in many kinds of cancer, such as intrahepatic cholangiocarcinoma (iCCA). Methods and Results: Formalin-fixed paraffin-embedded cancer tissues were acquired from the archive of the Department of Pathology, Chang Gung Memorial Hospital at Kaohsiung, Taiwan. In this study, we identified 23.8% cytoplasmic BAP1 overexpression and 20.8% nuclear BAP1 overexpression cases in 101 iCCA patients. There were significant correlations between cytoplasmic expression of BAP1 with tumor growth pattern (P = 0.005), T classification (P = 0.006), and clinical AJCC seventh staging (P < 0.001). In cell models (iCCA cell line RBE), overexpressed BAP1 were accumulated in cytoplasm. The translatome profile suggested that BAP1 overexpression may promote the migratory and invasive capacities of iCCA cells. In-vitro assays validated that BAP1 overexpression enhanced cell migration and invasion. Furthermore, we found that BAP1 overexpression induced epithelial-mesenchymal transition (EMT) in iCCA cells, showing E-cadherin and fibronection down-regulation and vimentin up-regulation. Conclusion: These findings indicated that cytoplasmic BAP1 overexpression increased migratory and invasive capacities in iCCA cells, at least in part, via enhanced EMT regulations. Based on these findings, cytoplasmic BAP1 expression could be a candidate predictor for metastasis of iCCA.

B-360

Usefulness of the ratios of CYFRA21-1 and p53 autoantibody immune complexes to their free antigens in lung cancer

Y. Hong, H. Kim, J. Lee, A. Oh, H. Kim. *Korea Cancer Center Hospital, Seoul, Korea, Republic of*

Background:

Autoantibodies against specific cancer-associated antigens are produced in the early stages of tumorigenesis. In lung cancer, the possibility of autoantibodies as diagnostic biomarkers has been proposed. This study was to evaluate the usefulness of novel DNA-guided sandwich immunoassay detecting CYFRA21-1 and p53 autoantibody immune complexes and their free antigens in diagnosis of lung cancer

Methods:

In total, 100 patients with lung cancer and 119 healthy controls were analyzed with 9G test™ Lung/Cancer (Biometrix Technology, South Korea) measuring plasma autoantibody immune complexes (CYFRA21-1 with anti-CYFRA21-1 autoantibody immune complex (CIC), p53 with anti-p53 autoantibody immune complex (PIC)) and their free antigens (CYFRA 21-1 and p53). The ratios of CIC to CYFRA21-1 (CIC/CYFRA21-1) and PIC to p53 (PIC/p53), and the multiplication of the two ratios, named as LC index were calculated.

Results:

The levels of CIC and PIC were significantly increased in lung cancer compared with those in healthy control (p = 0.0062 and p = 0.0026, respectively), while free antigens didn't distinguish between lung cancer and healthy control. The ratios CIC/CYFRA21-1 and PIC/p53 were significantly higher in lung cancer than those in healthy control (all, $P < 0.0001$). Diagnostic performances expressed as area under the curve (AUC) were higher in the ratios of autoantibody immune complexes to each free antigen (PIC/p53 and CIC/CYFRA21-1) than autoantibody immune complexes (PIC and CIC) (AUC; 0.887, 0.847 vs. 0.618, 0.608, respectively). The most excellent diagnostic performance was proven with LC index at diagnostic cut-off 3.60, which showed 81% sensitivity and 95% specificity with 0.945 AUC ($P < 0.001$). It was more sensitive to early lung cancer than to advanced lung cancer (sensitivity 87.5% vs. 72.7%).

Conclusion:

The ratios of CIC to CYFRA21-1 and PIC to p53 are useful biomarkers in lung cancer diagnosis and the combination of the ratios, LC index could be complementary tool for lung cancer screening with radiological tests.

B-361

Novel blood-based assay for early cancer detection demonstrates high precision and reproducibility on low tumor content

J. Charlton, C. Gilley, E. Brown, K. Gowen, M. Williams, C. DuRoss, J. Sun, J. Gregg. *Harbinger Health, Cambridge, MA*

Background: Early detection of cancer has significant potential to impact human health and society by decreasing cancer-related morbidity and mortality. While previous approaches to identify cancer-informative biomarkers are predominantly statistical, Harbinger Health has utilized foundational discoveries from developmental biology to design a targeted methylation assay for early cancer detection from cell-free DNA (cfDNA) extracted from plasma. Utilizing this biologically informed approach, we developed a fixed multi-layered logistic regression-based machine learning algorithm, trained with an in-house generated dataset of 1046 samples (621 cancer, 425 non-cancer) that predicts a binary classification (yes/no) for cfDNA samples processed through our assay. We have previously reported high sensitivity for multi-cancer detection, including for early-stage disease.

Methods: Here, we perform a comprehensive independent analytical validation of our assay and algorithm, encompassing 69 subjects: 19 with newly diagnosed treatment-naïve cancer (8 different cancer types) and 50 individuals with no history, diagnosis, or cancer symptoms. In total, we utilized 122 replicate samples to assess reproducibility and precision, 8 non-template controls (water) to determine limit of blank (LOB), and cohorts of matched biopsy and cfDNA to determine tumor content limit of detection (LOD).

Results: Precision was assessed within five different sub-studies, by comparing concordance of predicted binary cancer classification between replicate samples, giving results of 0.90 (0.95 CI: 0.764-0.959) for inter-run precision, 1.00 (0.95 CI: 0.796-1.000) for intra-run precision, 1.00 (0.95 CI: 0.871-1.000) for inter-operator precision, 0.96 (0.95 CI: 0.930-0.998) for inter-instrument precision and 0.83 (0.95 CI: 0.641-0.933) for inter-day precision. To determine LOB, we carried 8 non-template controls (water) through the entire assay and detected on average ~0.02% unique aligned reads

of a true sample on the same sequencing run. Finally, to assess tumor content LOD, we developed methodology that uses methylation signal to estimate the amount of tumor-derived DNA in each cfDNA sample and validated our estimates using whole exome sequencing, an orthogonal gold-standard approach. We then assessed the relationship between tumor content and classifier sensitivity using our training data of 625 cancer cfDNA samples and determined that our tumor content LOD whereby 95% true cancer samples were correctly predicted to be 0.037%.

Conclusion: Our assay shows high performance and high technical reproducibility. Our previously reported high sensitivity for stage 1 and stage 2 cancers, as well as extremely low tumor content LOD reported here supports our ability to perform early-stage multi-cancer detection, where the levels of circulating tumor DNA are low.

B-363

Solid Lipid Nanoparticles Mediated CBLC siRNA Delivery as a Platform to Monitor and Probe Biological Functions

K. R. Master, M. Bayachou. *Cleveland State University, Cleveland, OH*

Background: Nanoparticles have been used as a tool for delivering therapeutics since few decades. Researchers has been focusing on advances and modifications to improve the properties of these nanoparticles such as sustained drug delivery and bioavailability. This study focuses on modifying the conventional solid lipid nanoparticles (SLN) with Arginine-Glycine-Aspartic acid tripeptide (RGD). The peptidyl link in these SLN has an ability to bind to the integrin of the target cells, allowing solid adhesion to the cells for sustained drug delivery. The use of small interfering RNA (siRNA) allow better invasion through the cell membranes, silencing the intracellular targets. This study uses Casistas B-lymphoma lineage (CBLC) siRNA as the therapeutics for the treatment of non-small cell lung cancer (NSCLC). CBL family consisting of different proteins such as CBL, CBLB, and CBLC origins from a RING finger class. They are E3 ubiquitin ligases responsible for transferring ubiquitin from E2 to E3 substrate. They function as the negative regulator of Tyrosine Kinase activity in cell cycle signaling. CBLC is abundantly present in epithelial cells and so its expression is noteworthy in lung adenocarcinoma. CBL family also possess Tyrosine Kinase Binding domain (TKB) responsible to decrease the activated Epidermal Growth Factor Receptor (aEGFR). Although, CBLC was initially known to perform the same function as other CBL family members, reports suggest that it is a demethylated target. The expression of CBLC upregulates with the treatment of NSCLC by inhibiting agents for DNA methylation. This process increases the demethylation of the CBLC promoter upregulating CBLC. This causes CBLC to compete with CBL for binding to the aEGFR leading to polyubiquitination of aEGFR. Trafficking of aEGFR in the nucleus makes it more stable progressing lung cancer. CBLC silencing allows tumor cells to be sensitized to the therapy with tyrosine kinase inhibiting agents.

Methods: SLN were modified with RGDP followed by the loading of CBLC siRNA. To characterize the SLN within the diameter range of 20-100 nm, Scanning Electron Microscopy (SEM) was performed. The Energy-dispersive X-ray Spectroscopy was performed to confirm the presence of Phosphorous and Nitrogen in SLN coming from lipids and RNA. Dynamic Light Scattering (DLS) was done to verify the SEM results. To measure the efficacy of the SLN, the cells were treated with control SLN vs siRNA loaded SLN. The Sybr gold assay and invitro release study was done to confirm the release of siRNA from SLN. The human lung normal cell line NL-20 as control and lung cancer cell line A549 were cultured and treated with synthesized nanoparticles for knocking down CBLC gene. The assays such as MTT, western blot, flow cytometry and mass spectrometry-based proteomics were performed to confirm the knockdown of the CBLC gene.

Results: The SLN characterization confirmed the diameter in the expected range. The efficacy assays confirmed the encapsulation and release of CBLC siRNA. The results from cell studies showed reduction in tumor growth and viability confirming the knockdown of CBLC gene.

Conclusion: The data suggests significant effects for the use of synthesized nanoparticles as a therapeutic for the treatment of NSCLC.

B-364

Inhibition of Hypoxia-Inducible Factor-1a (HIF-1 a) Provides a Therapeutic Approach for Hepatocellular Carcinoma (HCC)

U. M. Alghamdi. *Cleveland State University, Cleveland, OH*

Background: The most frequent type of primary liver cancer in adults is hepatocellular carcinoma (HCC) which is the third-highest cause of cancer-related death worldwide. Globally, people die from liver cancer each year, with HCC accounting for around 90% of those deaths, making it a serious public health concern. Cirrhosis, hepatitis B, hepatitis C, and non-alcoholic fatty liver disease are all high-risk factors for HCC development. In addition, HCC is one of the most frequent solid tumors found in humans. Due to insufficient blood supply, these solid tumors usually are devoid of oxygen and nutrients. A reduction in oxygen concentration results in the formation of a hypoxic environment that downregulates the metabolic process and is fatal to cancer cells. To circumvent this barrier to growth, cancer cells produce hypoxia-inducible factor-1 (HIF-1), which alters their metabolic pathways and allows them to survive. HIF-1alpha (HIF-1α), a cytoplasmic subunit of HIF-1 can be over-expressed in hypoxic conditions. HCC cells cannot proliferate under hypoxia without a high level of HIF-1α expression, making it as a potential cancer treatment target. Using the LOPAC (Library of Pharmacologically Active Compounds) drug library, we have identified multiple drug molecules that inhibit the expression of HIF-1α. **Methods:** We constructed specific SK-HEP-1 cells, a human liver cancer cell line, expressing the luciferase reporter gene under the promoter of HIF-1α. By using the cells, we screened the (LOPAC) library. Other bioanalytical methods were used to confirm and analyze identified candidates. **Results:** We successfully identified several compounds that are able to inhibit HIF-1α expression by using luciferase activity assays and Western blot analysis (WB). Further investigation was conducted to determine the molecular mechanism and function for one of the drug candidates, termed as UZ-1, in inhibiting HIF-1α in HCC by using the MTT assay and WB. UZ-1, an antirheumatic agent, showed a strong inhibitory effect on the expression of HIF-1α in SK-HEP-1 cells. In addition, it suppressed the signaling pathways associated with HIF-1α, leading to the apoptosis of HCC cells. **Conclusion**: The UZ-1 molecule inhibits HIF-1α expression, as a result, and displays anticancer function in HCC cells through inducing apoptosis. Our result suggests that UZ-1 may be a potential drug candidate for the treatment of HCC.

B-365

Serum MAGE-A3 and MAGE-A4 Protein Levels as Potential Prognostic Biomarkers for Lung Cancer

D. Kong[1], R. Gonzalez[1], J. Yom[1], Z. Guo[1], T. Qu[1], X. Hu[2], Q. Ren[2], Z. Wu[2], E. Zewdu[1], H. Guo[1], B. Gilmore[1], Z. Zhao[1], X. Liu[1], W. Fu[1]. *[1]OriGene Technologies, Inc., Rockville, MD, [2]OriGene Wuxi Biotechnology Co., Ltd, Wuxi, China*

Background: Melanoma-associated antigen 3 (MAGE-A3) and MAGE-A4 are members of Melanoma Antigen Gene (MAGE) family, which have restricted expression to the testis and are abnormally expressed in cancer cells. MAGE-A3 and MAGE-A4 are overexpressed in lung cancer and have been shown to be associated with cancer progression and poor prognosis. Elevated MAGE-A4 protein level in lung cancer serum samples has been reported in the literature, however, it is unclear whether MAGE-A3 protein can be detected in serum samples of lung cancer patients.

Methods: We developed human MAGE-A3 and MAGE-A4 sandwich ELISA kits and used them to measure serum MAGE-A3 and MAGE-A4 protein levels in 21 lung cancer patients and 30 non-cancer controls.

Results: Serum MAGE-A3 protein was detected in all 21 lung cancer patients (range: 34 to 78871 pg/ml, mean: 5600pg/ml) and was significantly elevated compared to non-cancer controls (range: 23-308 pg/ml, mean: 140pg/ml) ($p<0.05$). Serum MAGE-A4 protein was detected in 5 of 21 (24%) lung cancer patients (range: 280 to 35096 pg/ml, mean: 1819pg/ml), and the samples with high serum MAGE-A4 concentration matched those with high serum MAGE-A3 concentration. Serum MAGE-A4 protein was not detectable in non-cancer controls.

Conclusion: Our findings suggest that serum MAGE-A3 and MAGE-A4 may serve as potential prognostic biomarkers for lung cancer. The detection of elevated serum MAGE-A3 and MAGE-A4 in lung cancer patients indicates their specificity for the disease. Larger studies with more samples, different cancer stages, and longer follow-up are needed to validate these results and establish the clinical utility of serum MAGE-A3 and MAGE-A4 levels as prognostic markers for lung cancer. If further validated, these biomarkers could aid in early diagnosis and better management of lung cancer.

B-366

Thyroglobulin Reflex to Manage Thyroglobulin Autoantibody Interference in an Academic Medical Center: A Comparison of Two Immunoassays and an LC-MS/MS Assay in the Presence of Thyroglobulin Autoantibodies.

M. Dee, N. Boyert, D. Payto, J. Colon-Franco. *Cleveland Clinic Foundation, Cleveland, OH*

Background Thyroglobulin (Tg) measurement is clinically important during the management of patients with differentiated thyroid carcinoma (DTC). Enzyme immunoassays (EIA) offer an affordable and highly automated method for measuring Tg. However, in the presence of thyroglobulin autoantibodies (TgAb), Tg levels may be falsely low in EIA assays due to interference. Tg testing using liquid chromatography tandem mass spectrometry (LC-MS/MS) is recommended when TgAb are detected. We implemented a reflex test to Tg by LC-MS/MS when TgAb are present and may cause interference in EIA tests. Reflex cutoffs need to be refined to minimize interferences while preventing unnecessary reflex testing. **Methods** Our objective was to compare two Tg EIAs and an LC-MS/MS assay in the presence of TgAb, and the prevalence and clinical performance of two TgAb EIAs. TgAb was measured on the Abbott Architect and Beckman Coulter DxI assays in 602 samples from patients with DTC and other suspected thyroid conditions between 11/29/2022 and 2/1/2023. In all samples, Tg was measured by two EIAs, the Siemens Immulite and Beckman Coulter DxI. When TgAb by the Abbott assay was >14.4 IU/mL, Tg was tested by LC-MS/MS in a reference lab. **Results** Fifty-seven specimens reflexed to LC-MS/MS testing based on the cutoff >14.4 IU/mL for the Abbott assay. Of these 57 samples, 42 samples would have reflexed by the Abbott and Beckman Coulter assay (cutoff ≥4.0 IU/mL) and 15 would not have reflexed using the Beckman Coulter assay. Fifteen specimens would have reflexed to LC-MS/MS using the Beckman Coulter cutoff, but not according to the Abbott assay. The TgAb positivity rate was 9.5% for both assays, and the overall reflexing agreement was 95.0% in the entire cohort of 602 results. For the 15 specimens that the Beckman Coulter assay would not have reflexed, LC-MS/MS and the EIAs demonstrated clinically matching results in 13 specimens. Twenty-nine of 57 specimens had undetectable Tg levels (<0.5 ng/mL) by LC-MS/MS, also had Tg <0.5 ng/mL by the Beckman Coulter assay, while 27 measured at below the limit of quantitation (<0.9 ng/mL) for the Siemens Immulite assay. Five results were discrepant near detectable ranges (detectable by LC-MS/MS and falsely low by EIA), with one result more closely agreeing to the Siemens EIA (a patient with no DTC diagnosis) and the other four more closely agreeing to the Beckman Coulter EIA. Linear regression comparing the Siemens Immulite (LC-MS/MS = 0.7 * Immulite + 8.7, r = 0.99) and Beckman Coulter DxI (LC-MS/MS = 1.2 * DXI – 0.6, r = 1.00) to the LC-MS/MS assay, demonstrated biased results but strong correlations. **Conclusion** The Beckman Coulter EIA demonstrated better agreement with LC-MS/MS results at or near lower limits of quantitation in patients with TgAb. While linear regression demonstrated strong correlation between assays, the five discrepant results indicate that TgAb interference impacts Tg results by EIA. The TgAb threshold for reflex testing should continue to be investigated.

B-367

Analytical validation of new CLIA based TPS assay as a biomarker in cancer management.

K. Jagarlamudi[1], Y. Damico[1], P. Pellegrini[1], M. Torstensson[1], Z. Xia[2], L. Xue[2]. *[1]AroCell AB, Stockholm, Sweden, [2]ZECEN Biotech CO., Ltd, Jiangsu, China*

Background: Tissue polypeptide-specific antigen (TPS) has been described as a potentially useful serum marker of tumor activity in adult epithelial tumors such as breast, ovarian, prostate, and other malignancies. Here we developed and analytically validated Chemiluminescence immunoassay (CLIA) for TPS on the CIA series analyzer and FOSUN series analyzer in collaboration with Jiangsu ZECEN Biotech Co., Ltd (Jiangsu and Beijing, China).

Methods:: The TPS-CLIA relies on two monoclonal antibodies generated against peptides that bind to cytokeratin 18 (CK18). The TPS-CLIA assay has been analytically validated and a reference range was established by using sera from blood donors (n=101), and potential applicability was demonstrated in sera from patients with different malignant diseases (n=54). The same serum samples were also analyzed with TPS-ELISA for comparison

Results: The TPS-CLIA had a limit of the blank of 6 U/L with a limit of detection of 10 U/L and a dynamic range of 10 -1200 U/L. The total assay precision was below 20% and no hook effect was observed up to 3265 U/L. Linear dilution of high endogenous TPS sera at 1:4 to 1:256 had shown a recovery in the range of 85-115%. No significant interference was observed with bilirubin, hemoglobin, and triglycerides (<10%, Table 1). The assay binds to CK18 and no cross-reactivity was observed with CK8 or CK19. The cut-off value (95% CI) based on the blood donor sera was 80 U/L (range = 10-116). There was a significant correlation between TPS-CLIA and TPS-ELISA assay (r=0.93; p<0.0001) in different malignancies. More analytical parameters of TPS-CLIA are summarized as shown in Table 1. *Table 1*. Analytical validation of TPS-CLIA

Parameter	Value/Range
Dynamic Range	10-1200 U/L
LoB	6 U/L
LoD	10U/L
Linearity	1:4 to 1:256 dilution, R≥0.99
Precision	Intra CV≤8%Inter CV≤15%
Accuracy	Spike recovery in the range of 85-115%
Cut off (95% CI)	80 U/l (Range 10-116)
Hook effect	No hook up to 3265 U/l
Method comparison	R^2 = 0.93 with TPS ELISA
Interference	Bilirubin 20mg/dL, Hemoglobin 500mg/dL, Triglyceride 2000mg/dL, Rheumatoid factor(RF)1500 U/L, Anti-nuclear antibodies(ANA)500 ng/mL, Human anti-mouse antibody(HAMA)1000ng/mL,
Cross-Reactivity	No cross-reactivity with CK8 and CK19

Conclusion: The new TPS-CLIA provides a unique, robust, and convenient method for measuring cytokeratin 18 levels in serum. This can improve the clinical applications of TPS as a biomarker in cancer management.

B-368

Non-invasive Longitudinal Analysis of Cancer Signal to Monitor Disease Progression and Responses to Treatment

C. Gilley, J. Charlton, M. Williams, R. Stevens, J. Sun, Z. Zhang, R. Witkowski, T. Shuber. *Harbinger Health, Cambridge, MA*

Background Multi-cancer early detection products are poised to change cancer treatment and survival. However, current applications lack the ability to translate analytical findings to clinical outcomes. Quantitatively tracking cancer signals is the next frontier for early cancer detection and data-driven intervention. Harbinger Health has pioneered a platform that combines novel epigenomic insights with artificial intelligence to detect cancer and monitor signal over time to track disease progression and/or response to treatment.

Methods We designed a comprehensive enrichment panel (17.8 Mb) which provides simultaneous methylation and mutational state for cancer and tissue-of-origin informative regions as well as a panel of tumor suppressor genes and oncogenic drivers. We also defined the most significant methylation motifs (MMs) that contribute to pan-cancer signal and developed an algorithm to estimate the amount of tumor-derived reads in a cell-free DNA (cfDNA) sample. To verify our methodology, we performed whole exome sequencing (WES) for a cohort of 46 patients with matched FFPE and cfDNA and compared variant allele frequency of somatic mutations to tumor content estimates from MMs. We used this tumor content (%TC) estimate to quantify signal over time and monitor dynamic changes in the tumor. Simultaneously, we tracked orthogonal mutations across treatment.

To clinically assess our quantitative analysis, we sourced longitudinal samples of 21 cancer patients over the course of treatment with four timepoints, averaging 38 days apart. Timepoint 0 (TP0) was before any treatment, while TP1, TP2, and TP3 were taken before subsequent treatment. For most timepoints, we had endpoint RECIST data which identified n=8 samples as progressive disease (PD), n=1 as stable disease (SD), n=9 as partial response (PR), and n=3 as complete response (CR). We also sourced 21 non-cancer subjects with two timepoints, 60 days apart. All samples across these timepoints were captured with the 17.8 Mb panel and analysis was done to assess longitudinal tracking - predict prognosis, identify minimal residual disease (MRD), and/or detect disease progression.

Results We confirmed that our %TC estimates derived using MMs were highly correlated to estimates derived using WES. We therefore utilized our methylation data to estimate %TC for all timepoints across treatment. The %TC ranged from 0.02% to 60%, with Stage IV disease showing the greatest tumor burden, as expected. Notably, %TC showed dynamic fold-changes during treatment, with differential levels between response groups and non-cancers. In a small subset that included three Stage IV pros-

tate cancer patients with high initial %TC, our biomarkers were able to identify one patient that was reported PR but showed an increase of 18.9% tumor content, indicating disease progression. This patient was deceased at last follow-up. [Additional data to be reported.]

Conclusion Our analytical approach of identifying %TC based on Harbinger Health's proprietary enrichment panel was successful in quantitatively monitoring cancer patients through treatment and disease progression. Our data can provide valuable insights to help clinicians make more informed decisions (e.g. increased monitoring or interventions) about patient care. Further development of techniques enriching specifically for these MMs are in progress.

B-370

Clinical utility of microRNA quantitative analysis using double quencher probes and methylation analysis by methylation sensitive restriction enzyme polymerase chain reaction for bladder cancer

A. Naruse[1], A. Kikuchi[1], K. Takagi[2]. *[1]Daiyukai Research Institute for Medical Science, Ichinomiya, Japan, [2]Daiyukai Daiichi Hospital, Ichinomiya, Japan*

Background: Histone modifications, DNA methylation, and microRNA (miRNA)-mediated gene silence are examples of epigenetic changes that regulate gene expression without causing sequence changes. The early phases of neoplastic transformation are characterized by the occurrence of these epigenetic changes, which are believed to be crucial for the development and progression of cancer. To increase the sensitivity and practicality of miRNA analysis, we developed a measuring technique using a ZEN double quencher into hydrolysis probe in reverse transcription quantitative polymerase chain reaction (RT-qPCR). Additionally, we developed a method for DNA methylation analysis employing methylation-sensitive restriction enzymes (MSRE) and methylation-insensitive restriction enzymes (MIRE) that can analyze a number of samples quickly. We examined the clinical usefulness of using these systems to understand the level of miRNA expression and methylation in bladder cancer.

Methods: In this study, 64 biopsied tissues from transurethral bladder tumor resection (TURBT) were used. For miRNA extraction, Cica genius™ RNA Prep Kit (For Tissue) II (KANTO CHEMICAL CO.,INC.) and a solution that improves miRNA extraction efficiency were used. Using stem-loop RT primers, complementary DNA was created from the isolated miRNA solution. Quantitative analysis was performed by RT-qPCR using a ZEN double quencher probe and miR-21,30a, 200c, and U6 were used as target. Genomic DNA (gDNA) was extracted from the TURBT sample using High Pure PCR Template Preparation Kit (Roche). Restriction enzyme digestion was performed using HapII (Takara Bio) as MSRE and MspI (Takara Bio) as MIRE; the latter has the same recognition site as HapII but is not affected by the presence or absence of methylation. Real-time PCR was performed on the LightCycler 96 (Roche) using Universal ProbeLibrary probes (Roche) targeting *ESM1*, *SFMBT2*, and *SEPTIN9.* From the obtained Cp value, each methylation rate was calculated. The Fisher's exact test was used to statistically analyze the relationship among miRNA, methylation data and other clinicopathological factors.

Results: For miR-30a, we observed a higher expression of miRNA was linked to more advanced T stage (P = 0.047). Additionally, ESM1 methylation exhibited a significant correlation with greater grade (P = 0.004) and T stage progress (P = 0.018). Data on miRNA abundance and methylation rate were combined in order to uncover a link with clinical data that was not apparent from either set of data separately. Samples with higher values for both miR-21 and ESM1 methylation rates were shown to have high grade (P=0.007) and progress to the T stage (P=0.006). Furthermore, samples with higher values for both miR-200c and SEPTIN9 methylation rates did not progress to the T stage (P=0.013).

Conclusion: The combined miRNA and methylation data revealed relationships with clinical results that could not be discovered with either data separately, highlighting the clinical usefulness of the current study's findings.

B-371

Two cases of multiple myeloma achieving complete remission but presenting residual M-protein by the EXENT® solution

O. Berlanga, J. Birtwistle, S. Allen, G. Malin, G. Lakos. *The Binding Site, Birmingham, United Kingdom*

Background: Disappearance of the monoclonal component is a condition for achieving complete response (CR), which requires confirmation by bone marrow biopsy for the absence of clonal plasma cells. Electrophoretic methods used for monitoring these patients lack sufficient sensitivity to identify low-level M-protein, potentially missing the presence of serological residual disease once the tests become negative. The EXENT® solution (in development by The Binding Site, part of Thermo Fisher scientific) uses matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) for the identification and quantification of M-proteins. The technique offers high sensitivity and specificity for identifying and tracking M-proteins. The aim of this study is to determine whether the superior analytical sensitivity of mass spectrometry conveys any clinical benefit to patient monitoring.

Methods: We present two multiple myeloma patients seen during routine practice. Baseline and longitudinal samples were available. Serum samples were retrospectively analysed with the EXENT® solution. Briefly, diluted samples were separately incubated with antisera specific for IgG, IgA, IgM, total-κ and total-λ conjugated to paramagnetic beads. Purified immunoglobulins were reduced and eluted from the beads, the solution mixed with matrix, spotted onto MALDI plates, and acquired by MALDI-TOF-MS to determine mass-to-charge (*m/z*), isotype and concentration of the M-proteins. Results were compared to serum protein electrophoresis (SPE) and free light chains (FLC; Freelite, The Binding Site). The EXENT solution responses were modelled on those from the International Myeloma Working Group. CR by MS refers to absence of the original monoclonal peak during follow-up.

Results: Case 1 is a 68-year-old white male presenting with 11.7 g/L monoclonal IgGκ on SPE and abnormal FLC ratio (3.06). After initiating therapy, FLC ratio normalized at month 2, whereas SPE became negative at month 8, at which time a CR was confirmed. The patient stayed in remission for over two-and-a-half years before relapsing at month 40, as determined by re-appearance of the M-protein on SPE (2.20 g/L). The EXENT solution also identified an IgGκ peak at baseline (*m/z*=11629, 11.9 g/L). The peak remained present until month 19, disappeared for over a year, then reemerged at month 36 (0.39 g/L) to indicate progression. Thus, the EXENT solution identified the presence of residual M-protein (<1 g/L) for an additional eleven months after standard monitoring methods indicated CR; and recognized relapse from CR four months before SPE.Case 2 is a 76-year-old Asian male presenting with 32.9 g/L monoclonal IgGλ on SPE and normal FLC ratio (0.43). The EXENT solution reported an IgGλ peak (*m/z*=11250, 44.3 g/L) at this time. SPE became negative ten months after starting treatment; and identified relapse from CR one year after. The EXENT solution remained positive throughout and was the only method detecting low-level M-protein (≤1.25 g/L) during the 1-year remission period.

Conclusion: EXENT demonstrates a potentially superior performance over electrophoretic methods for identifying residual serological disease during monitoring; and shows potential as a serum biomarker of early relapse in myeloma. The improved sensitivity of MS suggests it may delay and, in some cases, avoid the need for invasive methods to determine response to therapy.

B-373

The Development of a Logic-Gate Probe to Investigate Hypoxia in Cancer Stem Cells

C. B. Swartchick, J. Chan. *University of Illinois at Urbana Champaign, Urbana, IL*

Background:

Cancer stem cells (CSCs) represent only a small fraction of the tumor population, yet they present a profound challenge to cancer treatment. CSCs contribute to tumor complexity via their unique ability to self-renew, develop into multiple lineages, and maintain an unchecked proliferation potential. To identify CSCs, the cytoprotective enzyme aldehyde dehydrogenase 1A1 (ALDH1A1) is a well-annotated biomarker of cancer stemness which is associated with a poor prognosis in patients with gastric and lung cancers when overexpressed. Within CSCs, ALDH1A1 functions to regulate oxidative stress by transforming biological aldehydes into their corresponding non-toxic carboxylic acid counterparts and further is linked to the retinaldehyde signaling pathway. Beyond this enzyme, oxygen availability within the tumor microenvironment (TME) can also influence stemness properties. Specifically, hypoxia, is required to maintain the CSC phenotype by prolonging the undifferentiated state.

Methods:

To study the mechanistic underpinnings of complex relationship in vivo new technologies are needed as current approaches are insufficient. Indeed, probes have been developed to study ALDH1A1 and hypoxia in parallel, however, imaging approaches lack the requisite resolution to ensure they are reporting on the same CSC population. To this end, we have designed a powerful logic-gate probe that links the reporters of ALDH1A1 activity and oxygenation in a unique manner such that different readouts will result (color of light) depending on the TME environment.

Results:

Our lab has previously developed a near-infrared hypoxia-sensing fluorescent probe that is fundamental to our current logic-gate design. This component has been vali-

dated in 4T1 murine breast cancer cells, to yield a 1.6-fold increase in ratiometric fluorescence response when incubated under hypoxic conditions as compared to its normoxic controls. Additionally, the ALDH1A1 sensing component has been validated via purified enzyme to yield an increase in bioluminescent signal enabling its use for future *in vivo* studies.

Conclusion:

This technology has important clinical implications to understand the mechanistic underpinnings between oxygenation and cancer stemness. Further research is being conducted to further test the hypothesis that metformin (anticancer drug) kills cancer by altering oxygen metabolism which in turn depletes the CSC population. Furthermore, this probe has far-reaching implications in monitoring novel therapeutic cancer treatment.

B-374

Early Prediction of Recurrent Non-Muscle Invasive Bladder Cancer Using ATR-FTIR in Urine Samples

H. Kalil[1], S. Maher[2], A. Mohamed[3], A. El-Tobgy[4], M. Bayachou[1], A. El-Falouji[4]. *[1]Cleveland State University, Cleveland, OH, [2]Cleveland Clinic, Cleveland, OH, [3]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [4]Suez Canal University, Ismailia, Egypt*

Background: Non-Muscle Invasive Bladder Cancer (NMIBC) represents approximately 80% of all cases of bladder cancer. It consists primarily of low-grade tumors but has been associated with a high recurrence rate. The high recurrence rate is a significant challenge in its management and treatment plan. Recurrence can occur within months of initial treatment and can lead to more invasive treatment, including surgery combined with additional rounds of chemotherapy. Although the 5-year relative survival rate for patients with NMIBC is around 90%, the risk of recurrence is also high, with up to 70% of patients experiencing a recurrence within 5 years of initial treatment. For this reason, ongoing surveillance and monitoring are critical for people with NMIBC. Invasive cystoscopy, the current standard of care for NMIBC diagnosis and surveillance, can be uncomfortable and painful and carry the risk of complications such as bleeding, infection, and bladder perforation. The development of a fast and non-invasive biopsy-based assay would significantly improve the management of NMIBC. A liquid biopsy-based assay would provide a less invasive alternative that could be used to monitor the disease and detect recurrence while reducing the need for invasive cystoscopy. **Methods**: A fast liquid biopsy-based assay is used with urine samples to enhance the detection of cancer-specific biomarkers. Here, we describe a fast spectroscopic approach for detecting NMIBC recurrence in urine samples. Before the cystoscopy examination, urine samples from 62 previously diagnosed NMIBC patients were collected at their follow-up appointments. Fourier transforms infrared (ATR-FTIR) spectra were acquired from urine samples using the direct application. Utilizing parameter grid searching, spectral processing, and normalization were enhanced. Multivariate and principal component analyses (PCA) were employed to examine the technical variability. **Results**: Cystoscopy outcomes of 62 NMIBC patients (41 cancer-free, 21 recurrences) were documented and used as a reference to our non-invasive approach. We examined the performance of 35 machine learning models using a training set (70%) and a held-out test set (30%). A Regularized Random Forests (RRF) model attained an AUROC of 0.92 with a sensitivity of 86% and a specificity of 77%. The variable importance and predictive ROC spikes around 2912 cm^{-1} have confirmed the peak of lipid CH_2 asymmetrical stretching. An additional spike is also observed around 2980 cm^{-1}, reflecting the stretching vibrations of methyl hydrocarbon chains. Analyzing processed spectra clearly reveals spectral differences between recurrence and NMIBC-free samples. Together, our results suggest an altered lipid profile in urine samples of the recurrent NMIBC patients. **Conclusion**: To the best of our knowledge, this is the first study showing the CH_2 lipid asymmetrical fingerprint on the FTIR spectra in the range of 2800 to 3000 cm^{-1}, indicating the recurrence of NMIBC. Because of this explicit fingerprint, this liquid-biopsy spectroscopic technique can offer a promising non-invasive diagnostic tool for the early detection of recurrent NMIBC.

B-375

Mer-idpcr: a method for sequence-specific measurement of small rna expression and methylation in liquid biopsies

R. Horos, A. Daniel-Moreno, C. Bieg-Salazar, J. Skottke, J. Ceiler, M. Sanchez-Delgado, T. Sikosek, T. Rajakumar, B. R. Steinkraus. *Hummingbird Diagnostics GmbH, Heidelberg, Germany*

Background: Small RNAs are an important class of biomarkers in liquid biopsy for cancer diagnostics. New discoveries in the field of "epitranscriptomics" have uncovered further functional layers of tumor biology that are ignored by standard gene expression measurement methods. Therefore, there is a pressing need for sequence specific methods that can detect methylation stoichiometry on small RNAs purified from liquid biopsies in a diagnostic setting.

Methods: To detect small RNAs specifically, we designed structured oligonucleotide adapters that bear reverse complementary overhangs to both the 5' and 3' end of target RNAs. After nick-based ligation, reverse transcription under limiting dNTP conditions enables the differential processing of methylated versus unmethylated species. Finally, target specific TaqMan probes are used on a digital PCR based platform for final quantification and calculation of methylation stoichiometry.

Results: We show that mer-idPCR can detect target RNAs in a sequence-specific manner using synthetic cognate RNA oligos and specificity controls, such as nucleotide variants or non-target miRNAs. Detected RNA is quantified in a linear range with a limit of detection at 40 copies/µl of cDNA. Concurrently, the method can detect 2'-o-methylation in 0-100% range with a resolution of 10%. mer-idPCR can be applied to RNA extracted from various sources including serum, plasma or whole blood stabilizing tubes.

Conclusion: mer-idPCR can enable the high resolution, sequence specific quantification of target small RNA species, in addition to the measurement of 2'-o-methylation site status, in small RNAs extracted from peripheral blood. We show the adapter design requirements and discuss optimizations of adapter design and reverse transcription conditions that led to improved specificity, reproducibility, and linearity of this method. mer-idPCR enables the validation and discovery of novel biomarkers for cancer detection in liquid biopsies.

B-376

Performance of pathological anatomy immunohistochemical markers and serum tumor markers in prostate cancer.

I. C. Silva[1], G. C. Carvalho[1], F. A. Vilaca[1], D. R. Ramadan[1], S. Tufik[1], M. C. Feres[2]. *[1]Associação Fundo de Incentivo a Pesquisa - Afip, Sao Paulo, Brazil, [2]Associação Fundo de Incentivo - Afip, Sao Paluo, Brazil*

Background: The gold standard method for diagnosing prostate cancer is systematic prostate biopsy under ultrasound guidance and local anesthesia.Serum tumor marker tests (PSA, Free PSA and testosterone) and confirmatory or classificatory immunohistochemical (IHC) complement the conclusion of the patient's report. The maximum amount of information regarding the pathological anatomy and markers provided to physicians favors assertive decision-making in the conduct of treatment. However, the results of prostate biopsies after treatment with prostatectomy can differ significantly in the course of the disease. In this context, the authors' objectives were to analyze the performance of the dosages of these widely used serum markers against the IHC markers of biopsies, as well as their indicators of positive and negative predictive values (PPV and NPV) for prostatic neoplasms. The authors analyzed the level of agreement between pathological prostate biopsy results and immunohistochemical (IHC) testing for 34ßE12, p63, and alpha-methylacyl-CoA racemase (AMACR) to provide final pathological results for prostate cancer, as well as their relationship with data provided by biochemical tests for the markers PSA, Free PSA and Testosterone

Methods: Descriptive, retrospective study with a qualitative and quantitative approach, based on data obtained through IHC exams and available in a database of a large laboratory in São Paulo - Brazil. Data collection was based on anatomopathological results of male patients diagnosed with prostate cancer, aged between 40 and 69 years between August 2021 and December 2022 with a total of 1403 analyzes for biopsies using IHC combined markers such as 34ßE12 , p63 and AMACR, and a total of 144 analyzes for combined prostatic serum markers (total PSA, free PSA and testosterone). All analyzes were conducted using the R software version 4.1.0 (R CORE TEAM, 2021) and considered a significance level (α) of 5%.

Results: The Mann-Whitney test indicated that the distribution of the PSA variable (ng/dL) does not differ statistically between the 34ßE12 groups (W = 822.0; p = 0.224; r = 0.139 , p63 (W = 826.0; p = 0.361 ; r = 0.103), and AMACR (W = 610.0; p = 0.351; r = -0.106). Likewise, the Free PSA marker (ng/dL) did not differ statistically between

34ßE12 (W = 309.5; p = 0.473 ; r = -0.097), p63 (W = 319.5; p = 0.488; r =- 0.093 and AMACR (W = 372.0; p = 0.725; r = 0.048). The results of the ROC curve with PSA (ng/dL) as predictor of the biopsy result with the marker 34ßE12, p63 and AMACR the mean positive predictive values (MPVP) were 0.623 , 0.635 and 0.615 respectively. For Free PSA (ng/dL) with the markers respectively, the VPPM were 0.743, 0.750 and 0.455. As for testosterone, our sample was very small.

Conclusion: Our findings showed that all markers are important indicators for the diagnosis and monitoring of prostate cancer and that they complement each other. Free PSA showed good PPV for 34ßE12 and p63 markers and low PPV for AMACR.

B-377

Mirsnps as potential colorectal cancer biomarkers: an overview

C. Santos Silva, K. Alves dos Santos, K. Costa de Souza, V. Nogueira Silbiger, A. Ducati Luchessi. *Federal University of Rio Grande do Norte, Natal, Brazil*

Background: Colorectal cancer (CRC), also known as colon cancer, is a neoplasm that affects the colon (large intestine) or rectum. CRC stands out for being the third most frequent neoplasm in the world population, being the second cancer with the highest mortality rate. Single nucleotide polymorphisms (SNPs) located in miRNAs genes, known as miRSNPs, may be associated with dysregulated expression of miRNAs in neoplasms. The objective of this review was to highlight the SNPs that alter the biogenesis or processing of miRNAs associated with CRC as potential biomarkers of this disease, according to the literature. **Methods:** A search of original articles in the literature was performed in PubMed, Embase and Web of Science databases. The bibliographic search included scientific articles published between 2008 and 2022, with the objective of investigating associations between miRSNPs and CRC. Exclusion criteria were brief communications and other reviews on the subject. A total of 263 articles were detected in the preliminary search, of which 133 were excluded for being replicated, 34 for being reviewers, 3 for being paid and 40 for dealing with other subjects. Thus, of the remaining 53 studies, 35 were excluded after reading because they included studies that described SNPs located in miRNA genes and SNPs located in miRNA binding sites. Finally, 18 articles that included SNPs located in the regulatory regions of miRNAs were selected for this review. The list of genes generated for each of the targets was performed using the online tool miRBase and miRDB, for prediction of miRNA targets and functional annotations. The miRTarBase online database was consulted to select target miRNAs and possible interactions. By crossing the list of genes targeted by miRNAs to identify the biological pathways, those with the greatest biological relevance were chosen using the Enrichr online tool. **Results:** This review covered 18 studies related to SNPs located in regulatory regions of mature miRNAs that may influence the occurrence and progression of CRC. In order to characterize the metabolic profile induced by each target of the miRNAs selected in this review, it was possible to observe that miR-196, miR-211, miR-30a, miR-143, miR-27 and miR-146a are more strongly associated with positive regulation of transcription. Cytokine-mediated signaling pathway and regulation of cell migration were most strongly associated with miR-143 and miR-146a. Furthermore, it was identified that the upregulation of the apoptosis process was strongly associated with miR-30a, miR-143, miR-27a and miR-146a. MiR-211, miR-143, mi-27a and miR-146a were strongly associated with the upregulation of cell proliferation. Finally, with the objective of evaluating the correlation between the selected miRNAs. It was possible to observe a greater correlation between miR-27a and miR-30a. **Conclusion:** Studies related to miRSNPs have been of great interest in recent years in order to find promising and less invasive new biomarkers, sensitive and specific for the early detection of CRC. Our study aims to help better understand the pathways that cause the development of CRC and possible biomarkers to predict its development and consequently reduce morbidity and mortality and improve patient survival.

B-378

Cell-free plasma preparation workflow solution

L. Manley, S. Lopez, L. Kothandaramaswamy, H. Saunders, A. Cheng. *Thermo Fisher Scientific, Austin, TX*

Background: Liquid biopsy for the study and monitoring of various cancers has become a growing area of study, pre-analytical sample handling can impact the quality and profile of the circulating tumor nucleic acid. Preparing samples like cell-free plasma for downstream assays is imperative to maintaining quality in downstream applications for clinical research in oncology. To obtain cell-free plasma, blood samples must undergo centrifugation at 2000g for 10mins at 4C followed by a second spin for 30mins at 4C. This two-step spin process presents an issue with labs that don't have a refrigerated centrifuge suitable for higher speeds. **Methods:** Here, we perform a series of studies to investigate the impact of various spin conditions on cell-free nucleic acid recovery and quality. Four plasma centrifugation methods encompassing variables attributing to speed, temperature, and number of centrifugations steps, were evaluated from whole blood tubes obtained from six donors in K2EDTA upfront of nucleic acid isolation with MagMAX™ cell-free TNA nucleic acid isolation chemistry on a KingFisher™ magnetic particle processor. Yield, quality, and functional performance was evaluated from the extracted cell-free nucleic acid and compared across all plasma spin-down methods. **Result:** Study results indicated that centrifugation of plasma at lower speeds for a shorter period at room temperature had no negative impact to nucleic acid recovery and integrity. **Conclusion:** Here, we offer a plasma spin workflow solution to mid- to high- throughput clinical research labs to allow versatility within the laboratory setup. Reducing the need for refrigerated centrifugation allows clinical researchers to obtain high-quality cfNA suitable for their downstream applications in clinical oncology and genomics research.

B-379

Analytical Performance of Two Tumor Marker Immunoassays on the Atellica CI 1900 Analyzer

M. Quintanilla, A. Baldys, K. Thakur, G. Arrode-Bruses, J. Rhea-McManus, H. Leipold. *Siemens Healthineers, Tarrytown, NY*

Background: The Atellica® CI 1900 Analyzer is an automated, mid-throughput, integrated chemistry and immunoassay analyzer utilizing both Atellica CH and Atellica IM assays. This study was designed to evaluate the analytical performance of the Atellica IM Carcinoembryonic Antigen (CEA) and Alpha Fetoprotein (AFP) assays* on the Atellica CI 1900 Analyzer. **Methods:** The Atellica CI 1900 CEA and AFP assays use the same reagents and calibrators as the comparable Atellica IM assays. Precision and method comparison (MC) were used as performance indicators for the Atellica CI 1900 Analyzer. Precision studies were performed according to CLSI EP05-A3 using quality control, native, and contrived human serum samples. One aliquot of each sample pool was tested in duplicate in two runs per day ≥2 hours apart on each analyzer for ≥20 days. MC studies were performed according to CLSI EP09-A3. Individual native and contrived human serum samples were analyzed using the Atellica IM assays on both the Atellica IM and Atellica CI 1900 Analyzers. **Results:** Representative precision and MC results observed for each assay across indicated sample ranges are listed in the table. Slopes determined by the Deming linear regression model were approximately equal to 1. **Conclusion:** Evaluation of the CEA and AFP assays using the Atellica CI 1900 Analyzer demonstrated good precision and equivalent performance compared to the same assays on the Atellica IM Analyzer.

Precision	CEA Assay	AFP Assay
Sample range, ng/mL	1.59–79.18	3.8–898.4
Repeatability, %CV range	1.4–3.6	1.8–12.9
Within laboratory, %CV range	2.1–5.5	2.6–15.0
Method Comparison	**CEA Assay**	**AFP Assay**
Sample range, ng/mL	2.49–95.38	2.9–893.7
Regression equation for Atellica IM comparative assay	y = 0.98x + 0.07 ng/mL	y = 1.06x – 0.7 ng/mL

*The products/features mentioned here are not commercially available in all countries. Their future availability cannot be guaranteed.

B-380

Drift of Freelite on Optilite

M. H. Griffiths[1], V. Kulasingam[2], P. L. Cheng[2], X. Y. Wang[2], R. J. Schneider[3]. *[1]Diazyme Laboratories, Poway, CA, [2]Division of Clinical Biochemistry, University Health Network, Toronto, ON, Canada, [3]Abbott Laboratories, Abbott Park, IL*

BACKGROUND: Serum free light chains (sFLC) which include kappa (KFLC), lambda (LFLC) and their ratio (K/L) are used for diagnosis and monitoring of plasma cell dyscrasias (PCDs). In 2002, the Binding Site Freelite assay established reference intervals (RIs) for KFLC, LFLC and K/L. Since that time, renal RIs have been proposed and drift for the Freelite assays has been reported. The objective of this study was to evaluate healthy and renal insufficiency population RIs and examine retrospective data to ascertain the reported assay drift. **METHODS:** 260 serum samples were measured using the Freelite assay on the Optilite. Creatinine was measured on the Abbott Alinity and eGFR was calculated. Binding Site's RIs for KFLC, LFLC and

K/L were compared to samples with ≥60 eGFR and ≤59 eGFR samples were compared to the hypergammaglobulinemia population from a previous report in 2002. IFE was used to exclude samples with detectable monoclonal proteins. Retrospective data from University Health Network (UHN) was examined. **RESULTS:** The comparison results for the samples are in the tables below:

			Source			
Group	**Analyte**	**RI parameters**	**Optilite Product Insert (mg/L)**	**This study (mg/L)**	**Factor over 2002 data**	**Avg % over 2002**
eGFR ≥60	KFLC	2.5%	3.3	11.9	3.6	**340%**
		Median	7.3	21.9	3.0	
		97.5%	19.4	72.5	3.7	
	LFLC	2.5%	5.7	9.6	1.7	**170%**
		Median	12.4	18.0	1.5	
		97.5%	26.3	48.8	1.9	
	K/L	0%	0.26	0.40	1.5	**210%**
		2.5%*	0.30	0.80	2.7	
		Median	0.60	1.26	2.1	
		97.5%*	1.20	1.85	1.5	
		100%	1.65	2.90	1.8	
			Source			
Group	**Analyte**	**Data characteristics**	**Katzmann 2002* (mg/L)**	**This study (mg/L)**	**Factor over 2002 data**	**Avg % over 2002**
eGFR ≤59	KFLC	Range Lo	4.3	15.9	3.7	**240%**
		Median	19.6	53.5	2.7	
		Range Hi	273	351.2	1.3	
		% abnormal	52%	91%	1.8	
	LFLC	Range Lo	8.5	10.8	1.3	**100%**
		Median	28.8	36.6	1.3	
		Range Hi	307	153.4	0.5	
		% abnormal	58%	64%	1.1	
	K/L	Range Lo	0.38	0.57	1.5	**220%**
		Median	0.55	1.42	2.6	
		Range Hi	1.18	3.12	2.6	
		% abnormal	0%	33%	N/A	

The data suggest that Freelite has drifted up approximately 300% for KFLC, 150% for LFLC and 200% for K/L. Using 220,000 data points from UHN and applying these recalibration factors, the normal KFLC vs LFLC bivariate distribution appears centered to the well-known '0.26-1.65' K/L 100% diagnostic range.**CONCLUSIONS:** Drift of Freelite is confirmed with this study and recalibration factors for Freelite are proposed which are lower KFLC values by 300% and lower LFLC values by 150% for K/L to be centered to 0.26-1.65. Institutions should review their data with Freelite on Optilite and any clinical data to confirm this finding. Drift of this magnitude has the potential to cause false positives for kappa and false negatives for lambda in the diagnosis and monitoring of PCDs.

B-381

A Multiplex Blood-Based Assay Targeting DNA methylation in Peripheral Blood Mononuclear Cells Enables Early Detection of Breast Cancer

C. Wang, J. Li, T. Wang. *The Second Hospital of Shandong University, Jinan, China*

Background: The immune system can monitor the development of tumors, and the DNA methylation is involved in the body immune response to tumors. Therefore, the DNA from peripheral blood mononuclear cell (PBMC) that carries cancer-specific epigenetic aberrations may serve as a non-invasive marker for early detection of breast cancer (BC). **Methods:** Genome-wide DNA methylation profiling on PBMC DNA from BC patients and healthy controls (Cohort I, n=80) was performed in the discovery phase by Infinium 850K BeadChips. The candidate CpG sites were selected and validated in a multistep validation phase (Cohort II, n =200) via pyrosequencing and targeted bisulfite sequencing. After that, the multiplex quantitative methylation-specific PCR assay (mBC-MSP) was established to detect BC, and the performance of this diagnostic method was analyzed in a multicenter cohort (Cohort III, n=501). **Results:** A total of 289 differentially methylated CpG positions (DMPs) between BC patients and healthy controls were identified. Among them, eight significant DMPs markers were selected and further validated using pyrosequencing and targeted bisulfite sequencing, and four hyper-methylated DMPs (cg18637238, cg16652347, cg13828440, cg11754974) passed the final validation. Then, we developed a mBC-MSP assay based on the four methylated markers. This assay exhibited excellent performance in distinguishing BC patients, with an AUC of 0.925, sensitivity of 83.1%, and specificity of 90.4%. More importantly, compared with CA153, CA125 and CEA, the mBC-MSP assay had higher sensitivity for early-stage BC (stage 0, 88.2% vs. 0%, 0%, 5.9%) and minimal tumor with diameter ≤ 1.5 cm (91.7% vs. 0%, 2.1%, 0%). **Conclusions:** The mBC-MSP assay based on the four identified DNA methylation markers from PBMC can serve as a noninvasive, accurate, rapid, and high-throughput method for early diagnosis of BC, and it is more sensitive than traditional tumor markers for detecting early-stage BC and minimal tumor.

B-382

Accurate and early detection of colorectal cancer using a multilocus DNA methylation markers-based testing in peripheral blood mononuclear cells

C. Wang[1], p. li[2], y. xie[2]. *[1]The Second Hospital of Shandong University, Jinan, China, [2]The Second Hospital of Shandong University, jinan, China*

Background: An effective blood-based method for the diagnosis of colorectal cancer (CRC) has not yet been developed. Molecular alterations of immune cells occur early in tumorigenesis, providing the theoretical underpinning for early cancer diagnosis. Therefore, we aimed to develop an effective multi-molecular simultaneous detection method based on peripheral blood mononuclear cells (PBMCs) to improve the diagnosis of early-stage CRC. **Methods:** Candidate DNA methylation markers were first identified from PBMCs using the Infinium MethylationEPIC array in the discovery phase (Shandong cohort I, n=100) and further validated via pyrosequencing and targeted bisulfite sequencing in validation phase (Shandong cohort II, n=202). Then, a single-tube multiplex methylation-specific quantitative PCR assay (multi-msqPCR) for simultaneous detection of five markers was established in a pilot study. After that, a CRC prediction model (CPM) based on DNA methylation and multi-msqPCR method was construct and its diagnostic performance were evaluated using the area under the receiver operating characteristic curve (AUROC) in our multicenter cohort (n=595). **Results:** Five discriminative DNA methylation markers identified by Illumina 850K microarray were successfully validated to diagnose early-stage CRC. Multi-msqPCR showed a better discriminative performance and 10-time lower detection limit than single-molecule detection in early-stage CRC. The cross-validated AUROC for CPM for early-stage CRC was 0.91 (sensitivity=81.18%; specificity=89.39%), significantly higher than CEA (AUROC =0.62). CPM assay also yielded a high degree of discrimination for advanced adenoma (AA) cases (AUROC=0.85; sensitivity=63.04%; specificity=89.39%). Besides, detecting CPM in multiple cancer types implied a CRC-specific diagnostic value. Our follow-up data also demonstrated that CPM could detect early-stage CRC up to 2 years before current traditional diagnostic methods. **Conclusions:** CPM, in combination with epigenetic biomarkers and the multi-msqPCR method, was promising, cost-effective, and easily implementable for routine clinical diagnosis of early-stage CRC.

B-383

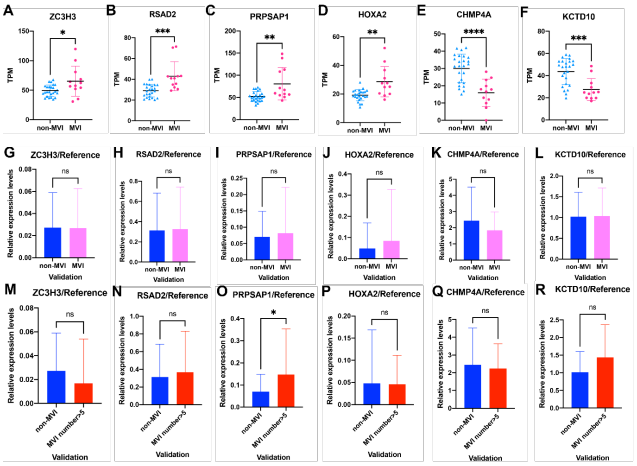
Exosomal PRPSAP1 in plasma predicts microvascular invasion in hepatocellular carcinoma

Z. Xin, J. Zhou, H. Chen, M. Song, L. Jiao, B. Ying. *West China Hospital of Sichuan University, Chengdu, China*

Background: Microvascular invasion (MVI), a critical predictor of postoperative tumor recurrence in hepatocellular carcinoma (HCC), is only detectable by histopathological examination of the surgical specimen. Prediction of MVI before surgery is of great significance to prognosis, especially by non-invasive means. Here, we report that exosome-derived mRNA in plasma can be promising biomarkers for MVI preoperatively. **Methods:** Patients with initially suspected HCC who were undergoing hepatectomy were prospectively recruited with preoperative peripheral blood collection and followed up. Exosomal mRNA profiling was performed using RNA se-

quencing in the discovery cohort, and the edgeR package in R software was used to screen out significantly differential expression exosomal mRNAs between MVI and non-MVI groups. Multiplex droplet digital PCR (ddPCR) technology was utilized to verify filtered exosomal mRNAs in the validation cohort. **Results:** A total of 131 HCC patients were enrolled with 37 in the discovery cohort and 94 in the validation cohort. RNA-sequencing data identified six candidate exosomal mRNAs (ZC3H3, RSAD2, PRPSAP1, HOXA2, CHPM4A and KCTD10) as potential predictors for MVI. In the validation cohort, expression levels of PRPSAP1 and HOXA2 were slightly higher and the expression level of CHMP4A was slightly lower in the MVI group than the non-MVI one, which were consistent with the discovery cohort though without statistically significant difference (P>0.05). Further analysis between MVI number>5 and non-MVI patients validated that the expression level of exosomal PRPSAP1 was significantly higher in MVI number>5 patients (0.147 vs 0.070, P=0.035). Altogether, exosomal PRPSAP1 in plasma was a promising biomarker predicting MVI for HCC patients. **Conclusion:** We validated for the first time that high level expression of exosome-derived PRPSAP1 can preoperatively predict MVI, especially with the number >5, to improve prognosis.

Key Words: Hepatocellular carcinoma; Microvascular invasion; Extracellular vesicles; Liquid biopsy



B-384

Genetic variations predict severe immunotherapy-related adverse events for PD-1/PD-L1 inhibitors

Z. Xin[1], L. You[1], F. Na[1], J. Li[1], M. Chen[2], J. Song[1], L. Bai[1], J. Chen[1], J. Zhou[1], B. Ying[1]. *[1]West China Hospital of Sichuan University, Chengdu, China, [2]The First Affiliated Hospital of Hainan Medical College, Haikou, China*

Background: PD-1/PD-L1 inhibitors have brought remarkable benefits but can cause profound immune-related adverse events (irAEs), especially life-threatening toxicities. The host immunogenetic background is likely to play a role in irAE susceptibility. Here, we identified the variants that could predict severe irAEs in patients with cancer.

Methods: Patients with solid tumors receiving PD-1/PD-L1 blockade were recruited and followed up. Genes considered pivotal contributors to tumor-immunity and autoimmune diseases were screened out via Protein-Protein Interaction network and Cytoscape. Consequently, thirty-nine variants in eighteen genes were genotyped using the multiplex genotyping assay. Association analysis between those variants and severe irAEs (G3-G5 grades) as well as irAEs-free survival was performed.

Results: Two immunogenetic variants as predictors of severe irAEs were identified. The A allele of *ADAD1* rs17388568 (OR=2.599, 95% CI=1.355-4.983, P=0.003) increased the risk of severe irAEs, while the G allele of *IL6* rs1800796 (OR=0.425, 95% CI=0.205-0.881, P=0.018) protected patients from severe irAEs. In multivariate Cox regression analysis, *ADAD1* rs17388568 (AA versus AG or GG: HR=0.11, 95% CI=0.025-0.49, P=0.004) and *IL6* rs1800796 (GG or GC versus CC: HR=3.10, 95% CI=1.315-7.29, P=0.01) were also independent variables for severe irAEs-free survival.

Conclusion: We identified two immunogenetic polymorphisms associated with severe irAEs in PD-1/PD-L1 blockade-treated tumor patients at the first time, and they may serve as promising predictive biomarkers.

B-388

Clinical performance of Xpert BCR-ABL Ultra Assay for isoform p210 quantitation

F. K. Marques, C. P. Mendonça, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Chronic myelogenous leukemia (CML) is one of the most common hematologic neoplasms. More than 95% of patients with CML have the distinctive Philadelphia chromosome (Ph1) that results from a translocation between the chromosomes 9 and 22, and consequently in the *BCR::ABL1* fusion gene. This fusion gene produces a tyrosine kinase with deregulated activity that plays a key role in the development of CML. Monitoring *BCR::ABL* transcript levels in patients on tyrosine kinase inhibitor (TKI) therapy using real-time quantitative PCR is standard of care in the management of CML patients. The test utilizes a quantitative, real-time reverse transcription polymerase chain reaction (RT-qPCR) to measure *BCR::ABL1* to *ABL1* percent ratios on the International Scale (%IS), in t(9;22) positive CML patients during monitoring of treatment with TKIs. In this context, this study evaluated clinical performance of the Xpert® BCR-ABL Ultra assay for quantitation of *BCR::ABL1* p210 transcripts. **Methods:** The clinical performance of assay was evaluated by comparison to an RT-qPCR assay routinely used in our institution, which also detects and quantifies the mRNA transcripts for the p210 translocation types and uses the ABL gene as an endogenous control. In total, 30 fresh peripheral blood samples of CML patients with the level of *BCR::ABL1* transcripts (international scale, IS) between >50% and undetectable were enrolled in this study. All steps of protocols were performed according to manufacturer´s instructions. **Results:** The comparison of two assay revealed consistente molecular response (MR) level. Linear regression analysis showed high correlation between the assays (R^2=0.974, P<0.0001) The slope of regression curve were not significantly different from the value of 1 (1.003; 95% CI, range 0.943 - 1.069). The evaluation of MR level was identical in 21/28 (75%) samples. Two samples were excluded from the study due to failure to control *ABL1* (Ct>18). The median of *ABL1* copies per sample was 66,011 copies (range 20,010 - 236,171) for conventional RT-qPCR assay. Xpert BCR-ABL Ultra reports %IS (MR), but this test does not report *ABL1* copy numbers. If the Xpert BCR-ABL Ultra test reports a positive or negative result and the *ABL1* Ct value is below 18, a minimum of 32,000 *ABL1* copy numbers is guaranteed in the reaction. The *BCR::ABL1* negativity was concordant in 2/8 samples. **Conclusion:** We showed that the results Xpert® BCR-ABL Ultra assay (MR) are in good correlation with the results of RT-qPCR conventional method. The Xpert® BCR-ABL Ultra assay simplifies the generation of results by integrating nucleic acid extraction, amplification, and quantification of the *BCR::ABL1* p210 transcripts in peripheral blood specimens. The assay turn-around-time is approximately 3 hours. This does not differentiate between b2a2 or b3a2 fusion transcripts.This assay is unable to detect *BCR::ABL1* p190 or other rarer translocations.

B-389

Validation of one-step in-house RT-qPCR method for detecting *MLL::AF4* fusion

F. K. Marques, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Rearrangements of mixed lineage leukemia gene (MLL) with 1 of >130 alternative partner genes is a recurrent cytogenetic finding in both acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) and is generally associated with a poor prognosis. Among the most common MLL rearrangement is t(4;11)(q21;q23), forming the MLL::AF4 (also known as *KMT2A::AFF1*) fusion gene. Unique among MLL rearrangements, MLL::AF4 is almost exclusively associated with pro-B cell ALL and is prototypical of infant ALL, where it carries a very poor prognosis. At least 10 different MLL-AF4 fusion transcripts have been found, due to break-points in different introns, some transcripts being more frequent in either adult or infant ALL. Fast and reliable diagnostic tests represent an important tool in clinical laboratories to detect these rearrangements and . In this context, this work aimed to validate a qualitative method to detect the main *MLL::AF4* fusion isoforms. **Methods:** We evaluated the performance of the Hs03043614_ft (e10e4, e9e4 and e9e5) and Hs03024412_ft (e11e4) inventoried TaqMan assays (Thermo Fisher Scientific). Total mRNA from negative and positive samples for each fusion was extracted by the TRIzol reagent according to the manufacturer's instructions. All assays were performed using TaqMan RNA-to-Ct 1-Step kit (Thermo Fisher Scientific). The inventoried *ABL1* assay (Thermo Fisher Scientific) was used as endogenous control. Using positive samples, negative samples and controls of each fusion were performed: sequence verification tests, standardization of RNA input, evaluation of reaction efficiency and determination of the detection limit (LOD). The performance of the assays was evaluated with *ABL1* control separately from the fusions. **Results:** Our results showed that both as-

says worked well for the detection of specific fusions. The best RNA input for the reactions was 200 ng. The efficiencies reactions were: a) with ABL1 in separate reactions: 92.6% and 110.0% for Hs03043614_ft and Hs03024412_ft assays, respectively; The literature recommends the limit of efficiency was between 90% and 110%. The LODs with *ABL1* in separate reactions were 10^{-3} for both assays, respectively. There was no unspecific amplification in negative samples. **Conclusion:** The TaqMan assays with RT one-step protocol evaluated are highly sensitive, fast, and reliable tools for detecting the most frequent MLL::AF4 fusion transcripts.

B-390

Next-generation sequencing panel to detect Homologous recombination deficiency (HRD) biomarkers in Brazilian ovarian cancer samples

A. B. de Lima, L. R. Leite, M. C. Freire, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Homologous recombination deficiency (HRD) has been used as a biomarker of PARP inhibitors (PARPi) sensitivity in ovarian cancer (OC), especially in high-grade serous (HGS) types. Initially, only likely pathogenic (LPV) and pathogenic variants (PV) in *BRCA1/2* were screened to define PARPi eligibility. However, LPV and PV are shown in less than 20% of OC, which restricted PARPi therapies to a small group. Advances in OC biology knowledge identified other markers that also contribute to HRD. Nowadays, is estimated that almost 50% of OC have HRD. When HRD is present, DNA damage is not properly repaired and the accumulated genomic changes over time are called Genomic Scars (GS). In this sense, Next Generation Sequencing (NGS) is important in GS detection due to searching different loci simultaneously, allowing an estimate of the GS score related to the HRD. So, this work aims to describe the profile of samples requesting an NGS test to detect HRD biomarkers. **Methods:** Were evaluated anonymized data of 76 formalin-fixed, paraffin-embedded (FFPE) samples with OC diagnosis. The samples' quality was evaluated with TapeStation 4200 and Qubit Fluorometer. Libraries were prepared with AmoyDx® HRD Focus Kit and sequenced with NextSeq 500. Data analysis was performed with ANDAS software. **Results:** The HRD-positive percentage was 59,2% (45/76), being mostly *BRCA* wild type. Almost 15% of all samples presented PV or LPV in *BRCA1* or *2*, which was more frequent in *BRCA1* (~82%). The allele frequencies (AF) detected ranged from 5 to 83%. Two of the detected variants (classified as LPV) are not yet registered in databases. The HRD-negative group represented 34,2% (26/76), and 6,6% of performed tests had inconclusive results. Some requests (data not included) were not performed due to insufficient material, low quality, or FFPE block age greater than two years, which are limiting factors of this kind of test. **Conclusion:** The availability of validated HRD testing options is essential to extend the reach to patients who may benefit from PARPi. In this analysis, most of the *BRCA1/2* PV or LPV had AF above 50%, which can help oncologists to suggest germline PV investigation for these patients and also their families. Besides, our data showed a slightly higher HRD-positive rate than reported in the literature, although other works have already described rates above 60%. It reinforces the importance to monitor the HRD status of Brazilian samples to obtain a more assertive profile to the clinical practice.

B-391

Is comprehensive cancer panel by next-generation sequencing (NGS) more efficient than cancer-specific NGS panel in the management of non-small cell lung cancer patients?

L. B. Alvim, F. L. Marinho, M. C. Freire, D. A. Zauli. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Genetic tests play an integral part in the diagnosis, treatment and prognosis of lung cancer patients. Comprehensive cancer panels using next-generation sequencing (NGS) are gradually becoming the standard tool and replacing the previous approach of single-gene or cancer-specific testing. In this study, we compared the performance of two commercially available NGS gene panels of different sizes concerning their clinical utility in the management of non-small cell lung cancer (NSCLC) patients. **Methods**: A total of 65 tumor samples were sequenced with both lung cancer NGS panel (Lung-panel), 12 genes, and comprehensive cancer NGS panel (Pan-panel), 161 genes, following the manufacturer's protocol. **Results**: The Pan-panel detected 159 variants in 93.8% (61/65) patient samples; 37.7% (60/159) of these variants were classified as clinically relevant (tier I or II) with 68.3% (41/60) containing therapy approved and 31.7% (19/60) eligible for a clinical trial. The Lung-panel covered 51 of the 159 (32.0%) variants reported by the larger panel in 72.3% (47/65) tumor samples and all genetic alterations detected (51/51) were indicated as clinically relevant with 78.4% (40/51) described therapy-related variants and 21.6% (11/51) with a genomically matched clinical trial. While the Pan-panel detected more variants, additional variants beyond those included in the Lung-panel had no impact on NSCLC patient management. Even more remarkably, the cancer-specific panel despite covering a smaller genomic region than the comprehensive panel (28.7 kbp versus 329.4 kbp) was more informative in the context of FDA-approved therapy or biomarkers included in NCCN or ESMO guidelines that predict response or resistance to therapies in this cancer type, 78.4% and 68.3%, respectively. **Conclusion**: Overall, this comparative analysis of the clinical utility of NGS panels of different sizes indicates that cancer-specific NGS panels containing carefully selected genes are as informative as larger panels when the primary goal is to identify clinically actionable mutations, particularly in patients with NSCLC. Furthermore, this approach presents a better balance among accurate identification of genomic variants, sensitivity, turnaround time, and cost-effectiveness for cancers with well-characterized molecular targets as shown in this study. Larger panels should be used in patients with rare and understudied forms of cancer or in academic reference centers where clinical and translational research is conducted.

B-392

Standardization of nucleic acid extraction method from thyroid fine needle aspiration (FNA) cells on stained and fixed slides for Next-Generation Sequencing protocols

L. A. Martins[1], R. S. Domingues[1], G. M. de Moraes[2], G. C. Penna[2], D. A. Zauli[1], A. B. de Lima[1]. *[1]Instituto Hermes Pardini, Vespasiano, Brazil, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Background: Thyroid Fine Needle Aspiration (FNA) cytopathology is the best method for histological classification of thyroid nodules. However, between 20-30% of thyroid nodules show indeterminate classification and are often referred for surgery due to the malignancy risk. Post-surgical histological reports indicate that 84% of these cases are benign, highlighting a high rate of unnecessary surgeries. In this sense, molecular approaches using next-generation sequencing (NGS) have been useful to confirm benignity or malignancy. As important as the choice of a powerful methodology to evaluate different genes simultaneously such as NGS, is the standardization of nucleic acid extraction methods to allow sufficient quality recovery of these molecules. Pre-analytical steps involved in FNA slide preparation include fixation and stain methods that contribute to the low quality and poor recovery of nucleic acid, representing a great challenge to NGS analysis. Thus, this work aims to describe a standardization of nucleic acid extraction method from FNA slides for NGS protocols. **Methodology:** Nucleic acid isolation from 30 FNA samples collected between 2016-2022 were performed by Trizol or RecoverAll kits, according to the manufacturer's instructions (ThermoScientific). Initially, cells' slides were counted in an optical microscope, considering cell clusters >10 (equivalent to 1 counter unit). Before extractions with Trizol, the slides were submerged in xylene for 15 minutes. For RecoverAll extractions, the varnished slides were previously exposed to dry ice for 5 minutes, while the slides containing coverslip were also exposed to dry ice and submerged in xylene for 15 minutes. In all cases, the slides were hydrated before scraping and then extracted. Subsequently, the extraction of whole slides and split slides was tested. To split slides, one-half of the slide was separated for RNA extraction, while the remaining half was for DNA extraction. Then, the DNA/RNA were quantified using the Qubit™ 4 Fluorometer equipment (ThermoScientific) and concentrated using Amicon® Ultra filters (Millipore). **Results:** The DNA and RNA quantifications range from 0.0720-1.77 ng/µL and 0.508-7.43 ng/µL, respectively. Among samples, 12% (DNA) and 62.5% (RNA) presented concentration below the quantification limit (too low). Four samples extracted with Trizol did not obtain recovery of nucleic acid and this methodology was discontinued. The use of Amicon® filter improved all samples' yield. The number of thyroid cell clusters was not proportional to the amount of DNA/RNA recovered. The split slides showed worst RNA/DNA recovery, being ideal to use at least two slides for each patient since co-extraction using the RecoverAll kit was not possible. **Conclusion:** The isolations of nucleic acid from FNA slides are essential to performing subsequent molecular tests, such as NGS. However, specific protocols for nucleic acid extraction from FNA slides are hardly found in the literature. This work showed the combination of methodologies that allowed the scraping of material from FNA slides by exposure to dry ice in samples with varnish or coverslip. The nucleic acid recovery capacity described for this type of sample indicates a way of possibilities to improve the FNA extraction methodology.

2023 AACC Annual Scientific Meeting & Clinical Lab Expo
Poster Abstract Author Index
(Numbers refer to Poster Numbers; see pages S2 to S214)

2023 AACC Annual Scientific Meeting & Clinical Lab Expo
Poster Abstract Author Index
(Numbers refer to Poster Numbers; see pages S2 to S214)

D

E

F

G

H

I

J

K

2023 AACC Annual Scientific Meeting & Clinical Lab Expo
Poster Abstract Author Index
(Numbers refer to Poster Numbers; see pages S2 to S214)

L

M

2023 AACC Annual Scientific Meeting & Clinical Lab Expo
Poster Abstract Author Index
(Numbers refer to Poster Numbers; see pages S2 to S214)

Q

R

S

2023 AACC Annual Scientific Meeting & Clinical Lab Expo
Poster Abstract Author Index
(Numbers refer to Poster Numbers; see pages S2 to S214)

T

U