

# 2022 AACC Annual Scientific Meeting & Clinical Lab Expo

## Poster Session Schedule

Posters of accepted abstracts were viewed in the Poster Hall of the of the McCormick Place Convention Center on Tuesday, July 26 and Wednesday, July 27.

Below are the topics and their scheduled times.

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### TUESDAY, JULY 26, POSTER SESSIONS

#### 9:30 am – 5:00 pm

General Clinical Chemistry . . . . .	A-001 – A-124 . . . . .	S2
Laboratory Management and Leadership . . . . .	A-125 – A-138 . . . . .	S38
Laboratory Stewardship and Patient Safety . . . . .	A-139 – A-147 . . . . .	S41
Molecular Diagnostics . . . . .	A-148 – A-240, A-304 . . . . .	S44
Special Patient Populations . . . . .	A-241 – A-272 . . . . .	S73
Toxicology and Therapeutic Drug Monitoring . . . . .	A-273 – A-303 . . . . .	S81

### WEDNESDAY, JULY 27, POSTER SESSIONS

#### 9:30 am – 5:00 pm

Analytical Techniques and Applications . . . . .	B-001 – B-126 . . . . .	S91
Data Analytics and Informatics . . . . .	B-127 – B-157 . . . . .	S128
Hematology/Coagulation . . . . .	B-159 – B-183 . . . . .	S138
Microbiology and Infectious Diseases . . . . .	B-187 – B-268 . . . . .	S145
Preanalytical and Postanalytical . . . . .	B-269 – B-298 . . . . .	S168
Precision Medicine . . . . .	B-300 – B-310 . . . . .	S178

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Tuesday, July 26, 2022

Poster Session: 9:30 am - 5:00 pm  
General Clinical Chemistry

**A-001**

**Evaluation of the Turbidimetric VITROS® wrCRP Chemistry Products Reagent\* on the Ortho VITROS 5600 Integrated System**

K. Ackles, T. DiMugno, N. Kinne, N. Wheeler. *Ortho Clinical Diagnostics, Rochester, NY*

**Background:** VITROS® wrCRP Chemistry Products Reagent\* is being developed for the measurement of C-Reactive Protein on the VITROS 5600/XT 7600 Integrated Systems and the VITROS 4600 Chemistry System. The VITROS wrCRP Assay\* uses a Standard Method (sCRP) as an extended range CRP method, and the Ultrasensitive Method (uCRP) as a high sensitivity CRP method. Measuring CRP is useful in the evaluation of inflammatory disorders. High sensitivity CRP assays may assess risk of coronary heart disease and detect early infection.

The VITROS wrCRP Assay\* is a turbidimetric ready-to-use liquid assay in a dual chambered package for use with serum and heparin plasma. The analytical performance of VITROS wrCRP Assay\* has been evaluated for method comparison, precision, extended analytical measuring range, and has ≥ 6 months calibration interval.

**Method:** The total within-lab precision and repeatability was calculated using an ANOVA on the VITROS 5600 System with quality control and patient pools using two replicates per day, twice per day over 22 days (total n=88) following CLSI EP05-A3 guideline. Serum patient samples spanning the measuring range for uCRP (n=123, 0.4-144 mg/L) and for sCRP (n=122, 0.5-307.2 mg/L) were evaluated on a VITROS 5600 System against the Siemens CardioPhase® hsCRP assay on the BN ProSpec® System and the Sentinel CRP Vario® assay on the Abbott Architect® System, respectively. The data was analyzed following CLSI EP09c guideline using a Weighted Deming regression.

**Results:** The total within lab precision from an ANOVA was less than 4.5% CV for all fluids on uCRP (mean 0.5 mg/L, 4.2% CV; mean 4.2 mg/L, 1.3% CV; mean 10.4 mg/L, 1.4% CV; mean 133.8 mg/L, 1.7% CV), and the repeatability was less than 4.0% CV for all fluids on uCRP (mean 0.5 mg/L, 3.9% CV; mean 4.2 mg/L, 0.8% CV; mean 10.4 mg/L, 0.9% CV; mean 133.8 mg/L, 0.9% CV). The total within lab precision from an ANOVA was less than 4.5% CV for all fluids on sCRP (mean 0.75 mg/L, 4.2% CV; mean 5.1 mg/L, 2.6% CV; mean 125.3 mg/L, 2.1% CV; mean 270.5 mg/L, 1.8% CV), and the repeatability was less than 3.5% CV for all fluids on sCRP (mean 0.75 mg/L, 3.4% CV; mean 5.1 mg/L, 2.5% CV; mean 125.3 mg/L, 1.1% CV; mean 270.5 mg/L, 1.3% CV). The method comparison study for the VITROS wrCRP Assay\* showed excellent correlation to comparative methods: VITROS uCRP = 1.05 \* BN ProSpec - 0.06; (r) = 0.999; VITROS sCRP = 0.98 \* Abbott Architect - 0.17; (r) = 0.998 on the VITROS 5600 System. The extended measuring range for the VITROS wrCRP Assay\* is 0.3-155 mg/L for uCRP and 0.4-310 mg/L for sCRP based on the assay linearity and limit of quantitation following CLSI EP06-A2 and CLSI EP17-A2 guidelines.

**Conclusion:** The data presented here demonstrate that the new VITROS wrCRP Assay\* on the VITROS 5600 System shows acceptable analytical performance to measure CRP using both the Standard method and Ultrasensitive method.

\*Under development

**A-002**

**The Distribution of NT ProBNP and respiratory virus PCR results in children with respiratory tract infections.**

S. Ahn, S. Lee, K. Lee, Y. Jeon, S. Lee, E. Lee. *Green Cross Laboratories, Yongin, Korea, Republic of*

**Background:**

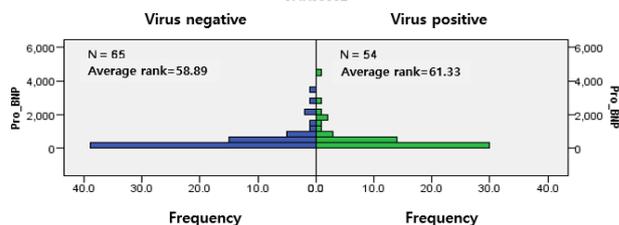
N-terminal pro-brain natriuretic peptide (NT-proBNP) is an important indicator of HF in children as well as adults. Some studies identified the role of NT-proBNP as biomarkers of bronchiolitis severity, in children with and without underlying congenital cardiac disease. Here we described the distribution of NT-proBNP and respiratory virus polymerase chain reaction (PCR) results in children with suspicion of respiratory tract infections.

**Methods:**

NT-proBNP and respiratory virus PCR results from 119 patients samples were collected. The samples were referred to Green Cross Laboratories from January 01, 2020 to December 31, 2021. NT-proBNP was tested with Cobas e601 (Roche, Grenzacherstrasse, Switzerland). Respiratory virus PCR tests were done with CFX96 Real-time system (Seegene, Seoul, Korea). Between group comparison was made with Mann-Whitney U test, and statistical analysis were done with SPSS.

**Results:**

Among 119 children, 54 children showed positive with respiratory virus PCR and 65 children were negative with respiratory virus PCR. Mann-Whitney U test proved that serum NT-proBNP levels were not significantly different between two groups. The average NT-proBNP levels from the children with respiratory virus PCR positive was 512.9 pg/mL (median: 252.5 pg/mL; range: 11.8-4,467 pg/mL), and the average level the children with PCR negative was 447.6 pg/mL (median: 208.4 pg/mL; range: 5.0-3,635 pg/mL). The overall detection rate of respiratory pathogens was 45.4% (52/119). Among them, human rhinovirus (33.7%, 28/83), adeno virus (27.7%, 23/83), and influenza B virus (12.02%, 10/83) were the predominantly detected pathogens in this study. Moreover, the co-infection was observed in 25 specimens. Rhino virus was the most common virus tending to occur in co-infection with other respiratory pathogens. **Conclusion:** This study reveals that NT-proBNP levels were not significantly different between patients with respiratory virus PCR positive and with PCR negative.



**A-003**

**Novel Diagnostic Validation of High Sensitivity Troponin Prior to Implementation**

E. Alexander, N. Pozzi, S. Jortani. *University of Louisville, Department of Pathology & Laboratory Medicine, Louisville, KY*

**Background:**

Analytical and clinical validation and correlation of high sensitivity troponin I (hsTnI) to the conventional 4<sup>th</sup> generation TnI (cTnI) assay has been extensively studied and shown exceptional concordance in the diagnosis of NSTEMI in large cohorts. However, diagnostic validation on smaller hospital level scales can be complicated through biases in sample collection logistics and physician interpretation differences. As implementation of high sensitivity troponin expands in the United States, new ways to quickly assess diagnostic efficacy in an individual hospital population may be required. Herein we report a novel method of simultaneous analysis of cTnI and hsTnI to evaluate diagnostic differences in small cohorts prior to full implementation.

**Methods:**

Automated analyzers were programed to run cTnI and hsTnI assays simultaneously on all ordered troponins over a period of 24 hours (N = 316 samples, 120 unique patients). Positive cutoffs for both assays were established as >99% reference interval (cTnI: >0.03 ng/mL; hsTnI: ≥15 ng/mL (female) or ≥20 ng/mL (male)). Patient troponin results and clinical presentation were evaluated by a group of cardiologists and emergency department physicians for the diagnosis of myocardial infarction. Additionally, the physicians were asked if the use of hsTnI would have impacted patient care in each clinical scenario.

**Results:**

Total negative MI diagnosis of this cohort for conventional TnI vs hsTnI were 62 (52%) and 77 (64%) respectively. There were 19 cases of discordant diagnosis; in 17 cases, hsTnI based analysis ruled out an MI diagnosis while in the remaining 2 cases resulted in a new diagnosis of MI. The team of physicians evaluating these cases indicated that of 120 patient clinical presentations, the use of hsTnI would have impacted patient care in 47 (39%) scenarios.

**Conclusion:**

hsTnI resulted in the same diagnosis for 101/120 (84%) patients over this 24-hour period compared to cTnI. The remaining 19 cases skewed towards ruling out MI when hsTnI was used. With this study we sought to validate the diagnostic efficacy of hsTnI

in our specific hospital population while minimizing biases in sample timing and individual physician interpretation. We propose similar analysis can be performed to evaluate the impact hsTnI may have on an individual hospital prior to implementation.

**A-004**

**Improved Analytical Measuring Interval and Hemoglobin performance for New Investigational Use Assay (IUO) Triglyceride Assay on the Abbott's ARCHITECT and Alinity c Systems**

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**Background:** Triglyceride assay is used to aid in diagnosis of diabetes, liver obstruction, and other endocrine disorders. The Adult Treatment Panel of the National Triglyceride Education Program (NCEP) recommends a lipoprotein profile every five years to screen for coronary heart disease (CHD) risk in adults 20 years and older. This panel includes total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride. The new Triglyceride IUO Assay demonstrates improved analytical measuring interval (AMI) and robust hemoglobin performance for serum and plasma samples on ARCHITECT and Alinity c systems.

**Methods:** Lipase enzyme hydrolyzes triglycerides to release glycerol, which then undergoes phosphorylation. The resulting glycerol-3-phosphate is oxidized by glycerol phosphate oxidase, which produces dihydroxyacetone phosphate and hydrogen peroxide. The peroxidase catalyzes the conjugation of N, N-Bis(4-sulfobutyl)-3-Methylamine (TODB) and 4-aminoantipyrine (4-AAP) in the presence of hydrogen peroxide. By complementing 4-AAP with TODB and measuring the resulting chromophore's absorption at 604 nm, the assay significantly improves the interference claim while maintaining a single-reagent format.

**Results:** The new TRIG IUO assay has AMI of 4 to 1505 mg/dL which results in up to 3-fold fewer results outside the AMI as compared to other commercially available TRIG assays, which reduces the burden on laboratories on additional dilution and repeat testing. The limit of detection (LoD) and limit of quantitation (LoQ) was  $\leq 2$  mg/dL and  $\leq 4$  mg/dL, respectively. Within laboratory imprecision was  $\leq 1.9\%$  for samples between 0.94 mg/dL to 14.29 mg/dL and  $\leq 0.012$  SD for sample at 0.12 mg/dL. This assay demonstrated linearity up to 1505 mg/dL. The assay has exceptional reagent onboard stability of at least 30 days with calibration curve stability of 30 days. The assay shows incomparable hemoglobin interference of  $< 1000$  mg/dL when tested at 100 mg/dL and 250 mg/dL Triglyceride in serum. Method comparison to Abbott's previous version of Triglyceride assay showed a slope of 0.99 with correlation coefficient of 1.00 for the serum concentration range of 0.08 - 14.67 mg/dL. The Triglyceride IUO Assay has an auto dilution feature for samples which extends the measuring interval to 6020 mg/dL. Accuracy of the assay showed bias ranges from 0.4% to 1.7% when evaluated against reference standard material (ACS Grade Glycerol).

**Conclusion:** New Triglyceride IUO Assay has been designed for use on the ARCHITECT and Alinity c systems with improved AMI and robust hemoglobin interference.

**A-005**

**Evaluation of the Ferritin assay using the Roche cobas e801 analyzer**

T. R. Allison<sup>1</sup>, S. P. Wyness<sup>1</sup>, S. L. La'ulu<sup>1</sup>, K. Doyle<sup>2</sup>, J. W. Rudolf<sup>2</sup>. <sup>1</sup>ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, <sup>2</sup>University of Utah Health, Department of Pathology, Salt Lake City, UT

**Background** Ferritin is an intracellular protein involved in iron metabolism and an indicator of total body iron stores. Measurement of serum or plasma ferritin concentrations are commonly used to diagnose and monitor deficiencies and overloads of iron in the body. The objective of this study was to evaluate the Roche Ferritin assay using the Roche cobas pro e801, including comparison to the Siemens ADVIA Centaur. **Methods** Method comparison was performed using residual de-identified patient specimens (n=73) spanning the analytical measuring range (AMR), analyzed on all instruments on the same day. The Siemens ADVIA Centaur XP Ferritin assay was used as the comparator method. Proficiency testing (PT) samples were analyzed on all analyzers as an additional measure of comparison and accuracy. Linearity studies were performed using 5 levels of manufacturer calibration verification materials in triplicate. Intra-assay and inter-assay precision were evaluated using a low and a high concentration quality control material. For inter-assay precision, single-use aliquots were analyzed in quadruplicate for 5 days, one run per day. Sensitivity was assessed using low concentration materials and approved diluent, according to the protocol described by EP Evaluator. Adult reference intervals were verified using serum specimens from self-reported healthy volunteer donors (64 male and 63 female). Expected values in the manufacturer's package insert are 13-150  $\mu\text{g/L}$  for females 17-60 years of age and

30-400  $\mu\text{g/L}$  for males 20-60 years of age. **Results** Method comparison using Deming regression demonstrated a slope of 1.372 (R=0.9881, bias 43.817%) for instrument 1 and a slope of 1.330 (R=0.9883, bias 39.657%) for instrument 2. Attempts to optimize repeat method comparison studies included calibrating all instruments on the same day and testing all samples within 4 hours. Yet, observed differences between the e801 and Centaur persisted despite these efforts. Analysis of proficiency testing (PT) material demonstrated acceptable results for both the e801 and Centaur, four of the five samples were within the prescribed ranges. Linearity was evaluated, with percent recoveries ranging from 95.4-102.1% for instrument 1 and 97.7-102.1% for instrument 2. Intra-assay imprecision ranged from 0.9-2.1%CV for instrument 1 and 0.7-2.9%CV for instrument 2. Inter-assay imprecision ranged 1.2-1.5%CV for instrument 1 and 2.3-3.8% CV for instrument 2. Sensitivity was verified for each instrument. Sex-specific adult reference intervals reported in the package insert were verified using 127 subjects. Nine subjects were excluded due to the prevalence of iron deficiency anemia, as determined by low ferritin, low iron, and high soluble transferrin receptor concentrations. **Conclusions** Acceptable analytical performance was observed for the Roche cobas pro e801 Ferritin immunoassay. Method comparison of the e801 to the ADVIA Centaur revealed notable differences in measured values. Analysis of PT on these platforms aided in evaluating accuracy.

Comparison of these methods for the ferritin assay is novel and not well-documented in the literature. While the reference interval was verified, several exclusions were necessary due to patterns consistent with iron deficiency anemia, suggesting the importance of evaluating other iron markers.

**A-006**

**Reevaluating the icterus index cutoff on the Roche Jaffé Gen 2 creatinine method**

Y. F. Alvarez, M. T. Kelliher, J. H. Hubbard, R. D. Nerenz, M. A. Cervinski. Dartmouth Hitchcock Medical Center, Lebanon, NH

**Background:** The aim of this study was to redefine the icterus index cutoff for the Roche Jaffé creatinine method using both conjugated and unconjugated bilirubin on three Roche cobas modules (c311, c501, and c701/c702), at laboratories across our hospital network. **Methods:** Interference was evaluated by adding conjugated bilirubin (as bilirubin conjugate, ditaurate) and unconjugated bilirubin to pooled remnant plasma. The effects of conjugated and unconjugated bilirubin were tested separately to assess the contribution of each species. The magnitude of interference was calculated as both absolute and percent error with total allowable error limits set at 0.1 mg/dL (8  $\mu\text{mol/L}$  or 8% (Figure 1). **Results:** Analysis of interference data across the three Roche modules did not show bias exceeding our total allowable error limits for plasma creatinine up to a conjugated bilirubin icterus index of 16.2 (~16.2 mg/dL or 277  $\mu\text{mol/L}$ ), or an unconjugated bilirubin icterus index of 18.5 (~18.5 mg/dL or 316  $\mu\text{mol/L}$ ), the highest concentrations tested (Table 1). **Conclusions:** Our results demonstrate that the Roche Jaffé method exhibits acceptable performance in the presence of bilirubin at icterus indexes above the manufacturer's current recommendations of 5 (~5 mg/dL or 86  $\mu\text{mol/L}$ ) and 10 (~10 mg/dL or 171  $\mu\text{mol/L}$ ) for conjugated and unconjugated bilirubin, respectively. We have updated the icterus index in our hospital system to 16 for conjugated bilirubin and 18 for unconjugated bilirubin.

Mean index	Mean plasma creatinine concentration											
	E801 (PT Laboratory 1)			C501 (Laboratory 2)			E801 (PT Laboratory 3)			C701 (Laboratory 4)		
	Concentration (Mean $\pm$ SD), mg/L (umol/L)	Bias, mg/L (umol/L)	% Bias	Concentration (Mean $\pm$ SD), mg/L (umol/L)	Bias, mg/L (umol/L)	% Bias	Concentration (Mean $\pm$ SD), mg/L (umol/L)	Bias, mg/L (umol/L)	% Bias	Concentration (Mean $\pm$ SD), mg/L (umol/L)	Bias, mg/L (umol/L)	% Bias
Low Creatinine + Unconjugated Bil	1.0 0.96(0.041355)	0.0012(0.0015)	0.12%	0.96(0.041355)	0.0012(0.0015)	0.12%	0.96(0.041355)	0.0012(0.0015)	0.12%	0.96(0.041355)	0.0012(0.0015)	0.12%
High Creatinine + Unconjugated Bil	4.5 4.37(0.142815)	0.06(0.18)	1.3%	4.37(0.142815)	0.06(0.18)	1.3%	4.37(0.142815)	0.06(0.18)	1.3%	4.37(0.142815)	0.06(0.18)	1.3%
Low Creatinine + Conjugated Bil	1.0 0.96(0.041355)	0.0012(0.0015)	0.12%	0.96(0.041355)	0.0012(0.0015)	0.12%	0.96(0.041355)	0.0012(0.0015)	0.12%	0.96(0.041355)	0.0012(0.0015)	0.12%
High Creatinine + Conjugated Bil	4.5 4.37(0.142815)	0.06(0.18)	1.3%	4.37(0.142815)	0.06(0.18)	1.3%	4.37(0.142815)	0.06(0.18)	1.3%	4.37(0.142815)	0.06(0.18)	1.3%

Table 1. Mean plasma concentration and standard deviation of creatinine obtained from the laboratories participating in the icterus index study. All participants used the same creatinine method but different analyzer models. Abbreviations: Bil, bilirubin. \* Creatinine results significantly different from baseline creatinine concentration using the Jaffe Kinetic Method (P<0.05 Tukey test). \*\* Samples not assayed due to a laboratory error, consequently bias and % bias could not be calculated for this set. Red highlighted cells denote creatinine methods falling outside our total allowable error (TAE) acceptability criteria ( $\pm 8\%$  or 0.1 mg/dL).

## A-007

**Clinical Utility of the Abbott FreeStyle Precision Pro POC Ketone Meter for Monitoring Pediatric Patients with Diabetic Ketoacidosis**

B. A. Andrews, E. E. Schuler, E. Webber, W. J. Smith, A. S. Krishna, A. L. Woodworth. *University of Kentucky, Lexington, KY*

**Background:** Diabetic ketoacidosis (DKA) is a life-threatening disorder characterized by hyperglycemia, electrolyte derangements, and metabolic acidosis caused by insulin insufficiency. Current consensus guidelines recommend measurement of blood ketones for both diagnosis and monitoring of DKA resolution. Recent literature endorses frequent monitoring of glucose, pH, electrolytes, and ketones during DKA treatment. Point-of-care (POC)  $\beta$ -hydroxybutyrate (BHB) testing may expedite diagnosis and treatment decisions while minimizing blood draws in pediatric patients. In collaboration with pediatric ICU (PICU) and Endocrinology providers, we evaluated the clinical utility of POC ketone testing via the FreeStyle Precision Pro in a standardized pediatric DKA treatment protocol.

**Objective:** To evaluate the clinical utility of POC-BHB testing as a part of a standardized treatment protocol in pediatric DKA patients.

**Methods:** A prospective study was performed utilizing patients admitted to the University of Kentucky Children's Hospital with a diagnosis of DKA (hyperglycemia > 200 mg/dL, ketonemia > 1.0 mmol/L, and venous pH < 7.3 or plasma  $\text{CO}_2$  ( $\text{HCO}_3^-$ ) < 15 mEq/L). POC-BHB testing was embedded in standardized DKA order sets and measured at initial diagnosis and every 4-8 hours with laboratory BHB (Lab-BHB) and other biochemical analytes. A preliminary assessment of 12 POC-BHB measurements from 7 patients was performed. POC-BHB was measured by enzymatic-amprometry with the FreeStyle Precision Pro meter (Abbott) in fresh capillary and/or whole blood specimens. Preliminary validations of the method included: linearity, precision, and patient comparisons using residual EDTA whole blood specimens from patients with simultaneous Lab-BHB tests sent to the UKMC clinical laboratory. POC-BHB results were compared to the Lab-BHB, Stanbio enzymatic BHB assay (Roche Cobas 501). Trending BHB results (POC and lab) were correlated with other monitored biochemical test results (Anion Gap, potassium,  $\text{CO}_2$ ). Routine chemistry testing was performed on the Roche Cobas 701. Anion Gap (AG) was calculated as  $[\text{Na}] - ([\text{Cl}] + [\text{plasma } \text{CO}_2 \text{ or } \text{HCO}_3^-])$ .

**Results:** Linearity studies demonstrated that, independent of strip lot, the ketone meter failed to report BHB results above 5.8 mmol/L. In DKA patients, overall correlation between Lab-BHB and POC-BHB was good [POC-BHB =  $0.89 * (\text{Lab-BHB}) + 0.58$ ,  $R = 0.98$ ], but showed a significant bias in results >5.8 mmol/L (POC-BHB =  $0.58 * (\text{Lab-BHB}) - 2.4$ ,  $R = 0.83$ ). Fingertick measurements obtained in pediatric patients correlated well with Lab-BHB ( $R = 0.97$ ,  $n = 12$ ), but none was >5.8 mmol/L. The trend in Lab and POC-BHB measurements correlated well with other chemistry analyses monitored during DKA treatment protocols.

**Conclusion:** The Freestyle Precision Pro Ketone meter accurately measures BHB in adult and pediatric DKA patients, but its use is limited. Limitations in linear range and correlation to lab-BHB necessitate careful implementation of the device. The meter accurately measures BHB at important clinical decision points of 3.0 mmol/L (ICU discharge) and 1.0 mmol/L (discontinuation of insulin infusion). In preliminary analyses, BHB trends correlate with normalization of important biochemical measures of DKA severity. The ability to monitor DKA via fingertick BHB analysis could allow for fewer blood draws in pediatric DKA patients, and a larger prospective analysis is ongoing to confirm this finding.

## A-008

**Creation of a Novel Serum B-Cell Maturation Antigen (sBCMA) Reference Standard**

J. Bailey<sup>1</sup>, N. Kennett<sup>1</sup>, P. Stubbs<sup>1</sup>, M. Rizzioli<sup>1</sup>, N. Li<sup>1</sup>, K. Dacanay<sup>1</sup>, J. Berenson<sup>2</sup>, E. Souther<sup>2</sup>, O. Berlanga<sup>1</sup>, S. Harding<sup>1</sup>, G. Wallis<sup>1</sup>, P. Hazarika<sup>1</sup>. <sup>1</sup>The Binding Site Group Ltd., Birmingham, United Kingdom, <sup>2</sup>ONCotracker, West Hollywood, CA

**Background:** Serum B-cell maturation antigen is a novel marker for B-cell dyscrasias. sBCMA levels are shown to be predictive of clinical outcome and associated with response to treatment in multiple myeloma patients. sBCMA levels are also reduced among those with primary immunodeficiencies such as common variable immunodeficiency and X-linked agammaglobulinemia. There is currently no clinically approved assay or an international standard to measure sBCMA. We have developed a sBCMA-Human Serum Albumin (HSA) fusion protein that has been quantified by amino acid analysis to use as Reference Standard in an ELISA to measure sBCMA in serum and plasma. **Methods:** sBCMA-HSA fusion protein was constructed from the sequence

of HSA and BCMA (N-terminal 54 amino acids; UniProtKB - Q02223) connected through a six amino acid linker. This was produced recombinantly by CHO cell culture and purified using a-HSA affinity chromatography and size exclusion chromatography. Amino acid analysis was performed at a UKAS accredited laboratory to ISO/IEC 17025:2017 standard. Total protein quantification of the Reference Standard was assessed using a BCA total protein assay with a BSA standard (Thermo Scientific). sBCMA concentration was also separately measured on a RUO commercially available BCMA ELISA (R&D Systems DY193 DuoSet) using both the provided kit standard and a commercially available BCMA-his tag protein (AcroBiosystems) as calibrator. The Reference Standard was assessed for purity by LC-MS (Waters Xevo-G2-XS) using tryptic digestion and the resulting peptide sequences were searched against human and Chinese hamster proteome databases using Progenesis QI software. SDS-PAGE analysis with Coomassie Brilliant Blue G250 staining and western blot with affinity purified polyclonal sheep a-BCMA HRP conjugated antibody (in-house) were also used to assess purity. Functional activity of sBCMA-HSA was tested on an in-house ELISA (currently under development) which utilises a polyclonal sheep a-BCMA sandwich. **Results:** Amino acid analysis resulted in a protein concentration of 3.683 g/L for the sBCMA-HSA fusion protein. The concentration of sBCMA therein was derived at 0.299 g/L as our Reference Standard. BCA total protein assay reported a concentration of 3.180 g/L. The value of sBCMA in the sBCMA-HSA fusion protein reported from the RUO BCMA ELISA (R&D Systems DY193 DuoSet) was 0.721 g/L versus the kit provided standard (BCMA-Fc homodimer) and 0.161 g/L versus a commercially available BCMA-his tag protein used as an alternative calibrator. LC-MS analysis reported 273 matching unique peptides for HSA and 10 for human BCMA. A minimum of 3 peptides for each discovered protein were confirmed through manual inspection of the raw data. No other peptides were found from either species' database. The sBCMA-HSA molecule produced good activity as a calibrator in an in-house polyclonal sheep sandwich ELISA for BCMA with an assay range between 15-3000 pg/mL. **Conclusion:** We have produced a sBCMA Reference Standard with application demonstrated on a polyclonal sheep a-BCMA sandwich ELISA. The Reference Standard demonstrates a high level of purity by several methods including LC-MS. Variation in the quantification of sBCMA on a commercial RUO ELISA by using different calibrators underscores the need for a Reference Standard with a traceably defined BCMA value.

## A-009

**Assessment of Anti-SARS-CoV-2 Antibody Status Among Laboratory Healthcare Workers in the City of Goiania, Central Brazil**

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**Background:** Healthcare workers (HCWs) were among the priority vaccine groups in Brazil receiving a two-dose schedule of either a whole virion SARS-CoV-2 (CoronaVac, Sinovac), an adenoviral-vectored nCoV-19 (AstraZeneca) or an mRNA vaccine (Pfizer-BioNTech). Since the beginning of the pandemic and according to availability from the Brazilian Ministry of Health, different booster regimens have been applied to HCWs in different regions of the country. The humoral immune response to SARS-CoV-2 can be evaluated through immunoassays that employ two main targets: the nucleocapsid (N) protein and the spike (S) protein. Antibody tests can be further classified into binding and neutralizing antibodies. Binding tests detect different immunoglobulin (Ig) classes, according to each manufacturer. On the other hand, virus neutralization tests (VNTs) evaluate the functional ability of antibodies to inhibit SARS-CoV-2 infection in vitro. Among the three types of VNTs, two are almost exclusively employed in the research setting. A third type of VNT is a competitive VNT (cVNT), commercially available in an enzyme linked immunosorbent assay (ELISA) format. Although the presence of binding antibodies and cVNTs should not be a marker of vaccine efficacy or immunity to infection, it can be useful for seroprevalence studies both in clinical and public health settings. **Methods:** This study was carried out with 158 vaccinated laboratory HCWs who signed an informed consent for SARS-CoV-2 antibody testing through 3 immunoassays. Binding antibodies were tested as follow: 1. Anti-S total antibodies measured by Elecsys™ Anti-SARS-CoV-2 S, using Cobas 6000-E601, RV: < 0.80 Negative and  $\geq 0.80$  Positive, 2. Anti-N IgG antibodies measured by Abbott anti-SARS-CoV-2 CMIA IgG, using the Abbott Architect i4000SR, RV: Index < 1.4 Non Reagent and  $\geq 1.4$  Reagent. Neutralizing antibodies were evaluated by the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript), VR: 0 to 20% Non Reagent; 20 to 30% Undetermined; > 30% Reagent. Chi square tests in SPSS v.26 were used for statistical analysis. **Results:** Among the 158 HCWs, 101 (63.9%) received AstraZeneca, 35 (22.1%) were CoronaVac recipients and 22 (13.9%) received the Pfizer-BioNTech regimen. Almost 80% (126/158) received a booster dose and 96/158 (60.7%) informed they have had a positive RT-

PCR test at some point during SARS-CoV-2 pandemic. Regarding the antibody status, all HCWs had anti-S detected (Roche) and 157 (99.3%) had neutralizing antibodies by the GenScript cVNT. Anti-N IgG antibodies were present in 58% (85/158) of the HCWs. Among anti-N positive HCWs, 62/85 (72.9%) informed SARS-CoV-2 infection and 20/85 (23.5%) had CoronaVac vaccination. There was a strong association between the presence of anti-N antibodies and previous SARS-CoV-2 infection ( $p < 0.01$ ) but not with CoronaVac vaccination ( $p = 0.05$ ). No significant increase in anti-N antibody frequency was observed among those who had received Pfizer booster dose ( $p = 0.98$ ). **Conclusions:** Because we studied a population with a known vaccination status and employed immunoassays targeted to two different antigens, we observed that this serological strategy can aid in the differentiation between past infection and vaccination and provides a more accurate interpretation of SARS-CoV-2 serologic results, especially in countries where different vaccine protocols are employed.

## A-010

### Comparison of eGFR Calculated by MDRD Equation and the 2021 CKD-EPI without Race Equation

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**Background:** To implement the 2021 CKD-EPI without race equation for GFR estimation at our institution, our current MDRD equation was compared with the new equation to examine the differences and to investigate the clinical implications.

**Methods:** We retrospectively analyzed 13,724 adult patients with serum creatinine results collected from Laboratory Information Systems. Patients were categorized according to self-identified gender and racial groups: "Asian/American Indian", "Black", "Hispanic/Latino" and "White." EP Evaluator EE12 was used to analyze the eGFR calculated by the two equations. Since MDRD equation was developed in renal disease patients, and not validated in eGFR  $> 60$  ml/min/1.73 m<sup>2</sup>, comparison was focused on patients with eGFR  $< 60$ .

**Results:** Compared with the eGFR calculated by MDRD equation, the 2021 CKD-EPI without race equation caused a negative bias of -3.0 (-9.0%) in 906 black female, and a negative bias of -3.4 (-10.0%) in 887 black male. There is an approximate 30% disagreement between the two equations in black people, which means 30% black people will be reclassified to a more severe stage of kidney disease. On the other hand, the 2021 CKD-EPI without race equation caused a positive bias of 4.2 (10.3%) in 1481 white female, and a positive bias of 3.0 (8.0%) in 1400 white male. There is an approximate 30% disagreement between the two equations in white people, which means 30% white people will be reclassified to a less severe stage of kidney disease. Similar to the white cohort, the 2021 CKD-EPI without race equation caused a positive bias of 3.4 (10.0%) in 86 Asian/American Indian female, and a positive bias of 3.3 (10.1%) in 47 Asian/American Indian male. There is about 20% disagreement between the two equations in Asian/American Indian people, which means 20% Asian/American Indian people will be reclassified to a less severe stage of kidney disease. The 2021 CKD-EPI without race equation caused a positive bias of 2.5 (10.4%) in 16 Hispanic/Latino female, and a positive bias of 4.3 (12.2%) in 42 Hispanic/Latino male. The reclassification of kidney disease stages was not performed in Hispanic/Latino patient due to small sample size.

**Conclusion:** Compared with the eGFR calculated by MDRD equation, the 2021 CKD-EPI without race equation caused a negative bias around -3.0 (-10.0%) in black people, which lead to 30% black people to be reclassified to a more severe stage of kidney disease. On the other hand, the 2021 CKD-EPI without race equation caused a positive bias around 3.0 (10.0%) in non-black people, which lead to 30% non-black people to be reclassified to a less severe stage of kidney disease.

## A-011

### Performance Evaluation of the Atellica® CH 930 Analyzer Lac<sub>3</sub>, CHE<sub>2</sub>, and Hapt<sub>2</sub> Assays

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**Background:** The analytical performance of the Atellica® CH Lactate<sub>3</sub> (Lac<sub>3</sub>), Cholinesterase<sub>2</sub> (CHE<sub>2</sub>), and Haptoglobin (Hapt<sub>2</sub>) assays on the Atellica® CH 930 Analyzer was evaluated. Lactate measurements are used in assessing circulatory function and oxygen status in plasma and differentiation between bacterial/viral meningitis in cerebrospinal fluid (CSF). Cholinesterase measurements are used in diagnosis and treatment of organophosphorus poisoning and certain liver diseases. Measurement of haptoglobin is used in the diagnosis of hemolytic disorders. The Lac<sub>3</sub> assay is based on oxidizing the lactate with lactate oxidase to result in pyruvate and

hydrogen peroxide. Lactate is measured proportionally by the formation of dye from hydrogen peroxide and a chromogen in the presence of a peroxidase. The CHE<sub>2</sub> assay catalyzes the hydrolysis of butyrylthiocholine (BTC) to butyrate and thiocholine, which reduces 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to 5-thio-2-nitrobenzoate (TNB). Cholinesterase amount is proportional to the absorbance. The Hapt<sub>2</sub> assay is based on the reaction between antibody and haptoglobin in a serum/plasma sample. Antibody reacting with human haptoglobin forms insoluble complexes which can be quantitated by the turbidity they produce. These new assays for the Atellica® CH Analyzer are liquid-stable and ready to use. **Method:** Performance testing included linearity, precision, and accuracy. Linearity was evaluated utilizing CLSI EP06-A using a nine-level dilution series. Assay precision was evaluated utilizing CLSI EP05-A2 over 20 days, with two replicates per sample twice per day. Assay accuracy was evaluated utilizing CLSI EP09-A3. Patient sample results for Lac<sub>3</sub> were compared to those of the Atellica CH Lac<sub>2</sub> assay for plasma and Dimension Vista® LA assay for CSF. Patient samples for CHE<sub>2</sub> and Hapt<sub>2</sub> were compared to the Atellica CH CHE assay and Dimension Vista HAPT assay respectively. **Results:** The Atellica CH Lac<sub>3</sub> assay is linear from 1.8-140.0 mg/dL (0.20-15.54 mmol/L), the CHE<sub>2</sub> assay from 1500-30,000 U/L, and the Hapt<sub>2</sub> assay from 10-700 mg/dL (0.10-7.00 g/L). Within-lab precision for the Lac<sub>3</sub> assay ranged from 0.6-1.2% CV in plasma and 0.8-2.3% in CSF. The CHE<sub>2</sub> assay ranged from 0.5-1.5% CV. The Hapt<sub>2</sub> assay ranged from 0.9-2.5% CV. The Lac<sub>3</sub> assay plasma comparison study yielded a regression equation of  $y = 0.95x + 1.5$  mg/dL ( $y = 0.95x + 0.17$  mmol/L) with  $r = 0.998$  versus the Atellica CH Lac<sub>2</sub> assay. The Lac<sub>3</sub> assay CSF comparison study yielded a regression equation of  $y = 0.95x + 0.1$  mg/dL ( $y = 0.95x + 0.01$  mmol/L) with  $r = 0.998$  versus the Dimension Vista LA assay. The CHE<sub>2</sub> serum assay comparison study yielded a regression equation of  $y = 1.12x + 321$  U/L with  $r = 1.000$  versus the Atellica CH CHE assay. The Hapt<sub>2</sub> serum assay comparison study yielded a regression equation of  $y = 0.97x + 1$  mg/dL ( $y = 0.97x + 0.01$  g/L) with  $r = 0.996$  versus the Dimension Vista HAPT assay. **Conclusions:** The new Atellica CH Lac<sub>3</sub>, CHE<sub>2</sub>, and Hapt<sub>2</sub> assays are liquid-stable and ready to use. These assays evaluated on the Atellica® CH 930 Analyzer demonstrated acceptable linearity, precision, and accuracy compared to on-market assays. \*The products/features mentioned here are not available for sale in the U.S. Their future availability cannot be guaranteed.

## A-012

### Development of an assay for the measurement of Total Immunoglobulin E (IgE) on Beckman Coulter Clinical Chemistry Analyzers

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**Background:** Total Immunoglobulin E (IgE) is a member of the immunoglobulin family of proteins that are produced by plasma cells in response to antigenic stimuli. IgE is unique however in certain structural aspects and the role it plays in allergic diseases. Measurement of total serum IgE is often used as a tool in the diagnosis and management of atopic diseases such as asthma, allergic rhinitis, atopic dermatitis and urticaria and has been used to distinguish atopic from non-atopic individuals presenting allergy-like symptoms. The new Beckman Coulter IgE assay\* is intended for use in the quantitative determination of total IgE concentration in human serum and plasma (lithium heparin, sodium heparin, K<sub>2</sub> EDTA, K<sub>3</sub> EDTA) samples on the Beckman Coulter AU/DxC AU clinical chemistry analyzers. **Methods:** Anti-IgE antibody-coated particles bind to IgE in the serum/plasma patient sample resulting in the formation of insoluble aggregates, measured by turbidity. The amount of particle aggregation correlates with the concentration of IgE in the sample. The following verification studies demonstrate the performance of the IgE assay on the AU/DxC AU chemistry analyzers. **Results:** Precision studies were conducted adhering to CLSI EP05-A3 guidelines utilizing 5 samples with 80 replicates each over 20 days on three DxC 700 AU analyzers. The IgE assay exhibits total imprecision of  $\leq 7.5\%$  with a total standard deviation (SD)  $\leq 7.0$  IU/mL at concentrations  $\leq 93.3$  IU/mL. The within run imprecision is  $\leq 7.0\%$  with a SD  $\leq 5.0$  IU/mL at concentrations  $\leq 71.4$  IU. The IgE assay demonstrated acceptable linearity throughout the analytical measuring range of 20 to 500 IU/mL following CLSI EP06-A. The sensitivity of the assay was verified in accordance with CLSI EP17-A2 and displayed a Limit of Blank (LoB) at  $\leq 10$  IU/mL, Limit of Detection (LoD) at  $\leq 15$  IU/mL and Limit of Quantitation (LoQ) at  $\leq 20$  IU/mL. A method comparison study following CLSI EP09c-ED3 compared the IgE assay on the AU/DxC AU Systems and a commercially available chemiluminescent immunoassay method. Serum patient samples ( $n = 136$ ) measured across the analytical range were analyzed using Weighted Deming regression and yielded a slope of 0.966, an intercept of 1.0 IU/mL and correlation coefficient  $R = 0.996$ . Interference studies following CLSI EP07-A2 guidelines demonstrated no susceptibility of the assay to interference from common endogenous substances and a panel of 21 exogenous drug substances. On-board stability and calibration frequency were assessed by monitoring IgE sample recoveries over 29 days with calibration at days 0 and 15. The on-board

stability was analyzed by measuring the recovery difference or % difference against day 0 as per CLSI EP25-A guidelines. **Conclusion:** The IgE assay on the DxC 700 AU, AU5800, AU680 and AU480 analyzers is a precise and accurate assay utilizing serum and plasma samples to aid in the diagnosis of IgE-mediated allergic disorders in conjunction with other clinical findings.

\*Pending clearance by the United States Food and Drug Administration and achievement of CE compliance. Not currently available for *in vitro* diagnostic use.

## A-013

### A Case for Updating Lipid Panel Assay Performance Recommendations

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**Background:** According to the US 2018 Multisociety Guideline on the Management of Blood Cholesterol, in patients 40 years or older without diabetes and with a low-density lipoprotein cholesterol (LDL-C) less than 190 mg/dL, decisions regarding the necessity for, and intensity of, preventative interventions, should begin with an estimation of risk by one of the pooled cohort equations (PCE), and may be further guided by consideration of risk-enhancing factors, additional testing and clinical judgement. Total serum cholesterol (TC) and high-density lipoprotein cholesterol content (HDL-C) are utilized in PCE, while elevated LDL-C and triglyceride (TG) concentrations are considered among the risk-enhancers. Thus, inaccurate lipid panel results may result in an incorrect assessment of risk, and mismanagement. The National Cholesterol Education Program (NCEP) recommends criteria for analytical imprecision and bias in lipid assays based on expert opinion, and on limitations as to what is analytically achievable by the state of the art. This is the weakest form of evidence according to the Milan Hierarchy of 2015. Furthermore, these performance recommendations have not been revised since 1995. We investigated the extent of risk misclassification that may occur when current NCEP performance recommendations are met, and explored new target recommendations that would reduce the misclassification rate. **Methods:** Lipid panel data for approximately 42,500 primary prevention candidates, aged 40-75 years, were extracted from the NHANES database. These "true" lipid values were used to classify the subjects into four risk groups based on the 2018 guidelines. Analytical bias and imprecision, at the recommended allowable limits, as well as biological variability as per the European Federation of Clinical Chemistry and Laboratory Medicine database, were introduced to the measured values in various combinations to determine the impact of these on misclassification. The errors were then reduced iteratively to examine the effects on misclassification rate. **Results:** Using the current NCEP performance criteria, risk misclassification of more than 10% of individuals may occur. Improved proportional bias by 1% in all assays, and imprecision to 3% in all assays would reduce the potential for misclassifications to less than 9%. This amounts to total error reductions from 9% to 8% for TC, from 13% to 10% for HDL-C and from 15% to 10% for TG. **Conclusion:** The current NCEP recommendations for analytical performance of standard lipid panel assays are outdated and allow for an unacceptable degree of misclassification and possible mismanagement of individuals with respect to cardiovascular disease risk. This error can be diminished by improving assay performance, and by reducing the impact of additional contributors to measurement uncertainty in the estimation of LDL-C.

## A-014

### Audit Of High Sensitivity Cardiac Troponin Requesting Patterns In Routine Clinical Use.

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**Introduction.** Routine measurement of troponin by a high sensitivity method (hs cTn) has been in routine use in the UK for 12 years and accelerated diagnosis recommended since 2014. We conducted an audit of requesting patterns against current protocols. **Methods.** The current protocol uses a single test on admission with patients considered for discharge if at low clinical risk and the hs cTnT is <3 ng/L. Patients with hs cTnT >50 ng/L are scheduled for admission. Patients with an initial troponin in the range 14-50 ng/L have a repeat test at 3 hours. If the repeat test is <14 ng/L with a delta (3 h sample - 0 h sample) of <=7 and low risk, patients are scheduled for discharge. Patients in the range 14-50 with a delta <=7 ng/L are further assessed. Patients with a delta >7ng/L are admitted. Cardiac troponin T (cTnT) was measured by the Roche high sensitivity cardiac troponin T assay hs-cTnT (Roche diagnostics), range

3 - 10,000ng/L, 10% CV 13ng/L, 99<sup>th</sup> percentile 14 ng/L. All test requests and results from August to November 2021 for cardiac troponin from the emergency department (ED) were extracted from the laboratory information system including date and time of result. Data was transferred to a relational database (Access, Microsoft corp) for analysis. Non-parametric statistics were used throughout using the Analyse It (www.analyse-it.com) add in for Excel. Results were divided into single tests/patient episode and serial tests/patient episode. **Results.** Data was obtained from 7352 requests on 6980 patients median age 55.8 years (interquartile range 37.8-73.7) 50.4% female. There were multiple attendances of 306 patients. There were 4869 single requests from 4593 patients, 996 (20.5%) <3 ng/L, 2664 (54.7%) in the range 3-50 ng/L, 270 (5.5%) >50 ng/L. Hence 26.0% of patients could be discharged or admitted using a single troponin. Most single troponin requests were repeated. Serial testing occurred in 2483 episodes in 2387 patients. 99 (2.7%) had both cTnT values 3 ng/L, 282 (11.4%) of the initial values were >50ng/L. 1260 had initial values in the range 3-14 ng/L (50.7%) with a delta <=7 ng/L in 1248 (50.3%). There was a delta <=7 ng/L in 809/873 of those in the range 14-50 ng/L. Even in patients with a clearly abnormal cTnT (>200 ng/L) a raised delta was seen in only 86/282. **Conclusions.** Use of the current protocol allowed identification of low risk in 31.4% of all patient episodes. Troponin requesting patterns in routine clinical practice differ significantly from recommendations and reflects the use of troponin as a catch-all test for any possibility of cardiac damage in patients presenting with other clinical conditions where a single test is seen as sufficient. Lack of repeat testing for values in the range 14-50 ng/L was surprising and requires further investigation.

## A-015

### Feasibility Of Implementing A 0-2 Troponin Algorithm

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**Introduction.** Recent guidelines have recommended rapid diagnosis based on repeat sampling at 2 hours from admission. We investigated the feasibility and diagnostic equivalence of repeat measurement at 2 hours by comparing the diagnostic classification achieved by measurement at 0 and 2 hours with a delta value of <=3 between samples to measurement at 0 and 3 hours and a delta of <=7ng/L between samples. **Methods.** From August to November 2021 all patients with chest pain where a diagnosis of acute coronary syndrome (ACS) was considered had a diagnostic protocol of measurement of cardiac troponin T (cTnT) on admission and at 2 and 3 hours from admission. Requests and results were extracted from the laboratory information system including date and time of result. Data was transferred to a relational database (Access, Microsoft corp) for analysis. Non-parametric statistics were used throughout using the Analyse It (www.analyse-it.com) add in for Excel. Cardiac troponin T (cTnT) was measured by the Roche high sensitivity cardiac troponin T assay hs-cTnT (Roche diagnostics), range 3 - 10,000ng/L, 10% CV 13ng/L, 99<sup>th</sup> percentile 14 ng/L. **Results.** 728 sets of serial samples were obtained on 711 patients, 40.1% female median age 61.8 years, (interquartile range 50.6-75). Comparison of classification showed agreement between the 2 hour and 3 hour delta in 593 cases (558 rule out, 35 rule in) and disagreement in 43. There were 37 cases that would have required further investigation, either by repeat sampling or admission. In 6 cases a positive 3 hour delta occurred with a 2 hour delta of 3 or less. 4 had values exceeding the 99<sup>th</sup> percentile on the admission sample so would have been retained for further investigation. The remaining 2 patients had co-existing clinical conditions that required further investigation. **Conclusion.** The routine use of serial sampling at admission and 2 hours was clinically safe and resulted in the same clinical decisions in the context of the busy ED environment for the population served by the hospital. However, this was at the expense of requiring further testing of some patients. The optimum strategy in this group remains to be determined.

**A-016**

**RACE-IT: Rapid Myocardial Infarction Exclusion Using an Accelerated High-Sensitivity Cardiac Troponin I Protocol in a Prospective Trial**

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**Background:** We assessed the safety of a rapid (0/1-hour) protocol compared to our standard protocol using high-sensitivity cardiac troponin I (hs-cTnI) for the exclusion of myocardial infarction (MI). **Methods:** In a stepped-wedge randomized trial, patients were evaluated for possible MI in 9 Emergency Departments (ED): 5 hospital-based and 4 free-standing EDs, in urban and suburban settings. Trial arms included the accelerated protocol and standard care; a new site implemented every 3 weeks. The hs-cTnI assay was from Beckman Coulter (99th percentile 18 ng/L). Patients were excluded from the study if any hs-cTnI was >18 ng/L within 3 hours of ED presentation or they were admitted to the hospital. In the 0/1-hour algorithm, MI was ruled out if hs-cTnI was <4 ng/L at time 0, or =4 ng/L at time 0 with the 1-hour hs-cTnI <8 ng/L. The algorithm recommended ED discharge if MI was ruled out by the 0/1-hour protocol. If not ruled out at 1 hour, a 3-hour hs-cTnI was collected. In the standard care arm, hs-cTnI was measured at 0 and 3 hours with values ≤18 ng/L used to rule out MI and recommend discharge if the HEAR score was <4. The primary outcome was adjudicated death or MI at 30 days. The analysis included a mixed effect model adjusting for ED site, time, sex, age, and race. **Results:** A total of 22,345 patients were studied, with 24 deaths and 26 MIs at 30 days. Overall, there was no statistically significant difference between the death/MI rates in the accelerated protocol and the standard care group (Table). **Conclusion:** The study demonstrated the 0/1-hour algorithm to evaluate for MI in the ED setting was safe to use when compared to standard care.

30 Day Adverse Events

	All (%)	Standardcare (%)	Rapid rule-out (%)	Adjusted odds ratio (95% CI)	p-value
Participants, n	22,345	9488	12,857		
Myocardial infarction/all-cause death, n (%)	50 (0.22)	18 (0.19)	32 (0.25)	0.82 (0.30-2.26)	0.71
All-cause death, n (%)	24 (0.11)	13 (0.14)	11 (0.09)	0.44 (0.18-1.04)	0.61
Non-cardiac death, n (%)	24 (0.11)	13 (0.14)	11 (0.09)	0.44 (0.18-1.04)	0.61
Myocardial infarction, n (%)	26 (0.12)	5 (0.05)	21 (0.16)	1.95 (0.50-7.99)	0.34
-Type 1 Myocardial infarction, n (%)	8 (0.04)	4 (0.04)	4 (0.03)	0.04 (0.00-0.57)	0.02
-Type 2 Myocardial infarction, n (%)	18 (0.08)	1 (0.01)	17 (0.13)	12.6 (1.70-94)*	0.01
*Only unadjusted OR reported due to challenge fitting model					

**A-017**

**CDC Clinical Standardization Programs for Free Thyroxine. Results of the Baseline Interlaboratory Study**

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**Background:** Thyroid function tests are the most used tests in the U.S. and are the only means to detect diseases of the thyroid. Concerns about the accuracy and reliability of thyroid function tests, especially free thyroxine (FT4), have been stated by the clinical laboratory community for many years. In response to these concerns, the CDC Clinical Standardization Program (CSP) has created a standardization program

for FT4 based on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference system and is partnering with IFCC to standardize FT4 testing globally.

**Methods:** There are no commutable, certified serum-based FT4 reference materials (RMs) available to assess the accuracy and reliability of FT4 assays. CDC has developed an accurate and sensitive reference measurement procedure (RMP) to assign reference values to individual donor sera. Assay manufacturers and research and clinical laboratories can use these sera to assess the analytical performance of their measurements, and monitor analytical performance over time. In addition, the CDC's FT4 RMP is used to assign reference values to materials used in EQA/PT programs to verify that standardization efforts at the developer level improve measurement accuracy and reliability at the end-user level.

**Results:** CDC CSP has conducted a study to define the differences among various types of commercially available and lab developed FT4 assays to inform its FT4 standardization program. The design of this study is based on the guidelines in the Clinical and Laboratory Standards Institute document EP-09-A2. A panel of 40 identified, fresh-frozen single donor patient serum samples was measured by participants and used the CDC reference method for FT4. Results from eight commercial assays and one laboratory developed test (LDT) were compared to the reference. Preliminary results indicate a negative mean bias for all commercial FT4 assays ranging from -34.77% to -5.48%. An equilibrium dialysis mass spectrometry-based assay had a mean bias of -4.13%. Preliminary results suggest that the observed bias can be effectively minimized through recalibration to the reference methods operated by the CDC CSP and the IFCC reference laboratory network.

**Conclusion:** The results of the interlaboratory comparison study will serve as a baseline for the CDC CSP and is used to customize samples and operation to address specific needs of manufacturers and LDTs.

**Disclaimer:** The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry. Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service, and the US Department of Health and Human Services.

**A-018**

**Anti-MDA5-Positive Dermatomyositis: a new clinical phenotype in patients with skin lesions**

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**Background:**

Dermatomyositis (DM) is an idiopathic inflammatory myopathy with a variable clinical spectrum. In recent years, a number of myositis-specific antibodies have been identified including anti-MDA5, anti-Mi-2, anti-NXP-2, anti-TIF-1γ, and anti-SAE-1/2 but there are also a number of myositis-associated antibodies including anti-Ro52, anti-PM/Scl and anti-U1RNP, which are not specific. Anti-MDA-5 antibodies, originally called anti-CADM-140 antibodies due to their association with clinically amyopathic dermatomyositis, were discovered in 2005. The clinical presentation of anti-MDA5 DM differs significantly from the other forms of DM, with three distinct clinical phenotypes, according to the predominance of skin-articular, pulmonary or vascular symptoms. We report one patient of DM with seropositive to the autoantigen MDA-5.

**Methods:**

In suspected cases of DM, patient sera were investigated using an indirect immunofluorescence assay (IFA) on a substrate combination of HEp2 cells and primate liver, with confirmation of results by monospecific tests. Since antibodies against the cytoplasmic antigens are sometimes not clearly detectable with IFA, parallel performance of the confirmatory test were realized using immunoblot assays (Euroimmun).

**Results:**

A 36-year-old woman who started with pruritic pearly plaques on the gluteus compatible with morphea, in the context of pregnancy, that improved with UVA treatment. Months later, physical examination revealed erythematous rash on the face, the anterior chest, violaceous plaques located on the dorsal part of the interphalangeal joints, called Gottron's papules and cracked palmar fingertips (mechanic's hands). Our patient referred pain in both wrists, ankles, elbows and knees with some functional impotence, dyspnea of great efforts, generalized alopecia in the last months and recurrent headache. Inflammatory muscle disease was minimal without elevation of muscle enzymes. Chest x-ray showed no clear interstitial infiltrate. Autoimmunity study was performed with positive antinuclear antibodies and showed a nuclear fine speckled

pattern at a titer of 1:160. Due to the dermatologic findings suggestive of DM and the mild muscle weakness, a specifi panel was conducted for differential diagnosis of DM and some forms of anti-synthetase syndrome. Myositis panel assay was positive for MDA5 antibody. Patient was treated with a combination immunosuppressive therapy based on prednisone and azathioprine. Actually, the patient shows slight clinical improvement and remains stable.

#### Conclusion:

Anti-MDA-5 DM is a rare systemic autoimmune disease, historically described in Japanese patients with clinically amyopathic dermatomyositis. DM encompasses a wide spectrum of disease with complex phenotypes, which makes diagnosis difficult, so there is a great need for better tools for diagnosis and prognosis. The discovery of myositis specific autoantibodies allowed a better definition of subgroups of patients in terms of clinical phenotypes, prognosis and response to treatment.

Multiparametric immunoblots are a good confirmatory method, as they enable mono-specific and simultaneous detection of many different antibodies, full automatability and easy interpretation.

## A-019

### Tiered Hemolysis Index Thresholds to Enhance Direct Bilirubin Reporting from Roche cobas Analyzers

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**Background:** Hemolysis is a common pre-analytical variable that leads to test cancellations and patient re-draws, delaying the resulting of accurate laboratory results. These patient safety issues are particularly problematic for pediatric patients. The Roche diazo reagent for direct bilirubin (BILD) commonly used on cobas clinical chemistry analyzers is sensitive to hemolysis. According to the package insert, BILD results are affected above a hemolysis index (HI) of 30 (corresponding to approximately 30 mg/dL hemoglobin) based on a bias of 10% at 0.3 mg/dL BILD. In this study, we aim to establish tiered HI thresholds for the Roche BILD reagent on a cobas c701 analyzer in order to minimize cancellations due to hemolysis.

**Methods:** Residual non-hemolyzed lithium heparin plasma was used to create sample pools targeting BILD concentrations of 0.5, 1.0, and 5.0 mg/dL. Concentrated hemolysate was prepared via freeze/thaw cycles using blood drawn from a healthy donor. Increasing volumes of the concentrated hemolysate (1 - 6.4 mL) were spiked into 500 mL of each sample pool to achieve HI ranging from 30 - 200. Each hemolysis spike was measured in duplicate using BILD and serum index reagents on a cobas c701 analyzer (Roche Diagnostics). Interferograms were generated to visualize the effect of increasing hemolysis on BILD results relative to an acceptable total allowable error (TAE) of 0.4 mg/dL or 20% and to establish HI thresholds where BILD results would be reported without a disclaimer (bias <50% of TAE), reported with a disclaimer (bias 50 - 100% TAE), or cancelled (bias >TAE). The clinical impact of these tiered HI thresholds was evaluated by retrospective review of middleware data (Instrument Manager v8.14, Data Innovations) collected over a one-year period.

**Results:** Increasing hemolysis induced a negative bias in all BILD pools studied. At 0.5 mg/dL BILD, bias was less than 50% of TAE at HI 140 and exceeded TAE at HI 200. At 1.0 mg/dL BILD, bias was less than 50% TAE at HI 50 and exceeded TAE at HI 110. At 5.0 mg/dL BILD, bias was less than 50% of TAE at HI 70 and exceeded TAE at HI 130. Of note, all of these empirically-derived HI thresholds were higher than the HI of 30 stated in the Roche BILD package insert. Retrospective analysis of one year of BILD results yielded hemolysis-induced cancellation rates of 17% overall and 31% in the pediatric population when following the manufacturer's recommended HI threshold of 30. Implementation of our tiered HI thresholds would reduce these cancellation rates by an order of magnitude to 1.7% overall and 3.2% for pediatric samples. A disclaimer alerting providers of a hemolysis-induced negative bias in reported BILD concentrations would be appended to 1.8% of all results and 4.3% of pediatric results.

**Conclusion:** The HI threshold recommended in the Roche BILD package insert is too stringent, leading to unnecessary test cancellations and re-draws due to hemolysis. Implementation of tiered HI thresholds significantly reduces BILD cancellations due to hemolysis, while ensuring that BILD results reported in slightly hemolyzed samples are reliable for clinical use.

## A-020

### Clinical performance of total-Tau and total-Tau/phospho-Tau ratios across platforms for the diagnosis of Creutzfeldt-Jakob Disease

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**Background:** Creutzfeldt-Jakob disease (CJD) is a rare but fatal prion disease. The definitive diagnosis of CJD can only be made postmortem. However, clinical symptoms, imaging studies, and laboratory tests can help support a diagnosis of CJD. While the real-time quaking-induced conversion (RT-QuIC) prion assay is highly sensitive and specific for CJD, it has a long turn-around time (TAT) and is not widely accessible. Moreover, the sensitivity of the RT-QuIC assay is decreased for certain clinical and molecular subtypes of CJD. CSF biomarkers including total-tau (t-Tau) and more recently phospho-tau (p-Tau) are adjunct tests used in the work of suspected CJD cases. Prior studies have indicated utilizing the ratio of t-Tau/p-Tau may have improved performance compared to t-Tau. However, a confounding factor in these studies is that these assays are not standardized and therefore diagnostic cut-offs are not directly transferable across testing platforms. The objectives of this study were 1) to determine platform-specific t-Tau cut-offs and 2) to determine if clinical performance of Tau ratios is superior to t-Tau concentrations alone for the detection of CJD.

**Methods:** Residual CSF samples from 108 CJD subjects with RT-QuIC results were included. Chart review was performed to classify CJD-positive cases as possible (n=3), probable (n=69), and definite (n=39) according to the CDC's Diagnostic Criteria for CJD. Residual CSF samples were also collected for 559 non-CJD subjects which included Alzheimer's disease (n=132), autoimmune encephalitis (n=67), frontotemporal dementia (n=35), Lewy body dementia (n=29), and other neurodegenerative conditions (n=296). t-Tau and p-Tau were measured in-house (Roche Elecsys) or by Athena Diagnostics (Innotest®). t-Tau was also measured by the National Prion Disease Pathology Surveillance Center (NPDPSC, Cleveland, Ohio). Optimal cutoff values for t-Tau and t-Tau/p-Tau to differentiate CJD from non-CJD were estimated using the Youden index. Sensitivity and specificity for t-Tau and t-Tau/p-Tau were determined. Statistical significance was determined using the Student's t test.

**Results:** t-Tau concentrations (mean±SEM; pg/mL) were significantly increased in CJD patients when measured by the NPDPSC assay (668±226 non-CJD vs 4847±364 CJD, p<0.0001), Athena (non-CJD 487 ± 245 vs CJD:3813±410, p<0.0001), and Roche (non-CJD:245±28.4 vs CJD:1770±98.0, p<0.0001) assays. p-Tau concentrations (pg/mL) were not significantly different between groups regardless of the assay used. The t-Tau/p-Tau ratio was significantly increased in CJD vs non-CJD by Athena (non-CJD:8.0±12.1 vs CJD:64.5±6.1, p<0.0001) and Roche (non-CJD:12.0±1.4 vs CJD:96.7±4.7, p<0.0001). Optimal t-Tau cut-offs varied across platforms. The NPDPSC optimized assay cut-off of 1893pg/mL had 69.7% sensitivity and 96.5% specificity (AUC:0.915, 95%CI: 0.868-0.962). The Athena optimized assay cut-off of 1608 pg/mL had 59.0% sensitivity and 96.3% specificity (AUC:0.885, 95%CI: 0.818-0.953). The Roche cut-off of 808 pg/mL had 69.2% sensitivity and 98.5% specificity (AUC:0.962, 95%CI: 0.937- 0.986). A t-Tau/p-Tau cut-off of 12.7 (sensitivity 86.8% specificity 99.9%, AUC: 0.914, 95%CI: 0.840-0.988) was optimal for Athena while 30.5 was optimal for Roche (sensitivity 84.6% specificity 99.9%, AUC:0.986, 95%CI:0.976- 0.995).

**Conclusion:** Platform-specific cut-offs for t-Tau concentrations are required to optimally differentiate between CJD and non-CJD. The use of t-Tau/p-Tau ratios has superior diagnostic performance for ruling out CJD.

## A-021

### Assessment of neutralization potential of convalescent and vaccinated individuals against mutated SARS CoV2 strains- using fully automated IVD assays

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**Background:** The global SARS CoV2 pandemic has so far infected around 6% of the world population and led to more than 6 million casualties. We developed antibody tests measuring the anti-Spike1-IgG titers and a fully automated Assay for the measurement of the neutralizing potential of anti-SARS CoV2 antibodies. Here we compare the level of the neutralizing potential of immune responses from mildly affected non-hospitalized CoViD 19 patients, severely affected and hospitalized CoViD 19 patients and fully vaccinated individuals against SARS CoV2 (WT and Variant alpha, beta and gamma).

**Samples and Methods:**

## Vaccinated Individuals

Samples (n=18) originated from healthy voluntary donors (mean age 50 yrs. (11 female 7 male) having received both doses of either Comirnaty (Pfizer) or Spikevax (Moderna) vaccine at average 14 days before donation

## Convalescent Long-term follow-up

Convalescent voluntary donors n=25, mean age: 45 yrs.; 10 female 15 male that had been infected with CoViD 19 within Q1 and Q2 of 2020. The volunteers gave repeated donations. Samples were provided by CSL Behring in an unified effort to develop an IVIG product to administer CoViD 19 immunity towards immunologically impaired individuals.

## Hospitalized

Samples from n=20 patients (mean age 67yrs; 13 females, 5 male) hospitalized in Q1 2020 within the first wave of CoViD-19 in Europe. The patients received treatment in the CHU de Montpellier. The patients were suffering from moderate to severe symptoms of CoviD19 and samples were drawn at 7 to 51 days (mean 32) post positive PCR result of SARS CoV2 infection.

## ARBLA

S1-RBD antigens of the wild type variant, also S1 RBD variants alpha beta and gamma were coated on irradiated polystyrene ELISA wells. Plasma samples were tested on the Phadia System at 37°C. Antibodies bound to the S1 RBD antigens and all variants are detected fluorometrically with a human angiotensin-converting enzyme 2 (ACE2)- $\beta$ -galactosidase conjugate and a 4-methylumbelliferyl- $\beta$ -D-galactosidase substrate. The ARBLA method follows the principle of a competitive inhibition assay, measuring the inhibition of the SARS-CoV-2 ACE2-receptor binding by antibodies present in diluted plasma samples.

**Results:** The results indicate two different levels of protection against an infection or reinfection against a SARS-COV-2 infection. An infection that caused only moderate symptoms, will only lead to a moderate titer of neutralizing antibodies. Even at a timepoint when anti S1 IgG titers are at a peak, the neutralizing potential remains relatively low- significantly lower than of hospitalized patients with a severe disease course. When comparing the relative inhibition values of vaccinated individuals in the face of mutant variant strains of SARS-CoV-2, significant differences can be observed.

**Conclusion:** An effective protection of neutralizing antibodies cannot be observed in the sera of convalescent plasma samples from patients of the first wave of CoViD19 that were only mildly affected by the disease. Regardless of the Variant tested the neutralization remains at a level of approximately 1/30 of the level that can be observed e.g. in mRNA vaccinated individuals at about 2 weeks after the 2nd Dose of vaccine. The fading of IgG antibodies limits the duration of the protection.

**A-022****Utility of Commercially Available Quantitative hCG Immunoassays as Tumor Markers in Trophoblastic and Non-Trophoblastic Disease**

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**Background:** The use of hCG as a tumor marker has been widely accepted among clinicians and laboratorians. Differences in iso- and glycoform recognition among hCG immunoassays is well-established, but comparison in tumor marker applications has not been reported. Here, we assess the utility of five quantitative serum hCG immunoassay platforms for use as tumor markers in patients with trophoblastic and non-trophoblastic disease. **Methods:** Remnant specimens from 150 patients with gestational trophoblastic disease (GTD; n = 18), germ cell tumors (GCT; n = 42), non-trophoblastic disease (NTD; n = 88), and other malignant disease (n = 2) were obtained. For each specimen, hCG was quantified by five analyzer platforms (Abbott Architect Total  $\beta$ -hCG, Roche cobas STAT hCG, Roche cobas Total hCG +  $\beta$ , Siemens Dimension Vista Total  $\beta$ -hCG, and Beckman Access Total  $\beta$ -hCG). Data were evaluated by statistical analysis using GraphPad Prism. **Results:** Concentrations of hCG were highest in patients with GTD (Median, 12029.0; IQR, 431.3–15994.3 IU/L), followed by GCT (Median, 70.9; IQR, 15.4–9580.0 IU/L) and NTD (Median, 2.69; IQR, 1.4–5.2 IU/L). Inter-method CV ranged from 0.7–168.8% for all specimens and was significantly increased in specimens from patients with NTD compared to GTD (p = 0.0011) and GCT (p = 0.0487). Specimens were grouped by malignancy to assess frequency of elevated hCG production above reference cutoffs. Rates of elevated hCG were highest in GTD (94–100% of specimens), followed by GCT (50–57% of specimens), and NTD (4–9% of specimens). In total, 88% (132/150) of specimens

displayed concordantly elevated or non-elevated hCG among the five immunoassay platforms. Of the 12% (18/150) of specimens with discordant hCG results, 13/18 were from patients with NTD (prostate, n = 3; colorectal, n = 2; ovarian, n = 5; lung, n = 2; cholangiocarcinoma, n = 1), 4/18 from GCT (testicular seminoma, n = 2; testicular non-seminoma, n = 2), and 1/18 from GTD (hydatidiform mole, n = 1). The Roche cobas Total hCG +  $\beta$  method detected the greatest number of specimens among those with discordant results (11/18, 61%) and overall (50/150, 33%). In general, similar rates of elevated hCG detection were found between evaluated methods (Abbott, 46/150; Roche STAT, 44/150; Roche Total, 50/150; Siemens, 47/150; Beckman, 46/150). **Conclusion:** Choice of hCG immunoassay is unlikely to change clinical outcome for the purpose of tumor detection. Harmonization between hCG methods is lacking and needed; serial testing for tumor monitoring should be performed using a single method. Serum hCG may be a viable tumor marker in select patients with NTD, however further studies are needed.

**A-023****Fluid Type Matters: A Descriptive Analysis of Five Body Fluid Analytes to Inform Processes in the Clinical Laboratory**

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**Background:** Measuring the concentration of albumin, total protein, lactate dehydrogenase (LDH), amylase, and lipase in body fluids (BFs) provides important information to aid in clinical decision-making. The range of expected values of these analytes in BFs (peritoneal, pleural, pericardial, synovial, or drain fluids) is not expected to match serum exactly and the distribution by fluid type is not well described. Quality control (QC) materials are designed to reflect clinically relevant concentrations found in blood. Likewise, there is varying suitability of measuring ranges for assays modified for use in BF testing. Laboratorians and manufacturers would benefit from knowing the distribution of BF types as well as the expected concentrations of each analyte compared to serum. The aim of this study was to characterize the range of BF analyte concentrations compared to serum and identify the distribution of fluid types by analyte.

**Methods:** A retrospective analysis was performed for the following analytes in BFs: albumin (reportable range (RR) with maximum dilution factor x2, 0.2–12.0g/dL), total protein (0.2–36.0g/dL, x3 dilution), LDH (10–9000U/L, x10 dilution), amylase (3–140,00U/L, x100 dilution), and lipase (3–300,000U/L, x1000 dilution). All 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 were extracted from an institutional database of electronic medical record data at all Mayo Clinic locations. Fluid types were categorized as peritoneal, pleural, pericardial, synovial, or “other” (not approved fluid type or not documented). Serum results were included for comparison. Descriptive statistical analyses (median and range) were performed with GraphPad Prism 9.0. Mann-Whitney test for nonparametric data was used to compare medians.

**Results:** Median (range, n) of albumin was 1.0g/dL (<0.2–4.1g/dL, n=5,253) with 87.5% peritoneal, 10.6% pleural, 1.4% pericardial, and 0.5% other fluids. Albumin in BFs was lower compared to serum with median=4.1g/dL (<0.19–8.5g/dL, n=950,522, p<0.0001). The total protein median=2.6g/dL (<0.2–36g/dL, n=10,366) with 35.8% peritoneal, 60.2% pleural, 2.8% pericardial, 1% synovial, and 0.2% other fluids. Total protein in BFs was lower compared to serum with median=6.6g/dL (2.7–16.2g/dL, n=100,232, p<0.0001). The LDH median=149U/L (<10–31,835U/L, n=7,809) with 17.8% peritoneal, 78.8% pleural, 3% pericardial, 0.2% synovial, and 0.2% other fluids. LDH in BFs was lower compared to serum with median=212U/L (<10–25,000U/L, n=145,810, p<0.0001). The amylase median=56U/L (<3–166,160U/L, n=4,593) with 85.7% peritoneal, 9.9% pleural, 1.4% pericardial, and 3% other fluids. Amylase in BFs was lower compared to serum with median=60U/L (<3–7,002U/L, n=20,217, p=0.0005). Finally, the lipase median=34U/L (<3–560,000U/L, n=976) with 87.1% peritoneal, 12.1% pleural, 0.1% pericardial, and 0.7% other fluids. Lipase in BFs was higher than in serum with median=30U/L (<3–20,288U/L, n=81,696, p<0.0001).

**Conclusion:** The least common BFs received for testing included synovial and pericardial. The most common BFs were peritoneal for lipase, amylase, and albumin and pleural for LDH and total protein. The observed range of analyte concentrations was significantly lower than serum for albumin, total protein, LDH and amylase whereas lipase had a higher concentration in BFs than serum. Laboratories should be cognizant of clinically relevant BF concentrations and how the assays and QC meet this demand.

## A-024

**Analytical and Clinical Evaluation of the Automated Elecsys IL-6 Assay on the Roche cobas e602 Analyzer**

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**Background:** Since the onset of the SARS-CoV-2, healthcare facilities have been under stress to maximize resources by efficiently distributing them to the patients who need them the most. Interleukin-6 (IL-6) is a proinflammatory cytokine that is associated with many inflammatory diseases, including those induced by acute respiratory infections such as corona virus disease 2019 (COVID-19). Monitoring IL-6 levels in patients with inflammatory illnesses, such as COVID-19, may aid in risk assessment by identifying those patients at greater risk of more severe illness and additional intervention. At present, there are no FDA-approved IL-6 assays available. This validation study evaluates the automated Roche Elecsys IL-6 electrochemiluminescent immunoassay (ECLIA) that has been granted emergency use authorization (EUA) by the FDA.

**Methods:** The IL-6 ECLIA assay was evaluated for precision, linearity, interference (by hemoglobin, bilirubin, triglycerides, and biotin) and clinical performance was compared to V-PLEX Human IL-6 immunoassay (Meso Scale Discovery).

**Results:** The IL-6 ECLIA assay is precise (intra-assay <3% CV, inter-assay <5% CV), exhibits linearity across a measurable range of 1.5-4790 pg/mL, and is tolerant of significant interferences ( $H < 2522$ ,  $I < 62$ ,  $L < 2101$ , biotin <30 ng/mL). Comparison with V-PLEX Human IL-6 immunoassay revealed a 295% bias in patient samples evaluated for IL-6 concentration ( $n=43$ , range=1.5-1891 pg/mL,  $y=2.95x + 32.7$ ,  $r^2=0.84$ ). Bland-Altman analysis revealed an absolute mean bias of 151 pg/mL (SD =266 pg/mL).

**Conclusion:** The clinical utility of the IL-6 quantitative assay is to evaluate the evolution of inflammatory response over time for risk assessment. The Roche IL-6 assay showed good analytical performance; however, the large systematic bias compared to another reference method precludes using various methods to monitor IL-6 response. The random-access nature of an automated IL-6 assay on the Roche platform makes the test readily available.

## A-025

**A peptide enrichment and mass spectrometry-based workflow for the absolute quantitation of SARS-CoV-2.**

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**Background:** SARS-CoV-2 particles contain proteins that are biomarkers of a COVID-19 infection. An approach capable of detecting such proteins is bottom-up mass spectrometry (MS). Proteolytic digestion of proteins generates peptides, which can be separated by liquid chromatography (LC). A workflow had previously been developed that achieved low on-column detection limits for spiked nasal fluid samples in viral transport media (VTM). The addition of a peptide enrichment step, such as Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA), could increase sample purity. This may reduce background, thereby improving detection limits and reducing LC run-times. Furthermore, SISCAPA could remove any need for sample clean-up and allow the concentration of samples, meaning lower quantities of nucleocapsid protein in nasal fluids could be detected.

**Methods:** A mass spectrometry-based SARS-CoV-2 absolute peptide quantification method was developed using a Thermo Scientific™ Vanquish™ MD LC system and a Thermo Scientific TSQ Altis™ MD MS. Recombinant SARS-CoV-2 proteins and stable isotope-labeled standards (SIS) were spiked into pooled nasal fluids, before being added to VTM. Samples were then precipitated, centrifuged, and enzymatically digested (Thermo Scientific SMART Digest™ Trypsin kit). The resulting peptides were enriched with peptide-specific SISCAPA antibodies and separated using a 2-minute LC gradient with a Hypersil GOLD™ C18 column (1.9  $\mu$ m, 2.1 x 50 mm), coupled with a single reaction monitoring method. All assays were performed in triplicate and data was analyzed using Thermo Scientific TraceFinder™ LDT software.

**Results:** Full workflow testing with four peptide-specific antibodies confirmed the antibodies specificity and ability to work in conjunction. Coupling a mass spectrometry-based method with peptide enrichment not only allowed robust data acquisition, but also the LC-MS run-time to be shortened from 4 to 2 minutes. Absolute quantitation of targeted peptides was then performed by including the corresponding SIS for each peptide to mitigate measurement uncertainty, confirm the retention times, and correct for any possible matrix effects. LODs and LOQs were determined for each peptide, with all % RSD and % CV below 15%, and R-squared values greater than 0.99. Clear chromatographic separation was observed for each nucleocapsid peptide with mini-

mal variance in retention times observed ( $\pm 0.01$  minutes). Almost identical retention times ( $\pm 0.01$  minutes) were observed between each peptide and corresponding SIS. LODs and LOQs were determined to be between 0.25 and 2.5 femtomole on column for the three best performing peptides.

**Conclusion:** SISCAPA allowed 4-fold concentration of samples and eliminated the need for any further sample clean-up. This greatly increased the detection limits when the amount of nucleocapsid protein on each nasopharyngeal swab is considered, meaning that the protein could be detected at lower concentrations or on swabs with less spiked nasal fluid present. The increased sample purity associated with peptide enrichment also facilitated the reduction of LC-MS run-times from 4 to 2 minutes.

## A-026

**Diagnostic Performance for Myocardial Infarction of the Whole Blood Siemens VTLi Point of Care High Sensitivity Cardiac Troponin I Assay**

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**Background:** We evaluated the diagnostic performance of a whole blood point of care (POC) high sensitivity cardiac troponin I (hs-cTnI) assay for acute myocardial infarction (AMI) using serial samples at 0 and 2 hours. The POC assay performance was compared to two FDA-cleared central laboratory hs-cTnI assays.

**Methods:** We enrolled consecutive patients presenting to the emergency department with symptoms suggestive of ischemia/acute coronary syndrome (SEIGE; clinicaltrials.gov [NCT04772157](https://clinicaltrials.gov/ct2/show/study/NCT04772157)). Serial hs-cTnI testing was based on clinical indication, shortly after presentation (baseline, 0 hours) and 2 hours later. Parallel measurements were made using fresh whole blood (lithium heparin) on the Siemens Atellica VTLi POC hs-cTnI assay (investigational), fresh EDTA plasma on the Abbott ARCHITECT i2000 central laboratory assay used in clinical practice, and a second central laboratory assay, the Siemens Atellica, using lithium heparin plasma after one freeze/thaw cycle. Clinical adjudications for AMI were determined according to the Fourth Universal Definition of Myocardial Infarction using sex-specific 99<sup>th</sup> percentile upper reference limits (URLs) for all assays. Clinical sensitivities and negative predictive values (NPV) were calculated using 99<sup>th</sup> percentile URLs.

**Results:** The study cohort consisted of 1089 patients, including 418 females and 671 males. The prevalence of AMI was 91/1089 (8.4%). At baseline (0 hours) the POC hs-cTnI assay had a sensitivity of 65.7% for females and 67.9% for males and NPV of 96.4% for females and 96.7% for males. With serial sampling, sensitivity improved to 82.9% for females and 80.4% for males, while NPV improved to 98.2% and 97.9%, respectively. For the two central laboratory assays, comparable diagnostics were observed, as demonstrated for serial hs-cTnI measurements: females - sensitivity of 91.2% for ARCHITECT and 83.3% for Atellica, and NPV of 99.0% and 98.3%, respectively; males - sensitivity of 87.5% for ARCHITECT and 80.4% for Atellica, with NPVs of 98.7% and 97.9%, respectively.

**Conclusions:** The POC whole blood Atellica VTLi hs-cTnI assay demonstrated comparable diagnostic sensitivity for myocardial infarction to two central laboratory hs-cTnI assays using 0-hour and 2-hour serial sampling protocols at the 99<sup>th</sup> percentile upper reference limits.

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## A-027

**Pituitary Macroadenoma: Is high-dose hook effect still a relevant concern for prolactin measurement?**

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**Background:** Accurate measurement of serum prolactin is essential for the diagnosis and management of prolactin-secreting pituitary tumors, or prolactinomas. Tumor size generally correlates with circulating prolactin concentrations. Prolactin is typically measured in clinical laboratories using two-site "sandwich" immunoassays.

Historically, high-dose hook effect was considered a pitfall of these types of assays leading to falsely low results in samples with extremely high prolactin concentrations. Endocrine Society (ES) Clinical Practice Guidelines recommend measuring prolactin after serial dilutions when there is a discrepancy between pituitary tumor size (>1 cm) and a normal or mildly elevated (up to 250 ng/mL) prolactin concentration to rule-out high-dose hook effect. Serum prolactin measurement in neat and diluted samples is a clinically orderable test (Prolactin, Macroadenoma, Serum) performed in the Central Clinical Laboratory at Mayo Clinic, Rochester, MN. However, the frequency of high-dose hook effect in clinical specimens using a contemporary prolactin assay has not been evaluated.

**Objectives:** The aim of this study was to evaluate the frequency of high-dose hook effect when measuring prolactin in serum specimens from patients being evaluated for suspected prolactin-secreting macroadenoma.

**Methods:** Prolactin was measured using the Elecsys Prolactin II immunoassay (Roche Diagnostics, Inc.) on Cobas e immunoassay analyzers (e602/e801). Physician orders for Prolactin, Macroadenoma, Serum and corresponding prolactin results (neat, x10, x100, and x400 dilutions) were abstracted from the laboratory information system between 8/12/2014-8/30/2021. The % difference between each corrected dilution result (diluted result multiplied by dilution factor) and neat result was calculated for every ordered test with % differences <20% considered acceptable prolactin recovery (rule-out hook effect). Percent differences >20% were evaluated further. ES practice guideline adherence (with respect to neat prolactin concentration  $\leq$ 250 ng/mL) was also reviewed. **Results:** A total of 4171 Prolactin, Macroadenoma, Serum test orders from 3449 individual patients were included in the analyses. There were 2253 females and 1918 males with median(range) age of 56(4-94) years and 43(4-97) years, respectively. The mean(range) of neat prolactin concentration was 178(<1 to 25,231) ng/mL in males and 89(<1 to 27,384) ng/mL in females. Diluted sample results below the assay's analytical measurement range (AMR) (<1.0 ng/mL) were excluded from % difference analysis (n=8740). The average(range) % difference from neat result for x10, x100 and x400 dilutions was -2.3(-33-43)% (n=3130), -3.95(-22-21)% (n=477) and -6.4(-23-21)% (n=166), respectively. Fourteen patients had diluted results with >20% difference from the neat prolactin result but upon review the differences were due to diluted results being near the low limit of the AMR. The neat prolactin concentrations were  $\leq$ 250 ng/mL in 94% of orders.

**Conclusions:** Our studies show that no high-dose hook effect was observed in clinically-ordered Prolactin, Macroadenoma samples over a 7 year period. The manufacturer's package insert claims no high-dose hook effect at prolactin concentrations up to 12,690 ng/mL. Our data confirmed this finding at prolactin concentrations up to 27,384 ng/mL. The Roche Elecsys Prolactin II immunoassay does not appear susceptible to high-dose hook effect suggesting that routine evaluation for hook effect during clinical lab testing is no longer warranted.

## A-028

### Correlation Between Hemoglobin Measured by Point of Care Instrument and Hematocrit Measured by the Central Laboratory

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Hematocrit (HCT) as well as hemoglobin (Hgb) measurements are major determinants of blood viscosity and oxygen deliver dynamics and they are widely used to facilitate immediate therapeutic interventions. Rapid measurement as well as accurate classification of patients are therefore important. Although HCT measurement is often preferred by clinicians and is on many treatment protocols and that HCT instruments are commercially available, there are concerns on their complexity and of compliance with quality measures and the significant manual procedural components. Hemoglobin POCT devices are easier to use and offer wider quality assurance measures. This study compared Hgb and HCT measurement using both point of care testing (POCT) and laboratory-based instrumentation to assess clinical utility.

**Methods:** Retrospective hemoglobin levels obtained by POCT (HemoCue, HemoCue America, CA), and corresponding hematocrit as well as hemoglobin levels obtained by laboratory-based instrumentation (Sysmex XN 9100, Sysmex America, IL) were obtained. Correlation and statistical analysis were performed using NCSS® statistical software.

**Results:** Retrospective and corresponding Hgb and HCT values were obtained from 118 patients undergoing apheresis therapy. Mean and range hemoglobin levels by POCT and laboratory-based instruments were 12.8 g/dL (6.4 to 21.6), and 12.9 g/dL (6.4 to 21.6) respectively. Hematocrit levels ranged from 17.9 % to 65.7% (mean 38.7%). Correlation between POCT and laboratory-based Hgb was 0.994 (P<0.005) whereas, correlation between POCT Hgb and laboratory HCT was 0.998 (P<0.005). 55% of patients had hemoglobin <13 g/dL as determined by both POCT and labo-

ratory-based instrument, whereas 54% of patients had calculated hemoglobin (HCT divided by 3). 20.3 % of patients had Hgb >17.0 g/dL when measured by laboratory-based instrument and by HCT derived Hgb values, whereas fewer patients (19.5%) were obtained when measured by POCT. 68.6% of patients had HCT < 45% (considered optimum for circulatory dynamics). Few patients (13.6 %) exhibited overlap of HCT levels with Hgb levels between 13.0 and 17.0 g/dL.

**Conclusion:** There was good concordance between Hgb, HCT, and HCT derived Hgb obtained by both laboratory-based and POCT instruments. Few patients exhibited discrepant and abnormal HCT levels which was eliminated when using HCT derived Hgb calculation. Hemoglobin POCT provides comparable utility to laboratory-based HCT measurements facilitating rapid assessment and ease of compliance with quality measures. Futures studies will examine different patient population as well as correlation with clinical parameters of hemodynamics and therapeutic interventions.

## A-029

### Impact of Removing Race Adjustment When Estimating GFR on Chronic Kidney Disease Staging

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**Introduction:** Determination of glomerular filtration rate (GFR) is essential in assessment of kidney function, particularly in patients with chronic kidney disease (CKD). Formulas were developed to calculate estimated GFR (eGFR), as direct measurement is cumbersome and not amenable to routine monitoring. CKD-EPI is the most widely used formula for eGFR, using parameters of serum creatinine, gender, race, and age. A race adjustment was added from an unfounded assumption that Black patients have more muscle mass, increasing their eGFR by about 16%. There is concern on the impact of "correcting" for Black race on patient management, utility of eGFR, and its possible contribution to healthcare disparities. A new CKD-Epi refit formula is published and is being implemented by many. This study examined the impact of eliminating race adjustment from the original CKD-EPI formula on CKD staging in patients at a large safety-net hospital in an academic medical center. **Methods:** 80090 serum creatinine values from 56676 adult patients (31.4% Black, 67.5% female, median age 51 yrs) were collected from the electronic medical record (Epic, Verona, WI) over a 16-month timeframe. Values were excluded if they lacked age, gender, self-identified race, or serum creatinine. eGFR was calculated using the CKD-EPI equation with and without race modifier, and CKD staging was performed using 2012 Kidney Disease: Improving Global Outcomes guidelines. **Results:** In our cohort, only 50.8% of Black patients are in CKD1, indicative of no CKD, compared to 67.9% of non-Black patients. Given that these two cohorts are demographically similar, this shows a discrepancy in kidney function even before removing the race adjustment. After removal of the race modifier, 28.1% of Black patients were reclassified into a more severe CKD stage. The most restaging occurred from CKD3A to 3B (39%). **Conclusion:** Many Black patients in our study were reclassified to a more severe CKD stage, with the highest percentage of patients restaged to CKD3B, which would have attracted clinical attention for nephrology referral. We recommend removal of the race adjustment in eGFR, given its basis in the incorrect belief in biological differences between races. It perpetuates systemic racism and discrimination in healthcare, and its removal will provide more equitable care and reduce healthcare disparities. Future studies would re-examine the data in the light of the new CKD-Epi "refit" formula and also examine the increase in resources required to provide adequate medical care to patients who will be placed into a more severe CKD stage.

## A-030

### Vitamin D Levels Among Patients Prior to and During COVID-19 Pandemic

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**Background:** Vitamin D is one of the most commonly deficient vitamins in the US, and its deficiency has been implicated in many conditions and their pathophysiology. Pertinently, low levels have been suggested to be associated with poor COVID-19 outcomes. The COVID-19 pandemic and associated lockdowns changed daily living patterns for many Americans, likely altering sun exposure, medical follow-up, and supplement compliance. We examined vitamin D levels among our patient population prior to and during the pandemic and lockdown. **Methods:** To account for seasonal variation, vitamin D levels were reviewed between August and November for each year 2019, 2020, and 2021. In total 22,903 tests were reviewed, and medians and standard deviations were calculated. Vitamin D levels obtained were also categorized

according to the American Endocrine Society guidelines as deficient (<20 ng/mL), insufficient (20-30 ng/mL), or sufficient (>30 ng/mL). Statistical analysis was performed to determine if there was a significant difference between vitamin D levels and status before and during the pandemic. Results: Median and (interquartile range) vitamin D values for the study period were 23.0 (16.4 to 31.9), 25.0 (17.9 to 34), and 25.4 (18. to 34.3) ng/mL for the year 2019, 2020, and 2021 respectively. There was a significant difference in vitamin D levels prior to (2019) and during (2020 & 2021) the COVID-19 pandemic ( $p < .001$ ). Additionally, it was found that before the pandemic 38.3% of tests were in the deficient range, while during the pandemic that number has decreased significantly to 31.5% ( $p < .001$ ). Conclusion: Our results indicate that patient's vitamin D status among our population improved during the COVID-19 pandemic. Increased free time and avoidance of public indoor spaces may have led to increased sun exposure, and less busy schedules may have aided patients in medical follow-up and supplement compliance. Additional research is needed to determine degree of impact of the factors listed above to our observed increase in Vitamin D levels.

### A-031

#### Detection of anti-AK5 autoantibodies in patients with limbic encephalitis in indirect immunofluorescence assay and line blot

I. Heckler. *EUROIMMUN US, mountain lakes, NJ*

**Background** Adenylate kinase 5 (AK5) is an intracellular protein specifically expressed in the brain. It catalyses the phosphorylation of nucleoside monophosphates, thereby playing a critical role for cellular signaling pathways. So far, autoantibodies against AK5 have been described in at least 25 patients (REF 1-4), all suffering from limbic encephalitis associated with severe amnesia. Additional symptoms were depression, asthenia, weight loss or seizures. As patients generally showed a poor response to immunotherapy, a rapid diagnosis is important. However, so far no commercially available test system for the detection of anti-AK5 autoantibodies exists.

**Methods** Anti-AK5 positive sera and/or anti-AK5 positive CSF samples of eight patients (P1-8) as well as sera from apparently healthy control individuals (n=60) were analysed using indirect immunofluorescence assay (IIFA) with cryosections of brain tissues and recombinant HEK293 cells expressing AK5 (RC-IIFA). In addition, the samples were subjected to a line blot with recombinant AK5 purified from *E. coli* (EUROLINE).

**Results** In IIFA on hippocampal cryosections, 7/7 CSF, but only 3/8 serum samples displayed a characteristic staining pattern of the neural cell bodies of the *gyrus dentatus* and the CA3 area of the *cornu ammonis*. All serum and CSF samples (P1-8) were positive in AK5 RC-IIFA and all samples but one serum reacted with AK5 in the EUROLINE. Serum samples from healthy controls (n=60) were negative in RC-IIFA while one sample was anti-AK5 positive in the EUROLINE.

**Conclusion** Anti-AK5 autoantibodies can easily be missed in serum samples using IIFA with tissue cryosections. Therefore, the common general IIFA screening step should rather be performed with CSF samples. Positive samples can be confirmed by either assay using the recombinant antigen. Alternatively, a combination of RC-IIFA and EUROLINE can be applied to determine the autoantibodies in case anti-AK5 associated limbic encephalitis is suspected.

**References** (1) Tuzun et al., *Dalmau, 2007* (2) Le-Duy et al., *2017* (3) Bien et al., *2019* (4) Muniz-Castrillo et al., *2021*

### A-032

#### Identification of NVL as a novel ANA target in systemic sclerosis

I. Heckler. *EUROIMMUN US, mountain lakes, NJ*

**Introduction** Antinuclear autoantibodies (ANA) are important diagnostic markers for many autoimmune diseases. In this study, we identified nuclear VCP-like protein (NVL) as a novel ANA target antigen and investigated the clinical association of NVL-specific ANA. **Methods** The index serum was analysed by indirect immunofluorescence assay (IFA), immunoblot, and by immunoprecipitation with HEp-2 cell lysates followed by mass spectrometry. The identified candidate autoantigen was recombinantly expressed in *E. coli* and HEK293 cells, exclusively, and applied in immunoblot, recombinant cell-based IFA and neutralisation assays. In addition, the prevalence of ANA against this antigen was studied in a cohort of patients with systemic autoimmune rheumatic diseases (n=488). **Result** The patient serum displayed a nucleolar pattern on HEp-2 cells and monkey liver by IFA, but did not react with 23 known nuclear antigens. NVL was identified as the target antigen of the patient's autoantibodies. The serum reacted in immunoblots with the purified recombinant antigen, whereas sera from healthy controls (n=15) were nonreactive. Pre-incubation of the patient serum with the antigen abolished the ANA reaction on HEp-2 cells by IFA. Ad-

ditionally, the patient serum reacted with the target antigen in recombinant cell-based IFA using HEK293 overexpressing NVL. A line blot based on purified recombinant antigen revealed anti-NVL reactivity in four patients with systemic sclerosis. **Conclusion** ANA against NVL present as a nucleolar pattern on HEp-2 cells by IFA and may be a novel marker for systemic sclerosis.

### A-033

#### Relevance of anti-PLA2R levels in therapy decision and prediction of therapy outcome using cyclophosphamide and steroids treatment in patients with membranous nephropathy

I. Heckler. *EUROIMMUN US, mountain lakes, NJ*

**Introduction** Detection of anti-phospholipase A2 receptor (PLA2R) antibodies in patients with primary membranous nephropathy (MN) supports diagnosis as well as disease monitoring. An individualised therapy approach was introduced for anti-PLA2R positive MN patients at the Radboud University Medical Center. Immunosuppressive treatment (cyclophosphamide combined with steroids) was stopped when anti-PLA2R results by indirect immunofluorescence testing (IIFT) became negative. Here, we evaluated the relevance of anti-PLA2R levels for therapeutic decisions and the outcome of MN, comparing qualitative and quantitative detection methods. **Methods** Stored serum samples were retrieved for beginning of treatment (baseline), decision point, and follow-up. Anti-PLA2R levels were determined qualitatively by IIFT as well as quantitatively by enzyme-linked immunosorbent assay (ELISA) and chemiluminescence immunoassay (ChLIA) at baseline as well as after treatment and correlated to immunological remission and persistence. **Results** For an evaluation of the relevance of anti-PLA2R levels at the start of therapy, serum samples of 60 patients were available. Patients sampled at the beginning of the therapy were grouped according to tertiles of anti-PLA2R levels determined by ChLIA (n[lowest tertile] = 20, n[middle tertile] = 20, n[highest tertile] = 20). Lower anti-PLA2R levels were seen in patients with relapsing disease. Higher anti-PLA2R levels were associated with more severe proteinuria. Patients in the lowest tertile of anti-PLA2R were more likely to develop immunological remission after 8 weeks of therapy. In total, 90% vs 75% vs 45% of patients in the tertiles turned anti-PLA2R negative after being treated for 8 weeks. Moreover, in this subgroup of patients who showed immunological remission after 8 weeks of therapy, only 11% of the patients in the lowest tertile needed renewed immunosuppressive therapy, while this percentage increased in the middle and highest tertiles (11% vs 53% vs 33%,  $p=0.033$ ). For the assessment of anti-PLA2R levels before and after therapy, samples at baseline and week 8 of treatment were examined. At baseline, 50/50 (100%) tested positive in IIFT and ChLIA as well as 48/50 (94%) in ELISA. After 8 weeks on treatment, immunological remission based on IIFT was found in 37/51 (73%) patients. At this point, the overall agreement compared to IIFT was 92% for ChLIA and 82% for ELISA. Yet the availability of quantitative levels provided further insight into this group of patients in whom treatment was discontinued. With ChLIA, anti-PLA2R titers >5 RU/mL after 8 weeks of therapy were found in 4% of IIFT negative patients with persistent remission, vs 55% of IIFT negative patients with early relapse ( $p=0.006$ ). **Conclusion** Individualised treatment of MN patients with cyclophosphamide and steroids has been recently introduced. In this respect, the quantitative determination of anti-PLA2R levels adds further value. Of the examined quantitative methods, ChLIA demonstrated the highest agreement with IIFT. Additional studies are needed to evaluate the impact on clinical decision making and outcome.

### A-034

#### Sensitivity and Specificity of a Novel Cell-Based Indirect Immunofluorescence Assay for Detecting Anti-MAG IgM in Patients With Peripheral Neuropathy

I. Heckler. *EUROIMMUN US, mountain lakes, NJ*

**Background** Peripheral neuropathy with antibodies against myelin-associated glycoprotein (MAG) is a rare autoimmune demyelinating disorder caused by pathogenic IgM against the human natural killer-1 (HNK-1) glycoepitope expressed on MAG. Here, the performance of a novel recombinant cell-based indirect immunofluorescence assay (RC-IFA) for the detection of anti-MAG IgM was studied and compared with two other serodiagnostic techniques. **Methods** The anti-MAG IgM RC-IFA (EUROIMMUN) is based on the HNK-1 glycoepitope recombinantly expressed in HEK293 cells. The assay was probed with serially diluted serum samples from 95 clinically characterized patients with anti-MAG neuropathy and control samples from 55 anti-MAG IgM-negative patients with peripheral neuropathy. Results were compared to an Anti-MAG Autoantibodies ELISA (Bühlmann) and an *in-house* tissue-based indirect immunofluorescence assay (TBA) using *nervus suralis* sections as antigenic

substrate. **Results** The RC-IFA (cut-off 1:100) demonstrated a clinical sensitivity of 98.9% and a specificity of 100%. The positive and negative percent agreement with the ELISA (cut-off 1,000 BTU) was 98.9% and 100%, respectively, and with the TBA (cut-off 1:10) 98.8% and 84.6%, respectively. Kappa statistics confirmed better qualitative agreement between RC-IFA and ELISA ( $\kappa=0.986$ ) than between RC-IFA and TBA ( $\kappa=0.848$ ). Spearman's analysis indicated a strong positive correlation between (semi)quantitative RC-IFA and ELISA results ( $r_s=0.879$ ,  $p<0.001$ ). Sera with anti-MAG values  $\geq 10,000$  BTU had a median RC-IFA titer of 1:10,000. **Conclusions** The novel RC-IFA allows the detection of antibodies against MAG using an easy and standardized technique, and presents a sensitive and specific alternative to the more time-consuming ELISA. Future studies will address the assay's suitability to support treatment monitoring.

### A-035

#### Strategy for the detection of anti-septin antibodies by indirect immunofluorescence assay

I. Heckler. *EUROIMMUN US, mountain lakes, NJ*

**Background** Septins are small GTP-binding proteins which are widely expressed in all tissues. Antibodies against septin-5 are associated with cerebellar ataxia (1) while antibodies against septin-7 are described in association with myelopathy and encephalopathy (2). Here, we present a strategy for the detection of anti-septin autoantibodies.

**Methods** Patient sera with similar staining pattern on hippocampal tissue sections were subjected to immunoprecipitation followed by mass spectrometry. All identified septins were cloned and expressed recombinantly in HEK293 cells either as complexes, individually, or as depletion complexes missing one septin. The recombinant cells were used in immunofluorescence assays (RC-IFA) for the analysis of serum samples.

**Results** All patient sera (n=18) displayed granular staining of the outer molecular layer of the rat hippocampus dentate gyrus as well as a staining of the rat cerebellar molecular layer in IFA. Immunoprecipitation with rat cerebellum lysate revealed septin-3, -5, -6, -7, and -11 as potential target antigens. RC-IFA with all patient sera produced cytoplasmic staining of recombinant cells co-expressing septin-3, -5, -6, -7, and -11, while only one of 99 controls was similarly reactive. Furthermore, patient sera either reacted with one septin only, several single septins, or the septin complex only. The recombinant cells expressing single septin-3, -5, or -7 do not have optimal characteristics regarding specificity or sensitivity. Incubation of patient sera with five different depletion complexes, each missing one of the five septins, confirmed the anti-septin specificity observed with the single septin substrates.

**Conclusion** RC-IFA with HEK cells expressing the septin complex shows the highest specificity and sensitivity for the detection of anti-septin autoantibodies and is suggested to be used for the screening of serological samples with a characteristic staining pattern on rat hippocampus. Antibodies against individual septins can be further determined by RC-IFA with single recombinant septins or depletion complexes.

**References** 1) Honrat et al., 2018 2) Zivelonghi et al., 2020, poster presentation

### A-036

#### Development and Characterization of Monoclonal Standards for Calibration Verification of Reportable Ranges of SARS-CoV-2 IgG Assays

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**Background:** SARS-CoV-2 diagnostics are critical to contain the spread of SARS-CoV-2. Furthermore, diagnostic testing of the serological immune response to help differentiate native infection from vaccination or for use as a surrogate to COVID protection through immunogenicity is of great interest. Several serological assays are available on automated chemistry analyzers to detect SARS-CoV-2 IgG antibodies. The body's ability to fight the virus and its relation to the amount of IgG antibody present at any one-time during infection, and recovery or vaccination will help clinicians determine if and how the IgG levels relate to long-term protection. To further advance this area of study, here we report novel analytical approaches to quantitation and epitope mapping of anti-SARS-CoV-2 monoclonal antibody for use in the calibration verification of COVID serological assays.

**Methods:** A SARS-CoV-2 monoclonal antibody was characterized using a Q-Exact Plus Orbitrap to check for product integrity and purity. Epitope mapping was carried out by hydrogen deuterium exchange mass spectrometry (HDX-MS) using a

Synapt G2Si coupled with a LEAP PAL system. VALIDATE® SARS-CoV-2 IgG was formulated using the monoclonal antibody in a human matrix into six levels of known concentrations. Limits were applied as a percentage of the total allowable error (TEa), specific for SARS-CoV-2. The standard additions method for assigning concentration values has been adapted for use with immunoassays to enable the quantification of endogenous analyte concentrations within serological test samples. The method tolerates the non-linear correlation between the total concentration of the analyte (calibrant and endogenous analyte) and the immunoassay signal output. The method has been implemented to quantify IgG within VALIDATE® SARS-CoV-2 IgG.

**Results:** The intact protein analysis of the deglycosylated mAb, heavy and light chain reveals an amino acid loss, likely the C-terminal lysine of the heavy chain, which is a common posttranslational modification of mAbs. Epitope mapping results showed significant deuterium uptake differences in the receptor binding domain (RBD) indicating the antibody as a high affinity binder. Minor structural differences could be identified in the N-terminal region of S1 and at the C-terminus of S2 of the spike protein. With a 1000-fold net dilution of the L5 member, the recovery of the endogenous IgG by standard additions was 102.1% (inter-assay CV=11%; n=3), and the uncertainty estimate for each endogenous IgG concentration derived within each experiment was <10% by Monte Carlo simulation (n=200). For the 1500-fold diluted sample, recovery of the endogenous analyte was 96.2% (inter-assay CV=16.4%; n=3), and the uncertainty estimate for each endogenous IgG concentration derived within each experiment was <8.2% by Monte Carlo simulation (n=200). VALIDATE® SARS-CoV-2 IgG, is stable for its claimed expiration and is intended to evaluate the assays' linearity through the manufacturers' reportable ranges or through an LDTs reportable range.

**Conclusion:** The calibration verification material has been epitope mapped and value assigned to include a measure of endogenous IgG that is reactive to SARS-CoV-2 protein. Analytical characterization, together with results spanning six levels of concentration tested across multiple platforms, provide clinical labs with a highly characterized, liquid stable tool.

### A-037

#### Intuitive Modification of the Friedewald formula for calculation of LDL-cholesterol concentration

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**Background:** LDL-cholesterol (LDL-C) is an established risk factor for cardiovascular disease and considered an important therapeutic target. It can be measured directly or calculated from results of other lipid tests. The Friedewald formula is the most widely used formula for calculating LDL-C. In this study, we modified the Friedewald formula for more accurate and practical estimation of LDL-C.

**Methods:** Datasets composed of measured triglyceride, total cholesterol, HDL-cholesterol, and LDL-C concentrations were collected and assigned to derivation set and validation set. The datasets were divided into groups according to the triglyceride concentration. In the modified formula, LDL-C was defined as total cholesterol-HDL-cholesterol-(triglyceride/adjustment factor). The differences between calculated LDL-C and measured LDL-C were obtained and the numbers with smallest difference when applied were selected as the adjustment factors for each group. For validation, measured LDL-C and calculated LDL-C using the Friedewald formula, Martin/Hopkins formula, and modified formula were compared and classified according to the ATP III classification.

**Results:** In the derivation set, the differences between calculated LDL-C and measured LDL-C were smallest when the factors were 4.8, 5.8, and 6.2 in the group with triglyceride level less than 100, between 101 and 200 and higher than 200 mg/dL, respectively. When the determined factors were used, the coefficient of determination ( $R^2$ ) between calculated LDL-C and measured LDL-C was higher in modified formula than in the Friedewald formula ( $R^2 = 0.9354$  and  $0.9252$ ). When the calculated LDL-C was classified according to ATP III classification, using the modified formula for LDL-C calculation showed the least underestimated cases among the three methods.

**Conclusion:** We modified the Friedewald formula by applying different factors depending on the triglyceride level. As a result of the study, it was confirmed that the triglyceride to VLDL-C ratio, which is used in the equation to obtain an accurate LDL-C value, was different from 5, the number conventionally used in the traditional Friedewald formula. In the case of clinical laboratories that calculate LDL-C values using calculation formulae, it is necessary to review the calculation formula currently used, and the use of improved calculation formulae such as those presented in this study can be considered.

**A-038****Performance Evaluation of the Dimension EXL LOCI BRAHMS PCT Assay**

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**Background:** Procalcitonin (PCT) is a protein produced in the body by parafollicular C cells of the thyroid, often in response to bacterial infection or tissue injury. PCT test results are used in conjunction with other laboratory findings and assessments to aid in clinical risk assessments and decision making. Siemens Healthineers has developed the Dimension® EXL™ LOCI® BRAHMS Procalcitonin (PCT) assay for use on all Dimension EXL Integrated Chemistry Systems.

**Methods:** The Dimension EXL LOCI BRAHMS PCT assay is a homogeneous sandwich chemiluminescent immunoassay based on LOCI technology. The assay measures the PCT concentration in both serum and plasma (lithium heparin, sodium heparin, K2EDTA, and K3EDTA), using 5 µL of sample. Assay sensitivity was determined by evaluating limit of blank, limit of detection, and limit of quantitation. Linearity was assessed across the measuring interval (0.05-50.00 ng/mL). Precision was evaluated using six pools prepared from human patient samples and three quality controls. Cross-reactivity and interferences were evaluated in the presence of 0.25 and 2.00 ng/mL PCT. Comparison to the B.R.A.H.M.S PCT sensitive KRYPTOR assay evaluated 555 samples across the assay range, with positive and negative agreement assessed at multiple cutoff concentrations.

**Results:** Limit of blank, limit of detection, and limit of quantitation were determined to be 0.03, 0.04, and 0.05 ng/mL respectively. The assay is linear from 0.05 to 50.00 ng/mL and has an extended measuring interval up to 1000.00 ng/mL. Repeatability CVs ranged from 1.5 to 4.3%, and within-lab CVs ranged from 2.7 to 7.1%. No significant interference ( $\leq 10\%$  bias) was observed with biotin (1200 ng/mL) or with over 40 other endogenous and exogenous compounds at PCT concentrations of 0.25 and 2.00 ng/mL. Cross-reactivity was less than 1% in the presence of common cross-reactants (human, cel, and salmon calcitonin; human katacalcin; alpha-CGRP; and beta-CGRP). Patient sample comparison between the Dimension EXL LOCI BRAHMS PCT assay and the B.R.A.H.M.S PCT sensitive KRYPTOR assay produced the following weighted Deming regression statistics: slope = 1.07, intercept = -0.01 ng/mL, correlation coefficient = 0.958, and n = 555 over a PCT concentration range of 0.02-49.03 ng/mL. Positive and negative agreement between the Dimension EXL LOCI BRAHMS PCT and B.R.A.H.M.S PCT sensitive KRYPTOR assays demonstrated  $\geq 85\%$  agreement at cutoff PCT concentrations of 0.10, 0.25, 0.50, and 2.00 ng/mL.

**Conclusion:** The Dimension EXL LOCI BRAHMS PCT assay demonstrates acceptable performance for measuring PCT in serum and plasma, with good agreement to the predicate assay, B.R.A.H.M.S PCT sensitive KRYPTOR assay.

\*Under development. Not available for sale

**A-039****Body Fluid Matrix Study for Selected Tests Performed on the Roche Cobas Pro c503 Module**

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**Background:** Occasionally, clinicians submit body fluid specimens for laboratory testing based on a specific concern or clinical situation. However, these specimen types are generally not included in the manufacturer's FDA-cleared instructions.

**Objective:** To determine if pleural fluid and peritoneal dialysate fluid are acceptable matrices for selected tests analyzed by the Roche Cobas Pro c503 module.

**Methods:** The tests to be validated were decided upon by collaboration with our Surgery, Nephrology, and Hematology/Oncology Services. The tests chosen were specifically amylase, glucose, lactate dehydrogenase, lipase, total protein, and triglyceride in pleural fluid and BUN, creatinine, and glucose in peritoneal dialysate fluid. To validate a fluid type, remaining fluid samples (n=5) and a pooled plasma sample were analyzed for the selected tests three times to obtain the mean expected concentrations. Some peritoneal dialysate fluid samples had to be diluted with saline (the approved diluent) due to very high levels of the analytes to bring them into the analyzer's measurement ranges. The fluid samples were mixed with the pooled plasma sample with the fluid portion content of 100%, 75%, 50%, 25% and 0%. All mixed specimens were analyzed for the selected tests and the results were compared with the expected calculated results based on the straight analyses of the fluid samples and the pooled plasma sample. The passing criterion is that the measured results were within 20% of

the expected calculated values. The results which had very low concentrations used a passing criterion of absolute difference based on the test precision and clinical significance.

**Results:** All mixture results were within either 15% or the absolute difference (+/- 3 units) was within the instrument precision limit obtained for the plasma specimen type.

**Conclusion:** Pleural fluid and peritoneal dialysate fluid were validated for analytical accuracy for the selected tests (amylase, glucose, lactate dehydrogenase, lipase, total protein, and triglyceride in pleural fluid and BUN, creatinine, and glucose in peritoneal dialysate fluid) on the Roche Cobas Pro c503 module.

**A-040****Pre-analytical Characterization of Nasal and Nasopharyngeal Specimen CoV2 Antigen Stability in Transport Media**

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**Background:** The arrival of the SARS-CoV-2 respiratory pathogen in 2019 prompted a global response to create novel assays that can identify and diagnose infection in patients. CoV2 Antigen (Ag) tests are utilized to determine the presence of CoV2 Ag in sinuses of infected patients. CoV2 Ag tests are typically immunoassays and can be processed as 'rapid' tests in 10-20 minutes or secondarily shipped to central laboratories for high-throughput processing. Different test methods and logistics to generate results require the need for characterizing Ag stability and storage methods to ensure accurate results. We identify differing stability profiles in both collection and storage methods when assessing detection of the nucleocapsid Ag protein. Nasal/nasopharyngeal (N/NP) patient specimens, specimen collection/inactivation, specimen handling and storage conditions affect Ag stability and detection accuracy. Specimen pre-analytics are an important consideration for the collection, transportation, processing, and testing of specimens for central laboratories and encompass logistical and analytical challenges that limits utility of this testing paradigm.

**Methods:** A comparison of native and artificially spiked N/NP samples on ARCHITECT i2000SR and Beckman Access 2 were assessed for stability effects at various storage/specimen collection conditions. The rAg and a high native (N/NP) positive specimen were spiked into a native (N/NP) negative specimen matrix for this study. rAg specimens stressed at room temperature (RT) 15-30°C (6 and 20 hrs). Negative and high (N/NP) positive specimens stressed at 2-8°C (24, 48, 72 hrs, and 7 days), and 15-30°C (1, 3, 6, and 24 hrs). All specimens utilized an internal method of viral detergent based inactivation. Stability is referenced as % difference in S/C and qualitative S/C throughout. RT stability of N/NP patient negative and positive specimens, individual and pooled patient specimens, native positive spiked, viral lysate, rAg, and frozen dry swabs in, Copan Universal Transport Medium (UTM), calibrator diluent, and negative patient pool was evaluated. Clinical specimen storage evaluated at 2-8°C, -20°C, -80°C, and for freeze thaw (F/T) impact.

**Results:** The stability and clinical performance (qualitative) were similar for ARCHITECT i2000SR and Beckman Access 2. Various specimen storage conditions demonstrated limited stability at RT. Pre-inactivated specimens had stability of < 6 hours, and post-inactivation < 1 hour. S/C % difference of < 20% is designated as stable. The presence of mucous in positive native specimens was shown to be a major driver for Ag instability. Fresh vs. frozen specimens demonstrate that 1 F/T did not adversely impact stability. Long-term storage, >30 days at -20°C did not demonstrate significant loss of detected Ag.

**Conclusion:** The comparative immunoassay study demonstrated that specimen performance is similar. A limited period of stability for specimens at RT, (+/-) inactivation, is an important factor in the methods of collecting and testing specimens. Specimens that are frozen yield more stable results. Research and testing, beyond rapid POC kits, need to be monitored and characterized to maintain the validity of clinical specimens and their results. Understanding and controlling the causes of specimen instability will help further optimize current assays and direct future research to mitigating these areas of risk.

**A-041****Development of Exploratory Algorithms using Plasma Biomarkers on ARCHITECT i2000 and c8000 Platforms, Demographic, and Radiomic Data to Aid in Risk of Malignancy Prediction of Indeterminate Pulmonary Nodules**

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**Background:**

Lung cancer is the worldwide leading cause of cancer mortality. The United States Preventative Services Task Force (USPSTF) recommendation for screening modality of an annual Low Dose Computed Tomography (LDCT) for high-risk groups has led to a subpopulation of lung cancer nodules that cannot readily be categorized radiologically as benign or malignant. These indeterminate pulmonary nodules (IPNs) that cannot be categorized represent a deficiency in current lung cancer screening. Algorithms of demographic and biomarker variables may be useful risk stratification tools in the quest to stratify IPNs that may have a high risk for malignancy. Early detection of cancerous IPNs allows for earlier stage treatment initiation and improved patient prognosis. In this pilot study, we summarize our algorithm approach, discuss future algorithm refinements, and future screening studies that will strengthen the sensitivity and specificity of the IPN risk stratification tool.

**Methods:**

A pilot study cohort including 141 subjects with IPNs (105 stage I cancer and 36 benign nodules) were collected by Rush University Medical Center (RUMC). The demographic variables of gender, age, sex, race, ethnicity, nodule size (mm), and smoking pack years, as well as the plasma levels of CA-125, SCC, CEA, HE4, ProGRP, NSE, Cyfra 21-1, hs-CRP, Ferritin, IgG, IgG1, IgG2, IgG3, IgG4, IgE, IgM, IgA, Kappa Free Light Chain (KFLC), and Lambda Free Light Chain (LFLC) run on the ARCHITECT i2000 and c8000 platforms were assessed for this cohort. The technique of adaptive index modeling (AIM) was used, which is a method that adaptively searches for optimal cutoffs for predictors to build an overall model.

**Results:**

Multivariable analyses of the aforementioned biomarkers and demographic variables yielded a reduced AIM algorithm consisting of plasma biomarkers CA-125, total IgG, IgA, IgM, IgE, LFLC run on the ARCHITECT i2000 and c8000 platforms, nodule size, and smoking pack years with improved performance (AUC 0.82, 95%CI 0.74-0.90) over the same analysis of the demographic variables (age, nodule size, and smoking pack years) alone (AUC 0.70, 95%CI 0.61-0.78).

**Conclusion:**

The reduced algorithm of plasma biomarkers and demographic variables may be helpful to aid in assessing the risk of IPN malignancy. This type of risk stratification tool would be helpful in early detection of lung cancer in high-risk subjects pending further screening studies and clinical validation.

**A-042****Research Use Only (RUO) IgG1 and IgG2 Subclass Assays on the Abbott Alinity c Clinical Chemistry Analyzer**

N. M. Jeanblanc, S. Vaidya, K. Ngo, S. E. Brophy. *Abbott Laboratories, Abbott Park, IL*

**Background:**

IgG1&IgG2 represent two of the four IgG Subclass molecules. The IgG1& IgG2 subclasses differ in their physical/chemical properties and have different serum concentrations that are age dependent. In particular, IgG1&IgG2 have been used as an aid to diagnose primary immune deficiencies as part of an overall clinical assessment. IgG1 mediates the immune response to soluble protein antigens; whereas IgG2 mediates the response to polysaccharide antigens. Although low levels of IgG1&IgG2 can be transitory, deficiencies are often associated with various diseases: IgA deficiency, bacterial infections of the respiratory/digestive tract, absence of an immune response following vaccinations, allergic/autoimmune diseases. Clinical treatment of an IgG1 & IgG2 deficiency could involve the following: antibiotics, vaccination, and IgG replacement therapy. Research Use Only (RUO) IgG1&IgG2 assays have been developed for the quantitative determination of IgG1&IgG2 in human serum/plasma on the Abbott Alinity c system.

**Methods:**

The newly designed IgG1&IgG2 assays (RUO) are both immunoturbidimetric assays utilizing monospecific polyclonal antisera for their respective capture reagents. Ag-

glutination occurs when the anti-human IgG1 or IgG2 polyclonal antisera bind the IgG1 or IgG2 antigen in a patient's sample. This agglutination reaction is detected as an absorbance change at 340 nm with the rate of change being proportional to the quantity of IgG1 or IgG2 in the specimen. Both the IgG1&IgG2 subclass assays are end-up reaction types that calculate concentration from a spline fit 6-point calibration.

**Results:**

The RUO IgG1&IgG2 assays have robust analytical performance on the Alinity c platform. The assays have a LoB/LoD/LoQ for IgG1  $\leq 62.5, \leq 143.0, \leq 441.9$  mg/L and for IgG2  $\leq 29.3, \leq 46.3, \leq 103.8$  mg/L respectively. Total within laboratory imprecision for IgG1 was  $< 7.0\%$  for samples  $\geq 1330$  mg/L, SD of  $\leq 91$  for samples  $< 1330$  mg/L and for IgG2 was  $< 7.0\%$  for samples  $\geq 300$ , SD of  $\leq 21$  for samples  $< 300$  mg/L. The IgG1&IgG2 RUO assays demonstrated linearity from LoQ to 20000 and 13000 mg/L respectively and have reagent onboard stability of at least 30 days with calibration curve stability of 7 days. These assays demonstrate robustness to RF interference up to 969 IU/mL for IgG1 and 198 IU/mL for IgG2. The IgG1&IgG2 assays also demonstrate robustness to common endogenous interferences;  $\geq 40$  mg/dL conjugated/unconjugated bilirubin;  $\geq 1000$  mg/dL hemoglobin,  $\geq 1500$  mg/dL human triglyceride,  $\geq 1000$  mg/dL glucose and  $\geq 15$  g/dL total protein when tested at 1000 and 6000 mg/L IgG1 and 250 and 3000 mg/L IgG2 analyte concentrations. Method comparison to commercially available on market IgG1&IgG2 subclass assays showed a slope of 0.98 and 0.95 and correlation coefficient of 0.97 and 0.97 for serum samples across the assay ranges. The analytical measuring interval (AMI) for the IgG1 subclass assay was observed to be from LoQ to 20,000 mg/L and IgG2 from LoQ to 13,000 mg/L.

**Conclusion:**

Analytically robust RUO IgG1&IgG2 subclass assays were developed for use on the Abbott Alinity c system with excellent precision and exogenous/endogenous interferences at lower/upper medical decision points with all data generated within one dilution application for ease of customer use.

**A-043****Evaluation of Reagent Lot-to-Lot Performance of Alinity i Tumor Marker Assays**

K. Johnson, K. Gignac, P. Roth. *Abbott Laboratories, Abbott Park, IL*

**Background:** Serological tumor marker assays are a valuable tool to aid in the prognosis and management of cancer patients. For patients being monitored with tumor marker assays, changes in values can have significant implications for therapy and intervention. It is therefore imperative that the analytical performance of these assays remain consistent from one lot of reagent to the next in order to give clinicians confidence that fluctuations in values are due to changes in the patient's tumor status and not to reagent lot-to-lot variability. **Objective:** The goal of this study was to evaluate the reagent lot-to-lot performance of eight tumor marker assays (CA19-9, CA15-3, CEA, AFP, Total PSA, Free PSA, CA125 and HE4) on the Abbott Alinity i system over a two-year period. **Methods:** Quality controls (QC) with values across the measurement range and human serum panels were tested on each new reagent lot manufactured over a two-year period. Assays were run on the Abbott Alinity i instrument and tested in multiple replicates and runs. Imprecision (percent coefficient of variation (%CV)) of each control and serum panel was calculated across all reagent lots. Bias (% shift from mean) of each serum panel was also calculated across all reagent lots. **Results:** The imprecision of each control and serum panel for each assay was less than 5%CV. The bias for each assay was less than +/-9% from the mean at each panel level with no identified trends or shifts over time. **Conclusions:** Each of the eight tumor marker assays evaluated showed consistent lot-to-lot performance on all controls and human serum panels over a period of two years.

**A-044****Reagent Lot Consistency of Three Common Cardiac Markers on Alinity i**

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**Background:** Cardiac Troponins and Natriuretic Peptides are markers commonly used by ER physicians and cardiologists when patients present with evidence of underlying cardiac conditions. High Sensitive Troponin I (hsTnI) is used to aid in the diagnosis and prognosis of patients presenting with signs of Acute Coronary Syndrome and has more recently evolved as a tool for risk stratification of asymptomatic patients and apparently healthy individuals. B-Type Natriuretic Peptide (BNP) and N-terminal-proBNP (NT-proBNP) are two markers used to aid in the diagnosis and

assessment of severity of Heart Failure. Reliable interpretation of these markers over an extended period (risk stratification and assessment of severity) is critical to drive optimal patient outcomes. Consistent performance from one lot to the next is important to give laboratorians confidence that they are delivering high quality results to physicians and patients.

**Objective:** The goal of this study is to evaluate the reagent lot-to-lot performance of hsTnI, BNP, and NT-proBNP on the Abbott Alinity i system over a one-to-two-year period.

**Methods:** Abbott internal manufacturing data for all lots released over one year (NT-proBNP) or two years (hsTnI and BNP) were compiled and analyzed. Assays were run on the Abbott Alinity i instrument. For all assays, internal controls and human serum panels were tested in multiple replicates and runs. Imprecision (percent coefficient of variation (%CV)) of each control and serum panel was calculated across all reagent lots. Bias (% shift from mean) of each serum panel was also calculated across all reagent lots.

**Results:** The imprecision for each assay across lots for all controls and serum panels was less than 6%CV. The bias for each assay was less than +/-10% from the mean at each panel level with no identified trends or shifts over time.

**Conclusion:** Each of the cardiac markers evaluated showed consistent reagent lot-to-lot performance on all control levels and human serum panels tested.

## A-045

### Method Development of Hemoglobin A1c in the Whole Blood of Non-Human Primates

*A. Kalb, Charles River Laboratories, Ashland, OH*

**Objective**The objective of this study is to validate hemoglobin A1c (HbA1c) measurement in Non-Human Primate (NHP) whole blood using the ADVIA 1800 analyzer.**Methodology**HbA1c was validated in NHP whole blood 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. Whole blood samples were collected into tubes containing K<sub>2</sub>EDTA anti-coagulant. Intra-assay precision was determined by analyzing two biological samples and the two quality control levels of Bio-Rad Diabetes 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 Diabetes 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, specimen samples were diluted and analyzed in duplicate. The LOQ was determined by diluting quality control 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 whole blood samples. Correlation between the two ADVIA 1800 analyzers was determined by assaying 10 whole blood 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 whole blood samples was tested at room temperature, refrigerated, and frozen (at -70°C) for various durations of storage.**Results**Results show that measurement of HbA1c in NHP whole blood 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 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 2.5<sup>th</sup> to 97.5<sup>th</sup> 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**HbA1c in NHP whole blood met the acceptance criteria for all required validation parameters. The ADVIA 1800 analyzer can be used for preclinical studies to test HbA1c in NHP whole blood.

## A-046

### δ-Tocotrienol in Combination with Resveratrol Improve the Cardiometabolic Risk Factors and Biomarkers in Patients with Metabolic Syndrome: A Randomized Controlled Trial

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**Background:** Metabolic syndrome (MetS) is a cluster of central obesity, hypertension, hyperglycemia, and dyslipidemia. It is a global health issue with an increased risk of cardiovascular disease. Recently, a few natural products have been reported with promising anti-inflammatory and anti-oxidative effects. We aimed to evaluate the effect of δ-tocotrienol and resveratrol mixture (TRM) supplementation on cardio-metabolic risk factors and biomarkers in patients with MetS. **Materials and methods:** A randomized controlled trial was conducted at the hospitals of NUMS Rawalpindi, Pakistan. Total 82 patients of MetS aged 18-60 years were enrolled based on IDF-2005 diagnostic criteria and randomly grouped into TRM (n=41) and Placebo (n=41). Patients in TRM group were given 400mg capsule (δ-tocotrienol 250mg; Resveratrol 150mg) and placebo (cellulose 400 mg) twice daily for 24 weeks. Biochemical tests were analyzed on ADVIA 1800 Chemistry® analyzer and biomarkers by ELISA methods. **Results:** In the TRM group, a significant reduction in waist circumference, blood pressure, mean (95% CI) of fasting plasma glucose -0.15mmol/L (-0.22, -0.09), serum triglyceride -0.32 mmol/L (-0.47, -0.16) and increment in HDL-C were observed as compared with placebo. TRM supplementation also improved biomarkers including high-sensitive C-reactive Protein -0.59 mg/L (-0.89, -0.29), interleukin -1.99 pg/ml (-2.51, -1.49), tumor Necrosis Factor -2.19 pg/ml (-2.56, -1.83), malondialdehyde -0.48 umol/L (-0.65, -0.31) and total antioxidant capacity 1.71 U/ml (1.30, 2.12). Metabolic syndrome and its components were significant improved with Hazard Ratio HR 2.60 (1.12,5.99) at 24 weeks. **Conclusion:** TRM supplementations improved cardio-metabolic risk factors along with biomarkers of inflammation and oxidative stress, without any significant side effects in patients with metabolic syndrome.

## A-047

### Biological Variation in Creatinine in the SPRINT Hypertension Trial

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**Background:** Biological variation (BV) of serum creatinine is important in hypertension where monitoring of creatinine is used for detection of chronic kidney disease (CKD). Most creatinine BV studies have observed participants for relatively short periods of time, often days or weeks rather than the intervals of time that reflect clinical practice, which typically involves annual assessment of patients. A recent systematic review and meta-analysis for the EFLM estimated creatinine BV to be 4.5%.

**Methods:** We performed a secondary analysis of the BV of creatinine in participants in the SPRINT hypertension trial. Participants were randomly assigned to one of two treatment arms with target systolic blood pressure goals of 140 mm Hg (standard arm) or 120 mm Hg (intensive arm) and followed for a mean duration of 3.26 years. Creatinine was measured at randomization, months 1, 3, 6, and every 6 months thereafter. All creatinine measurements were performed using an enzymatic method principle on commercial clinical chemistry analyzers (Roche, Indianapolis, IN). A linear mixed-effects model using random intercepts and random slopes was used to determine the total within-individual variation (i.e., combined BV and analytical variation) in creatinine by study-defined subgroups: randomization arm, sex, age <75 at randomization vs. ≥ 75, Black vs. non-Black, and the presence of CKD at baseline. Analytical variation (expressed as the CV%) in creatinine measurement was calculated from daily quality control measurements. Biological variation was calculated from the total within-individual variation and the analytical variation.

**Results:** A total of 9,361 participants with hypertension were recruited to the trial and included in this analysis. Of these, 2,646 had chronic kidney disease at baseline (defined as estimated GFR of <60 mL/min/1.73 m<sup>2</sup> using the MDRD equation). The participants' mean (SD) age was 67.9 (9.5) years, and 35% were female. Analytical CV was calculated to be 3.3% across the study time period. Total variation in creatinine by study-subgroup between randomization and 6, 12, 24, and 36 months was 8-9%, with slightly higher values being seen over longer time periods. There were small but statistically significant differences in biological variation among all subgroups except those based on age. BV was lower in the standard treatment arm, in males, in patients without CKD at baseline, and in non-Black participants. Among participants with the most stable renal function during the trial, BV was estimated to be 7.4%

**Conclusion:** Biological variation among SPRINT participants with stable renal function was considerably higher than the values reported in previous studies which had

much shorter follow-up. This suggests that reference change values for creatinine and the derived estimated glomerular filtration rate in patients undergoing treatment for hypertension should be larger than those for healthy subjects.

**A-048**

**Evaluation of Peak Shape Errors in HbA1c Measurement by HPLC to Improve Quality and Efficiency of Resulting**

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Background: Measurement of hemoglobin A1c (HbA1c) is essential for diabetes diagnosis and monitoring. HbA1c assays are generally well-standardized. However, hemoglobin variants may differentially impact HbA1c results across methods. We measure HbA1c by ion-exchange HPLC, and samples with certain chromatographic errors undergo medical director review. We usually send samples with peak shape errors to another laboratory for testing by an alternate method due to concern about a method-specific variant interference. To improve the turn-around-time and efficiency of this process, we started a quality improvement project aiming to identify error patterns that could help guide us in the need for sending out these samples.

Methods: In-house HbA1c is measured by ion-exchange HPLC (Bio-Rad D-100™). The analytical measuring range is 3.5-20%. Intra-assay precision was 0.5% at both low and high concentrations; inter-assay precision was 1.1% and 0.9% at low and high concentrations, respectively. Accuracy was evaluated by comparison to a previously validated Roche Integra assay; bias was -1.88%. Reference laboratory HbA1c is measured by capillary electrophoresis (CE) (Sebia Capillary). Upon initiation of the quality project, we tracked all samples sent out for reference laboratory testing due to peak shape errors (n=33).

Results: We observed three broad categories of samples: samples with 1) HbA1c peak tailing errors, 2) HbA1c broad peak errors, and 3) both HbA1c peak tailing and broad peak errors. Samples in the latter category were flagged for having elevated HbF (>23%) by CE, which was not observed on our HPLC method, suggesting an unknown interfering variant. The other two categories of samples could be further divided by the presence of additional error flags (A0 peak tailing and A0 broad peak errors). Some subcategories consistently received a CE result, while other subcategories had mixtures of samples with results or HbF interference. Again, the elevated HbF was not observed on our HPLC method. Unfortunately, we were unable to identify a visual pattern to distinguish samples that were able to be resulted using CE from the mixed subcategories. For those samples which had a result by CE, we compared the flagged HPLC result to the CE result. Ten out of the twelve samples had HPLC results that varied more than 6% from the CE result; the average percent difference was 8.8% (range -13.3-21.4%).

Conclusion: The comparative data suggests that peak shape errors accurately indicate a problem with HbA1c measurement by HPLC. However, by tracking samples to identify patterns, we were able to identify some error flag scenarios that will likely not benefit from CE testing. Therefore, these samples can be resulted with an interference comment more rapidly. Overall, it is important to consider the many possible clinically asymptomatic hemoglobin variants and their influence on HbA1c measurement. Hb Athens-Georgia is an example of a variant that may produce peak shape errors on HPLC, and published patterns fit some of the chromatograms reviewed in this project. It is critical to consider whether the measured HbA1c represents an accurate measure of glycemic control by being alert to peak shape errors that may signal a hemoglobin variant.

**A-049**

**Impact of Inappropriate Storage of Reagents on Test Results**

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**Background:** During reagent storage, temperatures may briefly exceed vendor-specified ranges. We challenged the performance of 10 laboratory tests by storing reagents at room temperature for longer than normal duration and assessed the impact on quality control (QC) and patient results (Table).

**Methods:** QC material and 10 patient serum samples were analyzed on ADVIA 1800 and Centaur analyzers (Siemens Healthcare Diagnostics, Tarrytown, NY), at 0hr when reagents were stored at 2-8 °C per vendor's specifications, and after reagents were stored at 22-28 °C for 6hr or 48hr. QC results were compared to established ranges (mean ± 2 standard deviations [SDs]). For patient results, a difference or % difference ≤1/3 of total allowable error (TEA) per Clinical Laboratory Improvements Amendments or College of American Pathologists proficiency testing evaluation criteria was considered acceptable.

**Results:** QC results for all tests except creatinine were within established ranges when reagents were stored at 22-28 °C for 6hr or 48hr. Patient results for 7 tests were acceptable with reagents stored at 22-28 °C up to 48hr. Unacceptable results were observed for creatinine (6hr & 48hr), thyroid stimulating hormone (6hr), and thyroxine (48hr).

**Conclusion:** Prolonged reagent storage at 22-28 °C had minimal impact on QC and patient results for most tests, suggesting that modest temperature fluctuations during storage should have even smaller impacts on results. If reagent storage is compromised for a long duration, QC monitoring alone may not be adequate to predict deviations in patient results.

Table. Effect of Prolonged Storage of Reagents at Ambient Temperature on Quality Control and Patients' Results

Test	Quality Control within Range	Average % Difference of 10 Patients' Results		TEA/3 (%)
	6hr & 48hr	6hr vs 0hr	48hr vs 0hr	
Glucose	Level 1: YES	-1.10	0.80	3.33
	Level 2: YES			
Calcium	Level 1: YES	0.20	0.90	2.00
	Level 2: YES			
Alanine Transaminase*	Level 1: YES	-0.60	8.20	6.67
	Level 2: YES			
Creatinine	Level 1: NO	16.30	15.30	5.00
	Level 2: YES			
Alkaline Phosphatase	Level 1: YES	-0.10	-1.40	10.00
	Level 2: YES			
Albumin	Level 1: YES	1.50	0.40	3.33
	Level 2: YES			
Immunoglobulin G	Level 1: YES	0.30	-0.30	8.33
	Level 2: YES			
Ferritin**	Level 1: YES	4.50	1.90	6.67
	Level 2: YES			
	Level 3: YES			
Thyroxine	Level 1: YES	-2.50	8.10	7.33
	Level 2: YES			
	Level 3: YES			
Thyroid Stimulating Hormone	Level 1: YES	6.20	QNS	5.00
	Level 2: YES			
	Level 3: YES			

TEA: Total allowable error; Bold: QC result outside 2SD range; hr, hour; Bold: Patient results average % Difference > TEA/3. \*: Average Difference < TEA/3; \*\*: Average of 7 patients' samples; QNS: Quantity not sufficient.

**A-050**

**Body Fluid Matrix Evaluation for the Roche cobas pro c503**

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Background: To investigate the accumulation of fluid in various compartments within the body, fluid specimens are collected and requested for analysis on automated chemistry/immunoassay instruments. Since these matrices are not commonly validated by the manufacturer, laboratories must perform additional studies to evaluate the performance of assays in these alternative matrices. Body fluid matrix studies (spiked recovery) were previously evaluated on the Roche cobas 8000 to exclude matrix interferences.<sup>1</sup> The present studies were performed to evaluate body fluid testing using the Roche cobas pro c503. Methods: A total of 45 analyte/body fluid combinations were investigated using the Roche cobas pro c503. The following analytes were evaluated: amylase, total bilirubin, cholesterol, glucose, lactate dehydrogenase, lipase, magnesium, rheumatoid factor, total protein, triglyceride, and uric acid. Following an IRB-approved protocol, the following residual fluids were obtained and de-identified after clinical testing: fecal, biliary/hepatic, cerebral spinal fluid, drain, pancreatic, pericardial, peritoneal, pleural, and synovial. Body fluid studies were performed after the assays were determined to exhibit acceptable performance characteristics for corresponding FDA-cleared matrices (e.g. serum, plasma, and/or urine). Method comparison was assessed using a minimum of 20 specimens per fluid/analyte combination, spanning the analytical measurement range (AMR). If needed, spiked specimens were prepared to cover the AMR, with spiked material not exceeding 10% of the total volume. Specimens were thawed, mixed, centrifuged, and tested on the cobas 8000 and c503 on the same day. Observed outliers were repeated on both analyzers and the repeat value was used for analysis. Samples outside the AMR were excluded. Mixed recovery studies (dilution linearity) were conducted using high (100%) and low (0%) pools which were mixed together to yield 25%, 50%, and 75% pools; all 5 levels were tested in triplicate. When necessary, high pools were created by spiking serum, commercial material, or other fluids at no greater than 10% of the total volume. Data analysis was performed using EP Evaluator 12. Results: Acceptable agreement was observed for the c503 compared to the cobas 8000 with slopes ranging from 0.87 (lipase in pancreatic fluid) to 1.05 (total protein in pericardial fluid), percent biases ranging from -11.4% (lipase in pancreatic fluid) to 2.97% (uric acid in peritoneal flu-

id), and correlation coefficients ranging from 0.965 (lipase in pancreatic fluid) to 1.0 (amylase in pleural fluid). Mixed recovery studies (linearity) demonstrated favorable performance with percent recoveries ranging from 88.9% (uric acid in drain fluid) to 114.2% (uric acid in synovial fluid), and slopes ranging from 0.97 (rheumatoid factor in pericardial fluid) to 1.07 (uric acid in synovial fluid). Conclusions: Studies to exclude matrix interferences are an important validation step prior to consideration of performing analysis on specimens from alternative matrices. The findings presented in this study demonstrate the analytical performance of body fluids using Roche cobas pro c503. Assays should be validated and matrix interferences should be excluded when using analyzers for body fluid testing, as such interferences may be instrument and assay-specific. <sup>1</sup>Owen et al. ClinBiochem 2015

**A-051**

**Is reflex testing feasible for outpatient thyroid testing?**

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**Background:** While guidelines recommend screening for thyroid dysfunction by testing thyroid stimulating hormone (TSH) first and then assessing free thyroxine (fT4) only if TSH is abnormal, our outpatient clinics routinely order both tests. To improve this reflex fT4 testing strategy, some advocate broader TSH decision limits. We decided to determine if testing TSH alone is feasible to rule out thyroid dysfunction in our outpatients and to see the impact of wider TSH decision limits (0.2 - 6.0 mIU/L) on case detection.

**Methods:** The reference intervals for thyroid hormones assayed on the Roche Cobas e801 analyzer are: TSH 0.4 - 4.0 mIU/L, and fT4 10-20 pmol/L respectively. Consecutive paired outpatient TSH/fT4 between October 2020 – February 2022 were retrieved from our Laboratory Information System (LIS). For results with duplicate/multiple values from the same individual, only the earliest values were retained. The results (n=5902) were stratified according to the fT4 values with respect to TSH (see Table).

**Results: Table. Distribution of outpatient TSH and fT4 values**

	All TSH (mIU/L)	< 0.2	0.2–0.39	0.4–4.0	4.1–6.0	> 6.0
All fT4 (pmol/L)	5902	146	136	4995	334	291
fT4 < 10	90	5	1	33	9	42
fT4 10–20	5434	89	115	4680	313	237
fT4 > 20	378	52	20	282	12	12

Of the 4995 individuals with normal TSH, 33 (0.67%) had low fT4 and 282 (5.6%) had high fT4. A TSH only strategy will miss 6.3% of abnormal fT4. Expanding the TSH decision limits to 0.2–6.0 mIU/L will miss 10 more subjects with low fT4 and 32 with high fT4.

**Conclusion:** TSH testing alone in our outpatient clinics will miss over 6% of abnormal fT4. Our clinicians are uncomfortable with this TSH first strategy to rule out thyroid dysfunction with our case-mix of patients. Until our computerized order entry system has the ability to capture clinical indications for test ordering it is unlikely that we can expect a good outcome with this TSH with reflex fT4 strategy. In addition, its use should be confined to new cases and for fresh test orders.

**A-052**

**Clinical impact of IgG subclass and C1q binding assay in donor-specific HLA antibody-positive kidney transplant patients**

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**Introduction.** Donor-specific HLA antibody (DSA) is associated with antibody-mediated rejection (AMR) and graft loss in kidney transplantation. Since not all DSAs harm allografts, additional assays such as IgG subclass and complement binding of DSA have been introduced. In this study, we evaluated the clinical impact of IgG subclass and C1q assay on biopsy-proven AMR and histopathology. **Method.** A total of 38 patients with DSA (MFI>2000) at the time of indication biopsy were included.

Twenty-eight (73.7%) patients were diagnosed with biopsy-proven AMR. The median time between transplantation and biopsy was 21 months. DSA and C1q binding were detected by LABScreen Single Antigen and C1qScreen assay (One lambda, USA), retrospectively. IgG 1-4 subclass assays were tested using a modified single antigen bead assay with replacing monoclonal secondary antibodies. **Results.** Of 38 DSA-positive patients, 22 (57.9%) patients had IgG subclass (MFI >500) (+) DSAs, and they had higher MFI values of DSA compared to those with subclass (-) DSAs [median (95%CI); 17754 (11568.4-20441.7) vs 3312 (2816.5-4018.6)] (P<0.001). Of the subclass (+) DSAs, 5 were multiple subclass (+) [IgG1 + IgG3 (n=2), IgG1 + IgG4 (n=2), IgG1 + IgG3+ IgG4 (n=1)] and the remaining were only IgG1 (+). C1q (+) was found in 36.8% (14/38) of patients, and C1q(+) had higher MFI values of DSA than C1q(-) [17754 (11306.1-22703.8 vs. 4009 (3145.3-5228.5)] (P<0.001). The IgG1 subclass/C1q results of 28 AMR patients were (+/+) (n=12), (+/-) (n=10), (-/+) (n=2) and (-/-)(n=14). In DSA positive patients, the sensitivity of subclass (+) and C1q(+) for AMR prediction was 67.9 (47.6-84.1)% and 39.3 (21.5-59.4)%, and the specificity was 70.0 (34.8-93.3)% and 70.0 (34.8-93.3)%, respectively. The combination of C1q and subclass assays increased sensitivity to 78.6%, and PPV to 88.0%, without loss of specificity. **Conclusion.** Determination of the IgG subclasses and C1q binding may be additional prognostic biomarkers for predicting AMR in DSA-positive kidney transplant patients.

**A-053**

**Effect of second hand smoke exposure on establishing urinary cotinine-based cut-off values for smoking status classification**

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**Background:** Regulations for banning smoking in public places and workplaces have spread worldwide in recent years. The recent consecutive Korean National Health and Nutrition Examination Survey (KNHANES) between 2008 and 2018 showed a trend toward significant decreases in self-reported tobacco smoke exposure and measured urinary cotinine values. The cut-off values of urinary cotinine, which is established for smoking status classification, might be affected by various factors such as sex, age, pregnancy, nicotine-containing food intake, ethnic variation of cotinine half-life and nicotine metabolism, and second-hand smoke (SHS) exposure.

**Methods:** We conducted to establish and to compare each optimal cut-off values for assessing the effect of SHS exposure on establishing urinary cotinine-based cut-off values for smoking status classification in the controlled population setting for racial and cultural diversity, using four KNHANES data consisting of 2008, 2011, 2014, and 2018 survey.

**Results:** Total of 18,229 Korean subjects with measured urinary cotinine who aged over 19 were enrolled. Self-reports of current smoking status show that the prevalence of current smokers decreased from 22.9% to 18.2% between 2008 and 2018. During this period, mean value of urinary cotinine in non-smokers decreased from 45.0 ng/mL to 32.8 ng/mL, while mean value show no remarkable decrease, in current smokers. The one distribution of urinary cotinine consisting of mainly non-smokers show the shift to the left close to limit of detection, whereas the other distribution consisting of mainly current smokers show no remarkable shift. When establishing the optimal cut-off based on ROC analysis, the cut-off values of urinary cotinine for distinguishing current smokers from non-smokers decreased from 86.5 ng/mL to 11.5 ng/mL. However, the cut-off values for distinguishing active smokers from non-daily smokers show no remarkable decrease.

**Conclusion:** Our study showed that decreased SHS exposure result in decreased the optimal cut-off value for distinguishing current smoker from non-smoker.

Optimal cut-off value of urinary cotinine established by AUC analysis				
Year	Classification	Optimal cut-off value of urinary cotinine (ng/mL)	Sensitivity (%)	Specificity (%)
2008	Current smoker	86.5	97.2	94.1
	Active smoker	86.5	97.2	94.5
2011	Current smoker	43.9	95.2	94.9
	Active smoker	107.9	95.4	95.2
2014	Current smoker	15.9	98.5	95.1
	Active smoker	110.5	99.3	94.1
2018	Current smoker	11.5	98.9	95.8
	Active smoker	77.9	99.5	93.8

## A-054

## Analytical Performance Evaluation of the Beckman Coulter HbA1c Advanced Assay

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**Background** Hemoglobin A1c (HbA1c) is one of the most important biomarkers used in the diagnosis and treatment monitoring of diabetes mellitus and is incorporated in many clinical guidelines. We evaluated the analytical performance of the Beckman Coulter HbA1c Advanced Assay (Beckman Coulter, Brea, CA, USA), which uses a turbidimetric immunoinhibition method incorporated into a fully automated, high-throughput system. **Methods** The stability, precision, linearity, and carryover of the Beckman Coulter HbA1c Advanced Assay were evaluated. Utilizing 120 residual patient samples, HbA1c measurements of the Beckman Coulter HbA1c Advanced Assay were compared with three other commercial HbA1c assays, the Tosoh HLC-723 G11 (Tosoh, Tokyo, Japan), ADAMS A1c HA-8190V (Arkray KDK, Kyoto, Japan), and Cobas Pro c503 (Roche, Basel, Switzerland). Twenty-six secondary reference samples with known HbA1c concentrations measured via an IFCC reference measurement procedure were used to evaluate the accuracy of the Beckman Coulter HbA1c Advanced Assay. Interference due to Hb variants was evaluated using 21 samples of 3 known Hb variants (Hb G-Coushatta, Hb Queens, and Hb Ube-4) commonly found in the Korean population. **Results** Sample stability was proven for up to 1 week at room temperature, and up to 1 month at temperatures below 4°C. Repeatability (% CV) for low and high concentrations ranged from 1.6%- 2.6% and 1.0%-1.4%, respectively, and within-laboratory precision for low and high concentrations ranged from 2.8%- 3.7% and 1.5%-2.1%, respectively. Linearity was proven over HbA1c concentration ranges of 28.9 mmol/mol (4.8 %NGSP) - 102.2 mmol/mol (11.5 %NGSP) for patient samples, and over 28.3 mmol/mol (4.74 %NGSP) - 182 mmol/mol (18.8 %NGSP) with a commercial linearity evaluation kit. No significant carryover was observed, and accuracy evaluation showed a mean bias of 3.63%. HbA1c results were generally comparable to other common commercial HbA1c assays. Furthermore, the assay showed no significant interference by Hb variants and yielded reliable HbA1c measurements. **Conclusion** The Beckman Coulter HbA1c Advanced Assay showed commendable analytical performance with good precision, linearity, accuracy, and minimal carryover. When compared to other routine HbA1c methods, the Beckman Coulter HbA1c Advanced Assay gave reliable, comparable results and was resistant to interference by common Hb variants. **Keywords** Beckman Coulter HbA1c Advanced Assay, Analytical performance, Accuracy, Linearity, Precision, Hb variant

## A-055

## Limited Utility of Free Triiodothyronine (fT3) Testing in the Majority of Patients with Physician-Ordered Testing

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**Background:** Thyroid hormone testing is crucial for the assessment of thyroid function. To improve utility, clinical laboratories often perform reflex thyroid hormone testing by assessing free T4 (fT4) concentrations only after an abnormal TSH is measured. However, standards for free T3 (fT3) reflex testing have not been established and it is often measured simultaneously with TSH and fT4. While the measurement of fT3 is crucial for the diagnosis of T3 thyrotoxicosis and is characterized by low TSH, normal/low fT4 and high fT3, its measurement without *a priori* knowledge of thyroid status likely leads to unnecessary laboratory testing and increased healthcare costs. Therefore, we examined the clinical utility of fT3 with the goal of identifying TSH and fT4 cutoffs that best identified T3 thyrotoxicosis.

**Methods:** TSH, fT4 and fT3 results from Barnes-Jewish Hospital and St. Louis Children's Hospital laboratories between January 2016 and October 2021 were retrospectively extracted using the laboratory information system. All lab testing was performed on a Roche cobas e602 according to the manufacturer's instructions. Lab values from patients younger than one-week and patients with missing age were excluded. Inclusion criteria were all patients with a TSH, fT4 and fT3 resulted within the same 24h period. When the same test was performed multiple times on the same date, the latest result was used. Reference intervals were 0.3 to 4.2 µIU/mL for TSH, 0.9 to 1.7 ng/dL for fT4, and 2 to 4.4 pg/mL for fT3. Frequency of detected T3 thyrotoxicosis was examined with potential reflex cutoffs at TSH < 0.3, and 0.01. Chart review was

performed on a subset of patients with laboratory results consistent with T3 thyrotoxicosis to determine the indication for testing. T3 thyrotoxicosis was defined as a low TSH, normal/low fT4, and a fT3 above the reference interval.

**Results:** 4,373 results with TSH-fT4-fT3 measured within 24h were obtained from 137,698 TSH, 54,894 fT4 and 6,475 fT3 tests. Of the 4,373 results, 70 (1.6%) results were consistent with T3 thyrotoxicosis. The most common reason for T3 thyrotoxicosis was hyperthyroidism on anti-thyroid medication (n=28) followed by hypothyroidism on thyroid medication (n=18). Only 20/4,373 (0.5%) results revealed a new diagnosis of hyperthyroidism. The likelihood of detecting T3 thyrotoxicosis increased with lower TSH cutoffs (<0.3 µIU/mL; 10.3% of results relative to <0.01 µIU/mL; 27.6% of results). No patient with newly diagnosed T3 thyrotoxicosis had a TSH >0.01 µIU/mL. A higher frequency of T3 thyrotoxicosis was observed in the outpatient setting (34% of results) relative to the inpatient setting (14% of results, p<0.001) if the TSH was < 0.01 µIU/mL.

**Conclusion:** fT3 testing had limited utility in the vast majority of patients with physician-ordered TSH-fT4-fT3 testing and utility was limited further in hospitalized patients. A fT3 reflex in which patients with a TSH < 0.01 µIU/mL and a normal or low fT4 may improve laboratory test utility without missing newly diagnosed cases of T3 thyrotoxicosis.

## A-056

## Analytical comparison of the Roche c702 pancreatic lipase assay against two third-party lipase assays on the Abbott Alinity

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**Background:** Currently, the Sinai Health System offers total amylase as the only blood test to aid in diagnosis and management of pancreatitis. Considering evidence supporting the use of pancreatic lipase over total amylase, we sought to evaluate analytical performance of pancreatic lipase, on the Roche c702 system.

**Methods:** Precision, a three-way method comparison, and linearity were assessed using a total allowable error of 20% set by Accreditation Canada Diagnostics (ACD). Samples from 76 patients that covered the analytical measuring range of the Roche assay were used to assess relative accuracy across three different lipase assays. Finally diagnostic agreement between lipase and total amylase was assessed through measuring both enzymes on 190 samples from 159 unique patients and correlating enzyme levels with clinical outcome.

**Results:** Total imprecision (%CV), assessed over 5 days, was 1.2% for lipase. Lipase measured by the Roche c702 assay showed good correlation compared to a Sekisui method on Abbott Alinity with R = 0.93 and a mean relative bias of 13.0%. The Roche method also showed good comparison to the Sentinel assay measured on Abbott Alinity with R = 0.97 and a mean relative bias of -28.4%. By contrast, the Sekisui method compared to the Sentinel method had an R = 0.95 but a large mean relative bias of 62.1%. The Roche lipase assay was verified to be linear across the 10-8500 U/L range. Within the 190 sample cohort for diagnostic agreement, there was good correlation between the total amylase and lipase with R = 0.85. Lipase was elevated above the expected range (13-60 U/L) in 28 out of 159 individuals and 6 of these patients were confirmed to have pancreatic disease. By contrast, total amylase was elevated in 38 of the 159 patients and 6 of these patients were confirmed to have pancreatic disease. **Conclusion:** Altogether, these studies justified moving forward with the implementation and further validation of the of the Roche pancreatic lipase assay given the strong clinical utility of pancreatic lipase in the diagnosis of acute pancreatitis.

## A-057

Comparison of the CKD-EPI 2021 Equation (eGFR<sub>cr</sub>, 2021) with Other Creatinine-based eGFR Equations in Chronic Kidney Disease Diagnosis and Staging

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**Background:** Many commonly used eGFR<sub>cr</sub> equations incorporate a race factor to account for non-GFR determinants of serum creatinine, but recent studies have raised the concerns of overestimating eGFR in African Americans. Some laboratories have removed the race modifier from these equations for all patients. The most recent guideline recommends a new race-independent equation (eGFR<sub>cr</sub>, 2021). Another new equation known as EKFC, developed for a full age spectrum eGFR calculation,

provides a race-free alternative. The objective of this study is to investigate the accordance between the eGFR<sub>cr</sub> 2021 equation and other methods without the race modifier in eGFR<sub>cr</sub> calculation, chronic kidney disease (CKD) diagnosis, and staging.

**Methods:** A total of 321 subjects (age 18–87, median 66 years; 57% male, 43% female; 8.72% African American) were selected to calculate eGFR<sub>cr</sub> using serum creatinine concentration (Roche Cobas 8000) based on the eGFR<sub>cr</sub> 2021, eGFR<sub>cr</sub> 2009-non-black (NB), MDRD-NB, and EKFC equations. Correlations of these equations and impact on CKD diagnosis and staging were analyzed.

**Results:** For patients with eGFR<60 mL/min/1.73m<sup>2</sup> by the eGFR<sub>cr</sub> 2021 equation, eGFR<sub>cr</sub> 2021 was highly correlated with eGFR<sub>cr</sub> 2009-NB, MDRD-NB, and to less extent with EKFC (Spearman r=0.9997, 0.9962, 0.9866, respectively). When eGFR>90 mL/min/1.73m<sup>2</sup>, a significant discordance appeared where MDRD-NB was much higher than eGFR<sub>cr</sub> 2021, and EKFC also demonstrated an obvious discordance in this range. To compare the impacts of these equations on CKD diagnosis, a subset of subjects (n=136) with eGFR=30–90 mL/min/1.73m<sup>2</sup> calculated by all equations were dichotomized at eGFR=60 mL/min/1.73m<sup>2</sup>. eGFR<sub>cr</sub> 2021 and eGFR<sub>cr</sub> 2009-NB were similar in CKD classification, but MDRD-NB and EKFC both classified more subjects as CKD (eGFR<60) compared to eGFR<sub>cr</sub> 2021 (Binomial test p=0.0009 and 0.0002, respectively). Another subset of subjects (n=161) with eGFR<60 mL/min/1.73m<sup>2</sup> calculated by all equations were selected to compare these equations in CKD staging (G3a: eGFR=45-59, G3b: 30-44, G4: 15-29, G5: <15). Both MDRD-NB and eGFR<sub>cr</sub> 2009-NB showed no significant statistical difference from eGFR<sub>cr</sub> 2021 in CKD staging (p=0.2269 and 0.2894, respectively). The EKFC equation resulted in a decrease of subjects in G3a but an increase in G3b stage compared to eGFR<sub>cr</sub> 2021 ( $\chi^2$  test p=0.0003), without causing a significant change in total subjects in G3 stage. Notably, all four eGFR equations showed a similar percentage of subjects in G5 stage (eGFR<sub>cr</sub> 2021 =MDRD-NB=19.3%, eGFR<sub>cr</sub> 2009-NB =EKFC=21.7%).

**Conclusion:** The new race-independent eGFR<sub>cr</sub> 2021 equation correlates well with other equations (eGFR<sub>cr</sub> 2009-NB, MDRD-NB, EKFC) when it calculates eGFR<60 mL/min/1.73m<sup>2</sup>. Discordance with MDRD-NB and EKFC is only observed if eGFR<90 mL/min/1.73m<sup>2</sup> by eGFR<sub>cr</sub> 2021, and such discordance is more evident in EKFC probably caused by the difference in equation design. The eGFR<sub>cr</sub> 2021 equation shows no significant difference in CKD diagnosis compared to eGFR<sub>cr</sub> 2009-NB, but it may classify fewer subjects as CKD than the MDRD-NB and EKFC equations due to an increased estimation of GFR. Within the range of CKD, this equation is not significantly different from other equations in staging, especially for the renal failure (G5) stage.

## A-059

### Effect of In Vitro Acetylation of Hemoglobin on HbA1c Measurement by the DCA Vantage Analyzer

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**Background:** Globally, approximately 32.2% of diabetic patients are affected by cardiovascular diseases. A large number of these patients are prescribed aspirin to reduce the risk of cardiovascular disease. Acetylsalicylic acid (ASA), the chemical constituent of aspirin, reacts with hemoglobin to form acetylated hemoglobin. Acetylated hemoglobin is reported to interfere with some HbA1c assays, depending on methodology. In this study, the effect of aspirin on HbA1c assays was determined using in vitro acetylation conditions. **Methods:** HbA1c values of whole-blood samples were measured before and after in vitro acetylation by incubating the samples with 0.35% acetylsalicylic acid (ASA) at 37°C for 22-26 hours. Two devices were used for HbA1c measurement: the HLC-723G8 HPLC analyzer (TOSOH) and DCA Vantage® analyzer (SIEMENS HEALTHINEERS). Bias was calculated based on the average HbA1c of the sample before and after incubation with ASA.

**Results:** Two samples with normal HbA1c levels (5.3% and 5.4%), one sample with a prediabetic HbA1c level (5.8%), one sample at the medical decision level (6.6%), and two samples with diabetic HbA1c levels (7.2% and 7.4%) were tested. The bias values for samples measured on the HLC-723G8 system after incubating with ASA exceeded the 7% limit in all cases, ranging from 7.5% to 10.3%, and the bias on the HLC-723G8 device for the samples incubating without ASA was less than 2%, indicating the interfering effect due to acetylation. Conversely, under similar in vitro acetylation conditions, the HbA1c values decreased on the DCA Vantage Analyzer, with bias values ranging from -4.56% to -0.86%. The bias values for samples on the DCA Vantage analyzer after incubating with ASA were <6% in all cases, indicating no interfering effect from acetylation.

**Conclusion:** The results indicate that in vitro acetylation interferes with the HLC-723G8 HbA1c assay. In contrast, results indicate that the DCA Vantage assay is not interfered with by in vitro acetylation of hemoglobin.

## A-060

### Effect of Labile Glycated Hemoglobin on HbA1c Measurement by the DCA Vantage Analyzer

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**Background:** Research indicates that labile glycated hemoglobin (L-A1c) concentration increases after meals and fluctuates with increasing blood glucose levels. Such increase is reported to cause false HbA1c results when using HPLC and boronate affinity measurement methods. In contrast, immunoturbidimetric HbA1c assays measure HbA1c-protein by specific immunobinding. Therefore, in theory, an increase in labile glycated hemoglobin is not expected to influence an immunoturbidimetry assay. In this poster, we report the effect of increased L-A1c concentrations on HbA1c results obtained on the immunoturbidimetry-based DCA Vantage® Analyzer and the boronate affinity-based AFINION™ 2 analyzer.

**Methods:** Seven different whole-blood samples with HbA1c levels ranging from 4.7 to 8.1% were tested at various elevated L-A1c concentrations (1-4%, 10-14%, >14%). The elevated L-A1c concentrations were achieved by glycosylating whole blood in various in vitro conditions. The HbA1c values of the control and the glycated samples were measured in five replicates on HLC-723G8 (TOSOH), AFINION™ 2 (ABBOTT), and DCA Vantage (SIEMENS HEALTHINEERS) analyzers. The average HbA1c level of the non-glycated sample on the HLC-723G8 analyzer was considered the reference HbA1c value for the control sample. The HbA1c percent bias value was calculated for each device at the elevated L-A1c concentrations compared to the reference value of the control sample. The acceptable bias limit was considered ±7%.

**Results:** The HbA1c percent biases for samples tested on the DCA Vantage Analyzer were within the 7% bias limit, ranging from -3.91% to +6.15% when compared to the reference HbA1c values of the respective control samples for all the samples tested. The percent bias for the samples tested on the AFINION™ 2 analyzer ranged from -6.00% to 28.85%; in five of seven samples tested, the bias exceeded the 7% bias limit.

**Conclusion:** The results of this study indicate that the DCA Vantage Analyzer reported HbA1c results within acceptable bias ranges with increasing L-A1c concentration in a sample. Conversely, the AFINION™ 2 analyzer reported high HbA1c results outside the acceptable bias range with increasing L-A1c concentrations. The results indicate that DCA Vantage Analyzer measurements can be made regardless of meal timing or blood sugar level, while the boronate affinity-based AFINION™ 2 assay may be compromised under the same conditions due to the presence of elevated levels of L-A1c.

## A-061

### Concordance of Chronic Kidney Disease Stage and Metformin Management Using CKD-EPI 2021 Race-Free Equation vs. CKD-EPI 2009 Equation to Estimate Glomerular Filtration Rate

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**Background:** Metformin is a first-line agent for the treatment of hyperglycemia in type 2 diabetics, however risk of adverse effects (e.g. lactic acidosis) necessitates close monitoring of renal function. The Kidney Disease: Improving Global Outcomes (KDIGO) organization clinical practice guidelines recommend using an estimation of glomerular filtration rate (eGFR) to assess renal function, categorize chronic kidney disease (CKD) stage, and to guide metformin management. Multiple professional organizations have recommended implementation of the new CKD Epidemiology Collaboration (CKD-EPI) 2021 race-free eGFR equation calculated from serum creatinine (eGFR<sub>cr</sub>) that does not disproportionately affect any one group of individuals. In order to assess the potential impact of implementation, a retrospective analysis was performed to determine concordance of CKD stage and metformin management categories using the CKD-EPI 2021 versus CKD-EPI 2009 eGFR<sub>cr</sub> equations.

**Methods:** Creatinine values, age, and race from 7,236 patients ≥18 years old with an active metformin medication between April 1, 2021 and July 1, 2021 were obtained from the LIS. eGFR<sub>cr</sub> was calculated using both the CKD-EPI 2021 and CKD-EPI 2009 equations. Concordance, defined as the number (%) of eGFR<sub>cr</sub> values that fell into the same CKD stage and metformin management categories using each equation, was evaluated in both black and non-black patients on metformin. KDIGO recommends continuing metformin use in patients with an eGFR<sub>cr</sub> ≥45 mL/min/1.73 m<sup>2</sup> (CKD-1 to -3a), reviewing use for potential dosage adjustments with an eGFR<sub>cr</sub> 30-44 (CKD-3b), and discontinuing use when eGFR<sub>cr</sub> is <30 (CKD-4 to -5).

**Results:** Concordance in CKD staging and metformin management was 84.3% using CKD-EPI 2021 eGFR<sub>cr</sub> versus CKD-EPI 2009 eGFR<sub>cr</sub> equations in this cohort of patients with an active metformin medication. Of the remaining 15.7% discordant results, 27.9% of black patients with CKD-3a progressed to CKD-3b and 28.4% of non-black patients improved to CKD-2. For patients in CKD-3b, 11.9% of black patients progressed to CKD-4 and 27.5% of non-black patients improved to CKD-3a. No trends were observed based on age or sex for reclassification.

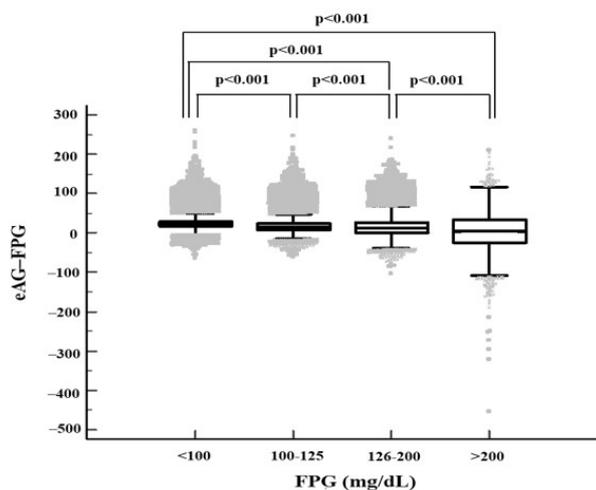
**Conclusions:** Implementation of the CKD-EPI 2021 eGFR<sub>cr</sub> equation will change CKD categorization and metformin management in select patients with moderate to severe renal dysfunction. Risk re-assessment, frequency of monitoring, dose adjustment, or discontinuation of metformin may be warranted for select black patients currently in CKD-3a and 3b based on CKD-EPI 2009 eGFR<sub>cr</sub>, whereas non-black patients currently in CKD-3b and CKD-4 may benefit from initiation of a metformin therapy or dose adjustment. Education of patient care providers regarding expected changes with CKD-EPI 2021 eGFR<sub>cr</sub> implementation is key for healthcare equity.

**A-062**

**Association between estimated average glucose and fasting plasma glucose levels for hyperglycemia management during health checkups**

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**Background:** Fasting plasma glucose (FPG) and glycated hemoglobin (HbA1c) are major indicators for diagnosing and managing hyperglycemia. This study aimed to determine the correlation between FPG and HbA1c-derived eAG and to analyze the factors associated with eAG during health checkups. **Methods:** This cross-sectional retrospective study consecutively selected subjects who had undergone health checkups at 16 health-promotion centers in 13 Korean cities during 2020. The subjects comprised 182,848 patients with normoglycemia, 109,555 with impaired fasting glucose (IFG), and 35,632 with diabetes. eAG was calculated using Nathan’s regression equation: eAG mmol/L [mg/dL]=1.59×HbA1c (NGSP, %)-2.59 [28.7×HbA1c (NGSP, %)-46.7]. Pearson’s correlation and multiple regression analysis were used to determine the correlation between eAG and FPG and the factors associated with eAG, respectively. **Results:** In all subjects, FPG was found to be fairly strongly correlated with eAG (r=0.811). When the subjects were divided into FPG subgroups, the strength of the correlation decreased among those with normoglycemia and IFG: FPG <5.6 mmol/L, r=0.210; FPG=5.6–6.9 mmol/L, r=0.413; FPG=7.0–11.1 mmol/L, r=0.573; FPG >11.1 mmol/L, r=0.524. There was a significant differences between eAG and FPG values according to FPG group, which were lowest in the diabetic group, especially in poorly controlled diabetic patients with FPG >11.1 mmol/L (P<0.001). The proportion of subjects with a higher value of FPG than eAG was 46.3% in poorly controlled diabetic patients, compared with only 1.5% in normoglycemic patients. Higher eAG level were associated with older age, female sex, higher FPG, and lower HDL-C and triglycerides (all P<0.05). **Conclusion:** The association between eAG and FPG differs depending on the hyperglycemia levels. FPG alongside HbA1c-derived eAG could be helpful managing both patients with diabetes and prediabetes, as long as the degree of hyperglycemia is considered in health checkups.



**A-063**

**Determination of Optimum Quality Control Testing Frequency for 27 Common Clinical Chemistry Analytes on Roche Cobas 702 platform**

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**Background:** Quality Control testing frequency is based on a number of factors such as analytical stability, error tolerance without impacting results, testing volume etc. Less than optimal frequency can result in erroneous results, which could lead to inappropriate clinical interventions. However, higher than optimum frequency can cause inefficiency in lab operations, put a strain on resources and negatively impact turnaround time (TAT) of reporting. Thus an ideal balance of QC testing frequency needs to be evaluated and determined based on the aforementioned factors. **Methods:** Evaluation of QC frequency requirements of 27 clinical chemistry assays on Roche Cobas 702 platform was performed. These analytes included Albumin ALP, Amylase, AST, BUN, Calcium, Chloride, Total Cholesterol, Creatine Kinase, Bicarbonate, Creatinine, D-Bilirubin, GGT, Glucose, HDL, LDH, LDL, Lipase, Magnesium, Phosphate, Potassium, Sodium, T-Bilirubin, Total protein, Triglycerides and Uric Acid. For all mentioned analytes a multi QC material (Bio-Rad Multiqual) was used over 12 hrs with 3 hr increments per day over 5 days with starting baseline at 0 hrs (3, 6, 9 and 12). Additional 2 days of QC and 3 days of native serum samples were tested for 5 analytes in the same manner as above (Creatinine Jaffe, Albumin, Phosphorus, Bicarbonate and Total protein) that either showed a drift at ≥9 hrs or failed our acceptance criteria. All assays were calibrated each day before starting the analysis of QC at 0 hrs. **Results:** The average absolute and percent change for the three levels of QC tested from the baseline to the rest of the timelines was evaluated using EP evaluator multiple instrument module. The timeline at which the results are within 2SD from baseline was chosen as acceptance criteria. The SD is either calculated from the achieved SD of the QC inter-assay analysis or from the ATE of the assays (Albumin:12.3%, ALP: 12%, Amylase:10%, AST:12%, BUN:12%, Calcium:4%, Chloride:3%, Total Cholesterol:6%, Creatine Kinase:30%, Bicarbonate:20%, Creatinine:8%, D-Bilirubin:20%, GGT:12%, Glucose:8%, HDL:12%, LDH:8%, LDL:15%, Lipase:20%, Magnesium:8%, Phosphate:8%, Potassium:3.2%, Sodium:3.7%, T-Bilirubin:12%, Total protein:5%, Triglycerides:12% and Uric Acid:12%). **Conclusion:** This study was conducted to evaluate the possibility to extend the current QC frequency of 3 hrs at our facility that was set up about 10 years ago. The data shows that most assays passed our acceptance criteria up to 12 hrs and few showed drift after 9 hrs (Albumin, Creatinine and Phosphate). Based on the testing volume at our facility and duration of testing performed (up to 12hrs) a QC frequency of 8 hrs was determined as optimum which improved the efficiency, TAT for resulting as well as reagent saving.

**A-064**

**Prevalence of Hemolyzed Blood Gas Specimens in Acute Care Settings**

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**Background:** The impact of *in vitro* hemolysis on laboratory results is well established due to contamination of plasma or serum with intracellular components from lysed red blood cells. While there is extensive literature on hemolysis rates in serum/plasma specimens, the prevalence of hemolysis in whole blood specimens in acute care settings is lacking. **Methods:** To assess the prevalence of hemolysis, whole blood samples left over from blood gas testing originating from multiple acute care settings (emergency department [ED], intensive care units [ICU], and medical units) were assayed for hemolysis index (H index) in plasma aliquots on the Abbott Alinity and Roche cobas analyzers. The samples were categorized as 1) no hemolysis - H index < 50; 2) mild hemolysis - H index 50-99; 3) moderate hemolysis - H index 100-199; and 4) gross hemolysis - H index 200+. **Results:** 544 residual blood gas specimens from 15 clinical units were tested, but only 8 units satisfied a minimum sample size (N = 10) and were represented in the final data analysis. Of the remaining 492 samples, 30 (6.1%) had mild hemolysis, while 10 (2.0%) samples had gross hemolysis. Analysis of samples by originating clinical units showed a trend, where samples originating from the Burn and Medical Units had significantly higher hemolysis rates than ED and ICUs. The blood gas samples from Surgical ICU (N = 31), Medical ICU (N = 111) and Cardiac ICU (N = 130) had mild hemolysis in 6.5%, 4.5%, and 3.1% of samples, respectively. Similarly, 3.3% of Medical Unit and 3.6% of Medical ICU samples were moderately hemolyzed, a rate comparable to ED (3.6%). **Conclusions:** A smaller number of samples from the Burn unit (19 of 492) might have skewed the

hemolysis rates in that unit. However, the overall prevalence of hemolysis demonstrates undetected hemolysis in whole blood samples sent for blood gas testing from acute care settings, potentially impacting patient results.

## A-065

### Performance Evaluation of the ADVIA Centaur NT-proBNP (PBNP) Assay

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**Background:** The Siemens Healthineers ADVIA Centaur® NT-proBNP (PBNP)\* assay is 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. In the outpatient (OP) and emergency department (ED) populations, measurements of NT-proBNP are used as an aid in the diagnosis and assessment of heart failure (HF) in patients with clinical suspicion of new-onset or worsening HF and for assessment of HF severity. **Method:** The ADVIA Centaur PBNP assay is a fully automated two-site sandwich immunoassay using direct chemiluminescent technology and two monoclonal antibodies. The Solid Phase contains a bound biotinylated monoclonal sheep anti-human antibody specific to NT-proBNP and conjugated to streptavidin magnetic particles. The Lite Reagent contains an acridinium-ester-labeled monoclonal sheep anti-human NT-proBNP F(ab')<sub>2</sub> fragment specific to NT-proBNP. A direct relationship exists between the amount of NT-proBNP present in the patient sample and the amount of relative light units detected by the system. **Results Analytical performance:** Time to first result is approximately 20 minutes. The assay requires 20 µL of serum or plasma and is linear from 35 to 35,000 pg/mL. Reproducibility determined using eight patient pools from 141 to 28,860 pg/mL of NT-proBNP yielded repeatability and reproducibility CVs ranging from 1.7 to 2.9% and 3.1 to 5.0% respectively. Interference testing showed no significant interference bias (<10%) in the presence of hemoglobin (1000 mg/dL), bilirubin (60 mg/dL conjugated and unconjugated), lipemia (3000 mg/dL INTRALIPID), and biotin (3510 ng/mL) at NT-proBNP concentrations of 123-137 pg/mL and 1612-1813 pg/mL. Cross-reactivity was ≤1% in the presence of common cross-reactants (CNP32, DNP, preproANP26-55, preproANP56-92, preproANP104-123, renin, urodilatin, and VNP). Cross-reactivity for 20 pg/mL endothelin was -5%. Limit of blank, limit of detection, and limit of quantitation were 12, 19, and 35 pg/mL respectively. **Clinical performance:** Clinical performance was determined for the OP population by comparing NT-proBNP results to the adjudicated diagnosis of new-onset HF using a single cutoff of 125 pg/mL. Sensitivity, specificity, positive predictive value, and negative predictive value were 86%, 64%, 34%, and 96% respectively. Clinical performance in the ED was determined by comparing NT-proBNP results to the adjudicated diagnosis of acute HF based on an overall rule-out cutoff value of 300 pg/mL and age-specific rule-in cutoff values of 450/900/1800 pg/mL for ages <50/50-75/>75 years. Likelihood ratio (LR+) estimated for the stratified NT-proBNP determinations of negative, indeterminate, and positive, yielded LR+ of 0.07, 0.55 and 3.53 respectively. NT-proBNP levels increased with increasing severity of HF based on NYHA and ACC/AHA classifications. **Conclusion:** The ADVIA Centaur PBNP assay demonstrates acceptable analytical and clinical performance for the measurement of NT-proBNP in serum and plasma and for use as an aid in the diagnosis and assessment of heart failure (HF) in patients with clinical suspicion of new-onset or worsening HF and for assessment of HF severity.\*The ADVIA Centaur PBNP assay is pending 510(k) clearance and not yet commercially available in the United States and other countries.

## A-066

### The value of high sensitive cardiac troponin I as predictive marker for cardiovascular complications and survivals in Korean adults with chronic kidney disease

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**Background:** It is well known that patients with chronic kidney disease (CKD) have elevated high-sensitive troponin I or troponin T (hsTnI or hsTnT) level and CKD is a strong risk of cardiovascular disease (CVD). However, it is not conclusive to interpret the chronic elevated cardiac troponin in CKD patients without acute CVD symptom nor sign. We investigated the predictive value and specific cut-offs of hsTnI for CVD

event in patients with CKD. **Methods:** We enrolled 120 adult patients with CKD, who visited Gachon University Gil Medical Center from May to July 2013, and 276 reference population. The last follow up date was 1<sup>st</sup>, April, 2019. Serum hsTnI was measured by using the chemiluminescent microparticle immunoassay (Architect STAT High Sensitive Troponin I; Abbott, Abbott Park, Illinois, USA/ Architect i1000s; Abbott). We assessed the data by *independent t test* and *Chi square* for comparison of two groups, *Pearson's correlation* for the relationship between hsTnI and renal function markers (creatinine, cystatin C), *ROC curve analysis* for optimal cut-off, and *Kaplan-Meier method* and *log-rank test* for survival analysis. **Results:** In the total 120 CKD patients, the median age was 62 years (range 20–85 years) with male–female ratio of 1:0.6 (male, 75; female, 45). The median hsTnI level was 7.1 pg/ml (range 2.3–120.4 pg/ml). The hsTnI level was significantly increased in CKD patients compared to the reference population ( $p < 0.001$ ). A positive correlation was found between hsTnI and renal function markers, such as creatinine ( $r^2 = 0.324$ ,  $p < 0.001$ ), and cystatin C ( $r^2 = 0.271$ ,  $p = 0.003$ ). The 5-year-event free survival (5-year-EFS) rate was 84.2% and 5-year-overall survival (5-year-OS) rate was 92.5% in 120 patients with CKD. In ROC analysis, the cut-off of hsTnI for 5-year-EFS was 7.2 pg/ml (AUC, 0.695;  $P = 0.002$ ; sensitivity, 57.43%; specificity, 73.68%). The cut-off of hsTnI for 5-year-OS was 7.7 pg/ml (AUC, 0.709;  $P = 0.021$ ; sensitivity, 63.06%; specificity, 77.78%). To apply each cut-off, the patients with elevated hsTnI showed significantly shorter EFS and OS than patients with non-elevated hsTnI ( $P < 0.010$  for EFS and  $P = 0.023$  for OS). In addition, the patients with elevated hsTnI showed significantly higher 5-year-EFS and 5-year-OS ( $P = 0.013$  and  $0.017$ , respectively). **Conclusions:** To consistent with previous studies, the hsTnI level was significantly increased in CKD patients than reference population, and it showed positive correlation with creatinine and cystatin C. The ROC and survival assessment showed the possibility of hsTnI to be a predictive marker for CVD risk or survival of patients with CKD. Additional studies with large population are necessary to understand the risk factors for CVD and to develop strategies to prevent of CVD events in patients with CKD.

## A-067

### CAP Quality Cross Check Materials are an Effective Approach for Biannual Instrument Correlations: A Pilot Study

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**Background:** Biannual instrument correlation studies must be performed for non-waived assays that are on multiple instruments under the same Clinical Laboratory Improvement Amendments (CLIA) license number. This is traditionally done by analyzing patient samples between instruments but may be challenging for institutions with multiple CLIA that want to perform these studies under Good Laboratory Practices (GLP). The aim of this study was to determine the feasibility of using College of American Pathologists (CAP) Cross Check Materials–Chemistry and Therapeutic Drug Monitoring (Program Code CZQ with 76 unique analytes) to assess instrument correlations between multiple analyzers, analyzer models and CLIA numbers for the basic metabolic panel (BMP) as a pilot.

**Methods:** Instrument correlation studies for the BMP were performed on nine Abbott Architects (c4000 (n=4), c8000 (n=2) and c16000 (n=3)) across three CLIA licenses using CAP CZQ material. The means, differences, and percent bias (% bias) for each individual level of CZQ (n=3) were determined for each individual analyzer and analyte (Table 1). Difference and percent bias (% bias) from the mean were calculated using the following equations 1.) difference = value-mean and 2.) % bias = ((value-mean)/mean)\*100. Acceptable difference and % bias was set using criteria provided by CAP or American Association for Bioanalysts (AAB).

**Results:** Three separate CLIA were compared in this analysis. Gray scale in Table 1 divides instrumentation into different CLIA licensure (no gray, light gray, dark gray). A few analytes and instruments in this study were outside the acceptable percent bias. However, all were determined to be within acceptability criteria based on concentrations.

**Conclusion:** Adoption of CAP Quality Cross Check materials provides a means to standardized correlation studies among multiple instruments across separate CLIA.

Assay	CCL Level	Mean	c1000A			c1000B			c1000C			c900A			c900B			c900C			c800A			c800B			c800C			
			Difference	% Bias																										
Cobas	CG10	12.11	0.11	0.9%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	6.43	0.05	0.8%	0.01	0.2%	0.01	0.3%	0.01	0.2%	0.01	0.2%	0.01	0.2%	0.01	0.2%	0.01	0.2%	0.01	0.2%	0.01	0.2%	0.01	0.2%	0.01	0.2%	0.01	0.2%	0.01	0.2%
	CG10	9.78	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
Ortho	CG10	8.9	0.05	0.6%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	10.0	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	10.0	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
Covance	CG10	11.11	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	11.8	0.05	0.4%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	11.8	0.05	0.4%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
Siemens	CG10	10.0	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	10.0	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	10.0	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
Parsiplex	CG10	11.1	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	11.1	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	11.1	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
Sofam	CG10	11.1	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	11.1	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	11.1	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
Urea	CG10	11.1	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	11.1	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%
	CG10	11.1	0.05	0.5%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.01	0.1%

A-068

Performance Characteristics of the Thyroid Stimulating Hormone 3-Ultra Assay on the Atellica CI 1900 Analyzer

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**Introduction:** The Atellica® CI 1900 Analyzer is a new integrated analyzer that uses Atellica IM and Atellica CH assay reagents. The purpose of this investigation was to evaluate the analytical performance of the Atellica IM Thyroid Stimulating Hormone 3-Ultra™ (TSH3UL) assay on the Atellica CI 1900 Analyzer. The Atellica IM TSH3UL assay is for in vitro diagnostic use in the quantitative determination of thyroid-stimulating hormone in human serum and plasma (EDTA and lithium heparin). Measurements of thyroid stimulating hormone produced by the anterior pituitary are used in the diagnosis of thyroid or pituitary disorder.

**Methods:** The Atellica IM TSH3UL assay employs anti-FITC monoclonal antibody covalently bound to paramagnetic particles, an FITC-labeled anti-TSH-capture monoclonal antibody, and a tracer consisting of another anti-TSH monoclonal antibody and acridinium ester (AE), both conjugated to BSA. A direct relationship exists between the amount of TSH present in the patient sample and amount of relative light units (RLUs) detected by the system. The following studies were performed to evaluate assay performance on the Atellica CI 1900 system: 10-day precision, limit of blank (LoB), limit of detection (LoD), 10-day functional sensitivity, and hook effect.

**Results:** Precision studies for six serum, three EDTA, and three lithium heparin samples using two lots of the Atellica IM TSH3UL assay on two Atellica CI 1900 Analyzers showed a repeatability coefficient of variation (CV) of  $\leq 2.1\%$  (TSH concentrations of 0.055-119.6 µIU/mL) and within-lab precision CVs of  $\leq 3.2\%$ . In detection studies, three lots of the Atellica IM TSH3UL assay on two Atellica CI 1900 Analyzers showed a LoB of 0.001 µIU/mL, LoD of 0.003 µIU/mL, and functional sensitivity of 0.003 µIU/mL. No hook effect was observed up to at least 3000 µIU/mL.

**Conclusion:** Study results demonstrated reproducible assay performance, robustness to hook effect, and detection capability suitable to provide accurate and precise measurements of TSH on the Atellica CI 1900 Analyzer.\*Product under development. Not available for commercial use or sale. Future availability cannot be guaranteed.

Unrestricted

A-069

An Alberta Lipase Correction Factor: Strong Correlation Between Serum Lipase Assays Across Vendors and Analyzer Types Will Enable Assay Harmonization Across This Canadian Province

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**Background:**

Laboratory testing in the Canadian province of Alberta is performed at laboratories operated by Alberta Precision Laboratories and DynaLIFE Medical Labs. The province is transitioning to a shared information system, therefore harmonization is a priority. However, analytes such as lipase are offered on 7 different analyzers from 3 different vendors and lack standardization. Lack of plasma lipase standardization leads to considerable result variation between different vendor's assays; this poses a challenge to reference interval harmonization and creates the opportunity for post-analytical error as patients move between care facilities. In this study, we evaluated the correlations between plasma lipase assays used in Alberta with the aim to develop

a series of correction factors to harmonize test results for plasma lipase across the province. To the best of our knowledge, there have been no previously reported correction factors for plasma lipase.

**Methods:**

Seven analyzers from three different vendors were used to measure plasma lipase on 40 leftover patient plasma samples; these samples spanned the analytical measuring range of the Roche Cobas analyzers (19.9 – 307 U/L). Each sample was thawed and measured in duplicate on the same day at various laboratories throughout the province on the following analyzers: Roche cobas c701, Roche cobas Pro, Ortho Vitros 350, Ortho Vitros 4600, Ortho Vitros XT3400, Siemens Atellica, and Siemens Dimension EXL. Pearson correlation coefficients and correction factors were determined by ordinary Deming regression analysis using Analyse-it software.

**Results:**

Overall, comparisons between the seven analyzers showed strong linear correlations but with large biases observed between the 3 vendors. The results are listed below:

Method Comparisons	Pearson Correlation Coefficient	Slope (95% confidence interval)	y-intercept (95% confidence interval)
<b>Roche Diagnostics Instrumentation</b>			
Roche Cobas Pro vs Roche Cobas c701	0.997	0.971 (0.944 – 0.997)	-3.08 (-5.52 – -0.642)
<b>Ortho Clinical Diagnostics Instrumentation</b>			
Ortho Vitros 350 vs Ortho Vitros XT3400	0.998	0.953 (0.924 – 0.983)	11.9 (0.182 – 23.7)
Ortho Vitros 4600 vs Ortho Vitros XT3400	0.999	1.02 (1.0 – 1.03)	2.60 (-4.90 – 10.1)
Ortho Vitros 4600 vs Ortho Vitros 350	0.998	1.07 (1.03 – 1.10)	-10.1 (-23.1 – 2.85)
<b>Siemens Healthineers Instrumentation</b>			
Siemens Atellica vs Siemens Dimension EXL	0.998	0.153 (0.150 – 0.156)	11.6 (10.0 – 13.1)
<b>Across Vendor Correlations</b>			
Roche c701/Pro vs Ortho Vitros 350/4600/XT3400	0.995	0.235 (0.228 – 0.243)	6.15 (2.74 – 9.56)
Roche c701/Pro vs Siemens Atellica	0.999	1.37 (1.35 – 1.39)	-17.0 (-18.7 – -15.3)
Roche c701/Pro vs Siemens Dimension EXL	0.999	0.209 (0.206 – 0.212)	-1.21 (-2.91 – 0.496)

**Conclusions:**

Strong within and between vendor correlations were observed for all plasma lipase assays ( $r > 0.99$ ). This will enable the implementation of provincial lipase correction factors for harmonized reported results and facilitate future reference interval harmonization.

A-071

A Pre-treatment free and Highly Selective Chromogenic Research Use Only (RUO) assay for Zinc deficiency on the Abbott Alinity c System

L. Ruvuna, J. Brady, N. Vondra, S. Ali, S. Brophy. *Abbott Laboratories, Abbott Park, IL*

**Background** Zinc is a critical nutrient for enzyme cofactors, metabolic functions, and immune system support. Zinc deficiency occurs in patients with inadequate nutrition and certain disease states such as Crohn's disease, Sickle Cell disease, liver, and kidney diseases. Labs often use Inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectroscopy (AAS) to test for zinc, which can require sending samples to a secondary location. Commercially available zinc assays provide a faster alternative, but many require additional preparation steps which can impact lab efficiency. This includes pre-treatment such as deproteinization and centrifugation of all samples, in addition to standard sample processing. Other offerings may require extensive reagent preparation, or recalibrations with each change in bottle. A new zinc research use only (RUO) assay was developed that is fully automated, without any sample or reagent pre-treatment steps, for the Abbott Alinity c System and provides a result within 10 minutes.

**Methods** Nitro-PAPS (2-(5-Nitro-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl) amino]phenol) is a dye that binds to heavy metals such as iron, cobalt, copper, and zinc. To utilize Nitro-PAPS as the capturing agent for zinc, chelators were added to the Reagent 1 to remove or mask other potentially interfering metals. Several chelators

were screened, and the concentrations optimized to balance assay performance and reduce metallic interferences. Nitro-PAPS enters the reaction as part of the Reagent 2 addition, and the Nitro-PAPS-Zinc complex is measured at 572 in an end up reaction. The concentration of zinc is proportional to the signal of the complex.

**Results** The observed precision was  $\leq 3.4\%$  CV when measured above 60  $\mu\text{g/dL}$  and 2.53 SD below 60  $\mu\text{g/dL}$ . The recovery of the NIST618a zinc standard was within 7%. The limit of detection was 6.2  $\mu\text{g/dL}$ , and the limit of quantitation was 15.3  $\mu\text{g/dL}$ . The linear range was up to 485  $\mu\text{g/dL}$ . The reagent on board stability was 14 days without recalibration. The interference levels for calcium, magnesium, and phosphate met the CLSI guidelines. Copper, Iron (II), Iron (III), and cobalt interference levels were  $\geq 500 \mu\text{g/dL}$ . A method comparison study vs a commercially available device demonstrated a slope of 0.9995 and correlation coefficient of 1.000. In addition, a slope of 0.9221 and a correlation coefficient of 0.999 was observed in a method comparison study with ICP-MS.

**Conclusion** A RUO zinc assay has been developed on the Abbott Alinity c System. The easy-to-use Abbott zinc assay demonstrated high analytical performance for precision, linearity, and sensitivity. Furthermore, the zinc assay demonstrates competitive metal ion interferences and high selectivity to Zinc.

**A-072**

**Clinical Impact of the Variability in eGFR values**

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**Background:** Estimated glomerular filtration rate (eGFR) is a calculation to estimate measured GFR, using serum creatinine, age, gender, and historically race. eGFR calculations can be based on serum measurements of creatinine or cystatin-C; creatinine methods can be enzymatic or picrate. The formulas vary, commonly used is CKD-EPI eGFR 2009 and less commonly CKD-EPI 2012 eGFRcys and eGFRcr-cys. Recently the NKF/ASN Task Force recommended the use of an equation without race variables (CKD-EPI eGFR\_refit 2021) for the primary determinant of eGFR; eGFRcys and eGFRcr-cys calculations were recommended “in complex clinical presentations.” In individuals with chronic kidney disease (CKD) the eGFRcys calculation can be comparable to that of eGFR with creatinine, confounders are inflammation, obesity, and diabetes. Serum Creatinine methods show interference with elevated glucose (picrate) and elevated hemolysis (enzymatic). The goal of this study was to assess method (serum creatinine picrate, creatine enzymatic, and cystatin-C) and calculation variability to determine how they impact CKD staging. **Methods:** The creatinine values were performed on an Abbott Alinity Chemistry instrument with the Alinity c Creatinine and Creatinine (Enzymatic) Reagents; the Gentian Cystatin-C Immunoassay was run on an Abbott Architect e8000. Precision studies were performed with Biorad Liquid Assayed Multiquant material. Six months of historical quality control (QC) results were assessed in light of the new method criteria ( $\leq 3.2\%$ ). A precision study was performed over 6 non-consecutive days. Remnant specimens (n=96) were collected; serum values from them were used in following eGFR calculations: CKD-EPI eGFR2009, CKD-EPI eGFRcys, CKD-EPI eGFRcr-cys, CKD EPI eGFR\_refit 2021. eGFR calculations were performed with both creatinine methods to determine overall variability; all calculations were compared to CKD-EPI2009 eGFR picrate calculation. CKD staging was determined utilizing the NKF scale for CKD. **Results:** The monthly historical CV for both picrate and enzymatic methods was  $<3.2\%$  for the high QC. The monthly CV of the low QC exceeded the 3.2% threshold on twice for both methods (3.8-4.6%). The intra-day precision was  $<1.2\%$  for picrate and  $<1.4\%$  for enzymatic methods; inter-day precision was  $<2.5\%$ . The creatinine based eGFR calculations were performed with each method. The different eGFR values were compared back to eGFR CKD-EPI 2009 Creatinine Picrate values (eGFR 2009-p). The eGFR CKD-EPI2009-enz was statistically different from eGFR 2009-p with a mean bias of 8 mL/min/1.73m<sup>2</sup> (two-sided t-Test p<0.0001). The eGFR CKD-EPI 2021\_Refit with picrate values was statistically different from eGFR 2009-p using the same values. Overall the changes were small (less than 10 mL/min/1.73m<sup>2</sup>). Four of the 96 samples changed by three stages, with the biggest change in eGFR being from 16 (eGFRcys) to 89 (eGFR 2021-enz). **Conclusion:** Utilization of different eGFR equations on the same sample can yield results that alters the CKD stage of an individual. Providers should be cognizant of the equations and the effect the equation may have on CKD staging. The equation could place the patient in a higher risk or lower risk of renal failure and affect the treatments recommendations. As such, establishment of an universal eGFR equation is paramount to reduce error in care.

**A-073**

**Comparison Between Electrochemiluminescence (COBAS e801/Roche) And Chemiluminescence (ADVIA Centaur XP/Siemens) Methodologies in the Dosage of Samples With Hyperprolactinemia**

C. O. Sabino<sup>1</sup>, D. Waltrick<sup>2</sup>, G. Zampieri<sup>2</sup>, J. S. Filletti<sup>2</sup>, B. S. Santos<sup>2</sup>, L. Rodrigues<sup>2</sup>. <sup>1</sup>DASA, Sao Paulo, Brazil, <sup>2</sup>Dasa, São Paulo, Brazil

**Background** The diagnosis of hyperprolactinemia always implies a laboratory investigation. To evaluate the efficiency of the analysis methodologies, we carried out a comparative study between two prolactin assays: Centaur/Siemens and Cobas/Roche. **Methodology** Patients who underwent prolactin measurement over a 3-month period on the Cobas/Roche and Centaur/Siemens platform whose measurements were within the linearity limit of each method were evaluated. Cobas: 40,287 women and 10,804 men had prolactin up to 470 ng/mL, in a total of 51,091 patients. Centaur: 48,496 women and 19,938 men had prolactin up to 200 ng/dL, in a total of 68,434 patients. Complementing the study, 43 samples of prolactin above the normality RV of the Cobas/Roche assay (23.3ng/mL) were selected. Dosing was then performed on the Centaur/Siemens platform. Subsequently, the samples were precipitated with PEG to verify the presence of macroprolactin. **Results** According to these data, it was found that around 30% of patients on the Roche platform and 6% on the Siemens platform had results above the reference limit of each methodology. The prevalence of hyperprolactinemia in general population is 0.1%. Macroprolactinemia accounts for about 10% of cases of hyperprolactinemia. In the PEG precipitation study, of the 43 samples with hyperprolactinemia measured on the Cobas platform, 9 samples were within normal limits for the Centaur/Siemens platform (20%). In the macroprolactin investigation of these 9 samples, 3 were positive (33%). Of the 34 samples altered in both methodologies, there was agreement in 28 cases (82%). 2 results were positive in Cobas and indeterminate in Centaur (6%). The incidence of macroprolactin in the Cobas/Roche trial was 19% and, in the Centaur/Siemens trial was 7%. **Conclusion** The Cobas/Roche assay presents more interference from macroprolactin, requiring research with PEG precipitation, a fact that must be considered by the laboratory when choosing the methodology.

ASSESSMENT 3 MONTH PERIOD - AUGUST TO OCTOBER 2021					
COBAS - ROCHE			CENTAUR - SIEMENS		
FEMININE	NUMBER OF PATIENTS	%	FEMININE	NUMBER OF PATIENTS	%
RESULT UP TO 470ng/mL	40287	100	RESULT UP TO 200 ng/mL	48496	100
RESULT UP TO 23.3ng/mL	29325	72.8	RESULT UP TO 29.2ng/mL	45701	94.2
RESULT ABOVE 23.3ng/mL	10962	27.2	RESULT ABOVE 29.2ng/mL	2795	5.8
MASCULINE	NUMBER OF PATIENTS	%	MASCULINE	NUMBER OF PATIENTS	%
RESULT UP TO 470ng/mL	10804	100	RESULT UP TO 200ng/mL	19938	100
RESULT UP TO 15.2 ng/mL	7442	68.9	RESULT UP TO 17.7 ng/mL	18787	94.2
RESULT ABOVE 15.2ng/mL	3362	31.1	RESULT ABOVE 17.7ng/mL	1151	5.8
TOTAL SAMPLES ABOVE VR PER MONTH	4775 SAMPLES		TOTAL SAMPLES ABOVE VR PER MONTH	1316 SAMPLES	
TOTAL SAMPLES ABOVE VR PER DAY	159 SAMPLES		TOTAL SAMPLES ABOVE VR PER DAY	44 SAMPLES	

**A-074**

**Determination of the functional linearity of BHCG assay**

C. Sabino, D. Waltrick, G. Zampieri, B. Santos, J. Filletti, J. F. de Souza, L. Rodrigues. DASA Medical Group, Sao Paulo, Brazil

**Background** Due to the need for precision in negative BHCG results, verification of the functional sensitivity of the Centaur/Siemens assay was performed. According to the test protocol, informed by the supplier on the package insert, the measurement range is: 2.0 to 1000mIU/mL and the analytical sensitivity is 2.0mIU/mL. Expected values: Non-pregnant women and men up to 2.0 mIU/mL. Postmenopausal women up to 6.0 mIU/mL. **Methods** Sample was used (pure, around 20 mIU/mL) with a possible result of reaching the sensitivity reported in the package insert after the sequence of dilutions following the proportions 10%/20%/30%/40%/50%/60%/70 %/80%/90%, in triplicate. Complementing the evaluation, a repeatability study was carried out with the linearity value proposed in the package insert and the one found in the work. **Results** The study demonstrated a minimum linearity value close to 4.0 mIU/mL. The repeatability evaluation was carried out with samples of 4.0 and 2.0 mIU/mL, with a coefficient of variation of 14% and 53% respectively (maximum allowed CV of 20%). **Conclusion** According to our studies, the lower limit of linearity of the BHCG assay of the Centaur/Siemens assay is 4.0 mIU/mL for non-pregnant women and men. This new limit reduces the need for repetitions in the investigation of indeterminate values.

STUDY OF DILUTIONS						
Samples	Dosage 1	Dosage 2	Dosage 3	Average Result observed	Result Expected	% Recovery
PURE	22,0	21,4	23,9	22,433	22,000	101,97
0,9	22,4	21	21,4	21,600	19,800	109,09
0,8	18,5	17,4	18,1	18,000	17,600	102,27
0,7	16,1	14,3	15,5	15,300	15,400	99,35
0,6	11,7	14,8	14,2	13,567	13,200	102,78
0,5	11,3	13	11,5	11,933	11,000	108,48
0,4	8,8	8,7	8,4	8,633	8,800	98,11
0,3	7,7	6,8	6,9	7,133	6,600	108,08
0,2	4,3	5,4	4	4,567	4,400	103,79
0,1	0,5	0,5	0,7	0,567	2,200	25,76

DETERMINATION OF FUNCTIONAL SENSITIVITY (LoQ)			
Determinations	Results	Determinations	Results
Dosage 1	5,2	Dosage 1	1,1
Dosage 2	4,7	Dosage 2	1,8
Dosage 3	5,1	Dosage 3	2,6
Dosage 4	5,2	Dosage 4	1,5
Dosage 5	5,4	Dosage 5	2,1
Dosage 6	4,1	Dosage 6	1,1
Dosage 7	5	Dosage 7	0,2
Dosage 8	4,2	Dosage 8	0,9
Dosage 9	4,3	Dosage 9	0,6
Dosage 10	3,3	Dosage 10	1,5
Average	4,65	Average	1,34
SD	0,66	SD	0,71
%CV	14,24%	%CV	53,26%

Acceptability Limit CV 20%

A-075

Performance Evaluation of Chek-Stix Liquid Quality Control across Siemens Healthineers Urinalysis Systems

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**Background:** Chek-Stix® Liquid Quality Control is a liquid, ready-to-use control material that requires no reconstitution and encompasses all Siemens-Healthineers urinalysis reagents and systems, including chemistry, sediment, and hCG tests. This QC is for the CLINITEK Status+, CLINITEK Advantus®, and CLINITEK Novus® systems and for the Atellica® UAS 800 Urine Sediment Analyzer.\* In this poster, precision and shelf-life stability characteristics are reported.

**Methods:** Reproducibility was evaluated by testing four lots of Level 2-positive quality control materials on the CLINITEK Novus, CLINITEK Status+, and CLINITEK Advantus analyzers. A total of 20 replicates (4 lots and 5 replicates per lot) were used to calculate the reproducibility for each analyzer. Reproducibility was determined by calculating the percent exact block agreement of recovered results against the assigned values for each analyte. This control has a shelf life of 22 months from date of manufacture when stored at 2-8 °C. Shelf life was determined by both accelerated and real-time testing. Testing was conducted with fresh product compared to product that was aged 6 months past the 22-month shelf life (~28 months) for all analyzers except for CLINITEK Novus analyzer, in which fresh product was compared to product aged 22 months.

**Results:** The reproducibility of all urine chemistry parameters was 100% exact block agreement across all instruments. Passing results from controls aged 28 months in real time demonstrate that the 22-month shelf life is adequate.

**Conclusion:** The data shows the new Chek-Stix Liquid Quality Control for urinalysis has a reproducibility of 100% exact block agreement. Data also indicated the shelf life of this quality control material is 22 months from the date of manufacture when stored at 2-8 °C and 30 days when stored at room temperature (up to 30°C). After opening, the Chek-Stix quality control material is stable until the expiration date stated on the label when stored appropriately and returned to 2-8 °C between uses.

\*The Atellica UAS 800 analyzer is not available for sale in the U.S.A. Product availability may vary from country to country and is subject to varying regulatory requirements.

Auto-Checks, CLINITEK, CLINITEK Advantus, CLINITEK Status, CLINITEK Novus, Multistix, POCelevator, RAPIDComm, Chek-Stix, Atellica, 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.

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

Chek-Stix® Liquid Quality Control is under development and not commercially available. Its future availability cannot be ensured.

A-076

Analytical Performance Assessment of a Newly Formulated, IFCC traceable Alkaline Phosphatase Assay for Abbott's ARCHITECT c and Alinity c Systems

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**Background:** Alkaline Phosphatase assay is used as an aid in the diagnosis and treatment of liver, bone, parathyroid and intestinal diseases.

**Methods:** Alkaline Phosphatase in a sample catalyzes the hydrolysis of colorless para-nitrophenyl phosphate (p-NPP) to give para-nitrophenol (yellow phenoxide form at alkaline pH) and inorganic phosphate. The rate of absorbance increase at 404 nm is directly proportional to the alkaline phosphatase activity in the sample. Optimized concentrations of zinc and magnesium ions are present to activate the alkaline phosphatase in a plasma or serum patient sample.

**Results:** Advantages of the new formulation are addition of an IFCC traceable calibrator, which yields a Total Allowable Error of 3.3% to 4.1%. Additionally, surfactant was incorporated to mitigate potentially interfering substances. Those include Acetaminophen 160 mg/L, Acetylcysteine 150 mg/L, Acetylsalicylic acid 30 mg/L, Ampicillin-Na 80 mg/L, Ascorbic acid 60 mg/L, Biotin 4250 ng/mL, Ca-dobesilate 60 mg/L, Cefotaxime 60 mg/dL, Cefoxitin 6600 mg/L, Cyclosporine 2 mg/L, Desacetylcefotaxime 6 mg/dL, Doxycycline 20 mg/L, Ibuprofen 220 mg/L, Levodopa 8 mg/L, Magnesium sulfate 50 mg/dL, Methyl dopa 25 mg/L, Metronidazole 130 mg/L, Phenylbutazone 330 mg/L, Rifampicin 50 mg/L, Sodium heparin 4 U/mL and Theophylline (1,3-dimethylxanthine) 60 mg/L, which all showed no significant interference (within ± 10%). The Analytical Measuring Interval (AMI) is 9 - 4522 U/L. Method comparison on ARCHITECT c to predicate Alkaline Phosphatase yields a slope of 1.00 and correlation coefficient of 1.00 for samples in the range of 14 - 4830 U/L.

**Conclusion:** The ARCHITECT c and Alinity c Alkaline Phosphatase2 assay is a robust, IFCC traceable assay with expanded interferent performance while maintaining linearity up to 4522 U/L, which reduces the need for dilutions and repeats.

A-077

Longitudinal Analysis of Characteristics Associated with Variable Antibody Response to BNT162b2 Vaccination Among Healthcare Workers over Ten Months

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**Objectives.** We sought to understand the demographic and clinical factors associated with variations in longitudinal antibody response following completion of 2-dose regimen of BNT162b2 vaccination. **Design.** This study is a 10-month longitudinal cohort study of healthcare workers and serially measured anti-spike protein IgG (IgG-S) antibody levels, using mixed linear models to examine their associations with participant characteristics. **Setting.** Large multi-site academic medical center in Southern California. **Participants.** A total of 843 healthcare workers met inclusion criteria including completion of an initial two-dose course of BNT162b2 vaccination, complete clinical history and at least 2 blood samples for analysis. Patients had an average age of 45±13 years, were 70% female, and 7% with prior SARS-CoV-2 infection. **Results.** Vaccine induced IgG-S levels remained in the positive range for 99.6% of individuals up to 10 months after initial 2-dose vaccination. Prior SARS-CoV-2 infection was the primary correlate of sustained higher post-vaccination IgG-S levels (partial-r<sup>2</sup>=0.133), with a 1.74±0.11 SD higher IgG-S response (P<0.001). Female sex (beta 0.27±0.06, P<0.001), younger age (0.01±0.00, P<0.001), and absence of hypertension (0.17±0.08P=0.003) were also associated with persistently higher IgG-S responses. Notably, prior SARS-CoV-2 infection augmented the associations of sex (-0.42 for male sex, P=0.08) and modified the associations of hypertension (1.17, P=0.001), such that infection-naïve individuals with hypertension had persistently lower IgG-S levels whereas prior-infected individuals with hypertension exhibited higher IgG-S levels that remained augmented over time (Figure 1). **Conclusions.** While the IgG-S antibody response remains positive for up to 10 months following initial mRNA vaccination in most adults, determinants of sustained higher antibody levels include prior SARS-CoV-2 infection, female sex, younger age, and absence of hypertension. Certain determinants of the longitudinal antibody response appear significantly modified by prior infection status. These findings offer insights regarding factors that may influence the 'hybrid' immunity conferred by natural infection combined with vaccination.

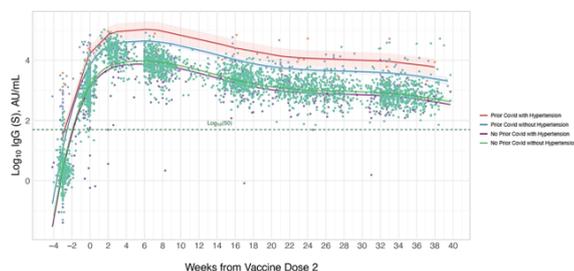


Figure 1: Longitudinal trajectory of IgG-5 antibody levels following completed BNT162b2 vaccination by prior infection and hypertension status. Multivariable-adjusted longitudinal trajectories are shown for individuals with a history of prior COVID-19 infection for those without prior COVID-19 infection, including an interaction for sex. Longitudinal estimates with 95% confidence limits (shaded areas) are adjusted for age and sex.

## A-078

### Improved hemoglobin interference performance on newly formulated Cholesterol assay for Abbott's ARCHITECT and Alinity c systems.

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**Background:** Cholesterol is a required structural component of cell membranes and necessary for other bodily functions such as production of bile, vitamins and hormones. Total cholesterol values are used to determine the risk of heart diseases and can serve as an indicator of liver and gall bladder function. A newly formulated cholesterol assay has been developed for the quantitative determination of cholesterol in human serum and plasma for Abbott's Alinity and ARCHITECT c Systems.

**Methods:** A newly formulated Cholesterol assay is a one-reagent photometric assay. Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase to cholest-4-ene-3-one and hydrogen peroxide. The hydrogen peroxide oxidatively couples with N,N-Bis(4-sulfobutyl)-3-methylaniline (TODB) and 4-aminoantipyrine to form a chromophore (quinoneimine dye) which is quantitated at 604 nm. The assay has a concentrated reagent formulation which improves the number of tests per wedge and minimizes frequent kit changes. The assay is traceable to the National Reference System for Cholesterol, the AbellKendall reference method in a CDC-Certified Cholesterol Reference Method Laboratory Network (CRMLN)

**Results:** This new Cholesterol assay has limit of detection of  $\leq 2.0$  mg/dL and  $\leq 3.0$  mg/dL for ARCHITECT and Alinity c systems, respectively. The limit of quantitation was  $\leq 5.0$  mg/dL and total within laboratory imprecision was  $\leq 3.0\%$  on both ARCHITECT and Alinity c systems. This assay demonstrated linearity up to at least 743 mg/dL following CLSI EP06-A guidelines. The assay has reagent onboard stability of 30 days with calibration curve stability of 30 days. This newly formulated assay shows significantly improved robustness to hemoglobin interference of 1000 mg/dL at cholesterol levels 150 mg/dL and 220mg/dL in serum. Correlation to previous version of the assay showed a slope of 0.97 with correlation coefficient of 1.0 for the serum samples across the range. The analytical measuring interval (AMI) was observed from 5.0 to at least 743 mg/dL for both systems. The assay has an auto-dilution feature which allows Reportable Interval (RI) of at least 3 to 2972 mg/dL. Accuracy of a newly formulated Cholesterol assay showed a bias of  $\leq 2.3\%$  when evaluated against Abell-Kendall reference method.

**Conclusions:** A new analytically robust Cholesterol assay was designed for use on Abbott's ARCHITECT and Alinity c Systems with exceptional hemoglobin interference performance.

## A-079

### Fast method of quantitative analysis of serum vitamin E ( $\alpha$ -tocopherol) using LC-MS/MS

M. O. Soares, F. P. Mazete, D. R. Ramadan, S. Tufik, E. K. Sugawara. *AFIP, São Paulo, Brazil*

**Background:** Vitamin E is the term for a set of chemical compounds with antioxidant properties. The most important form for humans is called  $\alpha$ -tocopherol. One of its main functions is the protection of polyunsaturated fatty acids, vitamins, hormones and antioxidant enzymes. Vitamin E deficiency is often associated with disorders of fatty acid metabolism that decrease lipid absorption. Symptoms can include edema

and hemolytic anemia (seen in premature and newborn babies) and injury to striated muscles (adults). Vitamin E is well tolerated, however, its excess can lead to reduced absorption of other fat-soluble vitamins. The aim of the study was to validate an easy and fast preparation method for the analysis of  $\alpha$ -tocopherol.

**Methods:** Validation was performed using a Xevo TQS micro tandem mass spectrometry sensitive liquid chromatography (LC-MS/MS). Analytical specificity is ensured using multiple reaction monitoring with fragmented ions that are exclusive to  $\alpha$ -tocopherol, quantifier ion 431.4 > 165.1 and qualifier ion 431.4 > 83. The procedure involves a small amount of 50  $\mu$ L of serum, followed by liquid-liquid extraction. The samples are then submitted to reverse phase separation on a Zorbax Eclipse Plus C8<sup>®</sup> analytical column (50 x 2.1 mm, 1.8 $\mu$ m). Mobile phase was A: 2 mM ammonium acetate/ 0.1% formic acid (aq) B: 2 mM ammonium acetate/ 0.1% formic acid/ MeOH, using a gradient with an initial ratio of 40:60 (v/v) with a flow rate of 0.3 ml/min. Quantitation is achieved by the comparison the responses of a given sample with the responses of calibrators with known concentrations. Linearity was observed in the expected concentration range from 2.0 to 64.0 mg/L and serum samples were evaluated six different concentrations and six times each at the same time point of the study. Albumin was used as a biological matrix for the study,  $\alpha$ -tocopherol standard (Sigma - Aldrich).

**Results:** The coefficient of determination (R<sup>2</sup>) of linearity was 0.993851. The method showed 97.16% of selectivity and interference of residual effect of less than 5%. In order 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 1.01, 2.53, and 1.89%, respectively. The accuracy of the method was checked by analyzing samples of known concentration and expressed as a percentage. A retention time (RT) of 2.8 min was obtained and the total analysis time was 3.5 min.

**Conclusion:** The method was fast and efficient for the determination of serum  $\alpha$ -tocopherol. The efficiency and selectivity allied to the technical robustness can be used in the diagnosis of nutritional disorders.

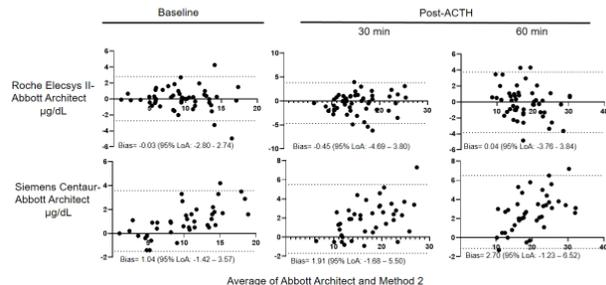
## A-080

### New Diagnostic Cutoffs for Adrenal Insufficiency after Cosyntropin Stimulation using Abbott Architect Cortisol Immunoassay

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**Introduction:** Accurate interpretation of the cosyntropin (ACTH) stimulation test requires method and assay-specific cutoff values of cortisol. Compared with the historical cutoff (18  $\mu$ g/dL) from polyclonal antibody-based immunoassays, lower thresholds (12.6-14.6  $\mu$ g/dL) were proposed for the Roche Elecsys II assay that uses a monoclonal antibody. However, cutoffs for other commonly adopted monoclonal antibody-based cortisol assays were not yet available. Here we compared cortisol measurements post ACTH stimulation on three immunoassays and established the peak cortisol thresholds specific to the Abbott Architect immunoassay. **Methods:** The standard-dose (250  $\mu$ g) cosyntropin stimulation test was performed in patients with suspected adrenal insufficiency (AI) (n=50). Serum cortisol was measured with Abbott Architect, Roche Elecsys II, and Siemens Advia Centaur cortisol assays. Deming regression and Bland-Altman analysis were performed to assess the agreement between Roche Elecsys II and Abbott Architect, and between Siemens Centaur and Abbott Architect assays. Results from Abbott Architect were also compared with those from liquid chromatography-tandem mass spectrometry (LC-MS/MS). Receiver operating characteristic (ROC) analysis was performed to derive new diagnostic thresholds for the Abbott Architect assay. **Results:** Cortisol measurements on the Abbott Architect were similar to those from LC-MS/MS and Roche Elecsys II, but significantly lower than Siemens Centaur (Figure 1). The optimized cortisol thresholds on Abbott Architect were 14.3  $\mu$ g/dL at 60 minutes after stimulation (sensitivity 100%; specificity 95%) and 13.2  $\mu$ g/dL at 30 minutes (sensitivity 100%; specificity 89%). In comparison to the historical threshold of 18  $\mu$ g/dL, applying the new cutoff may significantly decrease false positive results from ACTH stimulation testing. **Conclusion:** We recommend a cortisol threshold of 14.3  $\mu$ g/dL at 60 minutes post ACTH stimulation for the Abbott Architect assay. Applying assay-specific cortisol cutoffs will be essential to reduce misclassification and overtreatment in patients with suspected AI.

Figure 1: Bland-Altman plots of serum cortisol by Abbott Architect, Roche Elecsys II, and Siemens Centaur. LoA: limits of agreement.



## A-081

### Improved accuracy across the analytical measuring range for the newly formulated Calcium assay on Abbott's ARCHITECT and Alinity c Systems

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**Introduction:** Calcium is one of the most important minerals in the body that is needed for healthy bones and teeth and is essential for proper functioning of nerves, muscles, and heart. It is used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease, and intermittent muscular contractions or spasms. The newly formulated Calcium Assay has been developed with optimal Arsenazo-III dye concentration and pH for quantitation of calcium in human serum, plasma, or urine for the Alinity and ARCHITECT c Systems.

**Methods:** The Calcium assay is designed as a one-reagent photometric assay for quick turnaround time. Calcium binds to Arsenazo-III dye to form a blue-purple complex that is measured at 660 nm and is proportional to the calcium concentration in the sample. In this study, various calcium formulations with varying pH were manufactured and evaluated for performance on Alinity c.

**Results:** The newly formulated Calcium Assay with optimal Arsenazo-III dye concentration and pH has been designed with improved accuracy to the NIST 956 standard. The concentrations of NIST 956 Standard Levels 1 and 2 used to assign calibrator values read back within  $\pm 1\%$  of the target NIST concentrations. As a result of accurate calibrator value assignment, the overall % recoveries of Maine Standards Validate linearity samples, GC1 1100ab, and serum samples tested across the measuring interval of the assay (3 mg/dL - 18 mg/dL) are  $\leq 2\%$  when tested with multiple lots of Arsenazo-III dye. In addition, % deviations of linearity of samples tested near the medical decision points of the assay, 9 mg/dL and 12 mg/dL, are  $\leq \pm 1.4\%$ . Minimal lot to lot variability of Arsenazo-III dye was also established by testing serum samples (range: 3 mg/dL - 10 mg/dL) resulting in  $\leq 0.7\%$  difference for three reagent lots. The imprecision of samples tested between 2 mg/dL and 19 mg/dL resulted in  $\leq 2.1\% CV$ .

**Conclusions:** An analytically robust Calcium Assay was designed with optimal Arsenazo-III dye concentration and pH to be used on Abbott's ARCHITECT and Alinity c Systems with superior accuracy across the analytical measuring range of the assay.

## A-082

### Stability Performance Impact of Clinical Chemistry Enzyme Assays Prepared in Stainless Steel Containers

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**Introduction:** Stainless Steel (SS) containers are commonly used to manufacture clinical chemistry reagents. In this study, we investigated the shelf-life reagent stability impact of SS and other containers, glass, and polypropylene, used in manufacturing of two Clinical Chemistry reagents that contain enzymes in their formulations, Creatine Kinase (CK) and Enzymatic Creatinine (CreEnz).

**Method:** Reagents for CreEnz and CK were prepared in SS, glass, and polypropylene containers. To test the shelf life stability of reagents, a test method that stresses the reagents at a high temperature (37°C) for days to weeks was employed. For this accelerated stability model, two levels of commercially available controls were tested on reagents stored at 2-8°C on an automated clinical chemistry analyzer. Reagents

were then stored at 37°C and were used to test the controls at various time points. The %difference from baseline was calculated for each time point to assess the impact of containers.

**Results:** For the CK assay, reagents were prepared in glass and SS containers, and for the CreEnz assay, reagents were prepared in glass, SS, and polypropylene containers. Accelerated stress study at 37°C was performed and %difference analysis was done to evaluate reagent stability in different container types. For the CK assay, the %difference of SS conditions exceeded the acceptance criteria on Day 14 compared to the glass condition lasting to Day 18. For the CreEnz assay, the %difference of SS conditions exceeded the acceptance criteria on Day 2 compared to the polypropylene condition lasting to Day 41. **Conclusion:** Manufacturing CK and CreEnz reagents in SS decreased their shelf-life stability as demonstrated in a thermal stress model. It is likely that enzymes present in the reagents may be hindered by interactions with components of SS (e.g. iron, chromium), thereby resulting in reduced reagent stability. Our study investigated two assays that employ enzymatic reactions; however, the negative impact of SS may be generally applicable to other similar enzyme comprising clinical chemistry assays. For example, studies with the Lactic Acid reagent showed similar findings.

## A-083

### Analyte Dependent Performance Characteristics of Immunoassays for the Detection of U1RNP Antibody in the Evaluation of Connective Tissue Disease

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**Background:** Antibody to U1RNP is an important serologic marker for the diagnosis of mixed connective tissue disease (MCTD) and can also be seen in patients with systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and idiopathic inflammatory myositis (IIM) referred here as U1RNP-associated connective tissue disease (U1RNP-CTD). Analytes for detecting anti-U1RNP antibodies may include purified extracts (sm/RNP), recombinant proteins, synthetic peptides, or dominant epitopes of the three main proteins [RNP68 or RNP70, RNPA (33 kD), and RNPC (22 kD)] either singly or in any various combinations using different types of immunoassays. Despite the use of different analytes or combination of antigens, the nomenclature, reporting and interpretation of anti-U1RNP antibodies remains obscure. This investigation was performed to determine the clinical impact of using RNP68/C and Sm/RNP analytes in patient under clinical evaluation for connective tissue disease (CTD).

**Methods:** Two multiplex assays, Sm/RNP (TheraDiag) and RNP68/C (BioPlex) were used to test 498 consecutive patient serum specimens referred to ARUP Laboratories evaluation from the University of Utah Health. Available specimens positive in any of the two multiplex assays were tested for Sm/RNP by BioPlex (n=42) or ELISA (n=47) methods. Results were evaluated for antibody positivity by analyte and method of detection, and impact on clinical diagnoses through retrospective chart review for positive patients was performed.

**Results:** Of the 498 patients, 47 (9.44%) were positive in the RNP68/C (BioPlex) and 15 (3.01%) in the sm/RNP (TheraDiag) immunoassays, all 15 Sm/RNP antibody-positive patients were positive in the RNP68/C assay. The frequency of Sm/RNP in the BioPlex and ELISA was 15/42 (35.71%) and 17/47 (36.17%), respectively. Of the 47 patients, U1RNP-CTD was diagnosed in 16, other CTD in 6, and no CTD in 25 cases. The prevalence of antibody was 100% (RNP68/C), 92.31% (Sm/RNP, BioPlex), 81.25% (Sm/RNP, TheraDiag), and 87.5% (Sm/RNP, Inova) in those with U1RNP-CTD. For other CTDs and no CTD, the highest prevalence was observed with the RNP68/C, all others had comparable performance.

**Conclusion:** In this investigation, the Sm/RNP assays show comparable performance characteristics when compared to the RNP68/C immunoassay. Additional studies to characterize RNP68/C for optimal interpretation are warranted.

## A-084

## Validating Extension of Serum Creatine Kinase Clinically Reportable Range through Automated Dilution Using CLSI Approach

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**Background:** Creatine Kinase (CK) is a biomarker commonly used to assess muscle damage, such as in myocardial infarction, rhabdomyolysis and cerebral diseases. Particularly in severe conditions of muscle necrosis, intracellular muscle constituents including CK and myoglobin are released into the blood circulation, with the latter increasing burden to the kidneys leading to potential kidney failure if not appropriately treated.

In severe muscle disease or injury, it is not uncommon for CK values to exceed greater than 50 times the upper limit of normal values (10,000 to 20,000 U/L). Our laboratory reports CK values up to 20,000 U/L. Unquantifiable elevated CK values exceeding laboratory's clinically reportable range (CRR) of 20,000 U/L accounts for 0.25% (n = 179) of 71,585 reported CK results in 2019, and 0.57% (n = 393) of 68,752 reported CK results in 2021. Serial CK values can be useful in determining and monitoring the course of muscle disorder and its treatment effectiveness. Monitoring of patients with unquantifiable elevated CK levels thus becomes a clinical conundrum. The laboratory has received increasing feedback from our hospital's clinicians on requiring "absolute" CK values in such clinical events. This has prompted the laboratory to review the CRR of CK.

The laboratory embarked on a systematic approach based on Clinical and Laboratory Standards Institute (CLSI) guidelines to extend the CRR through automated dilutions on the laboratory's automated chemistry analyzers.

**Methods:** CK tests are performed on both Beckman Coulter AU5800 and Roche Cobas c502 using imidazole buffer method standardized against International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Extension of CRR was evaluated using the CLSI guidelines EP34Ed1E - Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking. Five patient serum samples with CK results above 70% of the analytical measuring range (AMR) of each analyzer were analyzed neat, in triplicates using automated on-instrument dilution, and in triplicates using manual dilution. On-board instrument dilutions were performed at dilution factors 10 and 50 using 0.9% NaCl saline as the diluent. Accuracy of dilution was assessed by comparing neat and average recovery of the dilutions.

**Results:** Automatic on-board instrument dilution on Beckman AU5800 showed a mean recovery of 106.1% (range: 105.3% to 106.6%) at factor 10 dilution, and mean recovery of 109.7% (range: 106.2% to 111.8%) at factor 50 dilution across all samples. Analysis on Roche c502 showed a mean recovery of 104.3% (range: 103.7% to 105.0%) at factor 10 dilution, and mean recovery of 102.0% (range: 101.4% to 102.4%) at factor 50 dilution. All recovery results were well-within our laboratory's acceptance criteria of 27.5%.

**Conclusion:** We have successfully evaluated the automatic on-board instrument dilution of CK with higher dilution factors, allowing for the extension of CRR for CK up to 100,000 U/L on both Beckman and Cobas platforms. Extension of the reportable range of CK values will thereby aid clinicians in monitoring the effectiveness of treatment on patients with high levels of CK. This systematic approach can also be employed to other analytes of interest requiring an extension of CRR.

## A-085

## Clinical Performance Evaluation of the cobas pulse System

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**Background:** The demand for improved analytical performance of glucose testing in point-of-care hospital environments is increasing. The cobas pulse is a new strip-based blood glucose monitoring system (BGMS), intended for prescription point-of-care testing in professional healthcare settings. This study was conducted to evaluate the performance of the new cobas pulse BGMS, which includes the cobas pulse instrument, test strips, QC kit, and linearity kit, with multiple patients from intensive critical and non-critical care settings, using venous, arterial, capillary, neonatal arterial, and neonatal heel stick whole blood samples.

**Methods:** There were a total of 17 external sites (14 US and 3 European sites) and 128 self-trained operators performing testing. The study protocol was approved by each site's Institutional Review Board/Ethics Committee in accordance with FDA and local regulatory requirements, and written informed consent was obtained for each subject before enrollment. Samples were analyzed using the cobas pulse and a commercially available point-of-care device (Nova StatStrip Glucose Meter) as the predicate device. These results were compared to those of the plasma-hexokinase test on the cobas 6000, which was used as the comparator method. Accuracy was assessed according to FDA guidance document 1755 acceptance criteria. The method comparison involved 2678 samples from 1577 patients with and without diabetes, from diverse hospital and outpatient departments, including patients receiving intensive medical intervention, to best reflect the intended use environment of the instrument. In addition to glucose, specimen pO<sub>2</sub>, hematocrit, and sodium concentrations were measured and each subject's medical diagnoses and medications were collected. Surveillance Error Grids (SEGs) were used to assess the significance of the difference between the cobas pulse system result and the comparator sample result for each sample type.

**Results:** Accuracy requirements were met for split-sample arterial vs plasma arterial and venous vs plasma venous comparisons. The neonatal arterial data, which were analyzed separately, also met all acceptance criteria for accuracy. Neonatal heel stick vs plasma heel stick samples met the first accuracy requirement (i.e., at least 95% of results within 12 mg/dL, or 12%); 96.6% of results were within 15 mg/dL or 15%, with a 75 mg/dL breakpoint. The performance of the cobas pulse system with heel stick samples (and for all other same sample comparisons) was better than that of the predicate device. The results of the cobas pulse system for capillary whole blood compared to plasma from a concomitant venous reference sample were equivalent to those obtained with the predicate device. Bench study results confirmed the highly accurate performance of the cobas pulse system with samples contrived to achieve extreme glucose concentrations, as well as with samples contrived to simulate high hematocrit and low glucose values characteristic of neonate samples. No significant interference

from hematocrit, oxygen, or sodium values has been identified. **Conclusion:** The results obtained in this multicenter evaluation confirm the accuracy of the cobas pulse system when used in the multiple professional health care environments and patient populations outlined in the intended use. From the SEGs, none of the results showed clinically significant medical risks.

### A-086

#### Multi-Hospital Laboratory Derivation of Beckman High Sensitivity Troponin I Reference Change Value

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##### Background:

Reference change values (RCV), the smallest changes in a laboratory test that signal a statistically significant change are usually derived from estimates of analytical variation ( $CV_A$ ) and biological variation ( $CV_I$ ). Serial differences between inpatient consecutive measurements can be transformed into Taylor series of variation vs time with the intersection at time=0 ( $y_0$ ) equal to the total variation (analytical + biological + preanalytical). With small preanalytical variation,  $y_0$ , expressed as a percentage of the mean, is equal to the variable component of the RCV calculation:  $(CV_A^2 + CV_I^2)^{1/2}$ . We present the RCV summaries of hsTnI results from Beckman Dxl 800 using the standard deviation of duplicates (SDD) approach (Clin Chem 68:4 1-9 2021).

##### Methods:

We determined the between day RCV of patient data for hsTnI and compared them to literature. We analyzed 418 consecutive days of Beckman Coulter Dxl 800 hsTnI data from 9 urban and suburban hospitals in Edmonton, Alberta. The serial patient hsTnI values (n=16071 pairs) were transformed into 95% 2-sided RCV. RCV from 24 hour were calculated from SDD approach.

##### Results:

The 24 hour RCV is around 30% for clinically relevant hsTnI levels (<10 ng/L to <60 ng/L).

##### Conclusion:

Our RCV values for hsTnI of 30% are consistent with the literature throughout the relevant hsTnI concentration. Estimating RCV for hsTnI may help to inform proper troponin delta values based on patient data for rapid rule in/out acute myocardial infarction.

### A-087

#### Multicenter Evaluation of SARS-CoV-2 Serology Assays Shows Differential Response Between Inpatients, Outpatients, and Long Term Care Residents

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**Background:** The measurement of antibodies against SARS-CoV-2 has garnered high interest, but its utility remains to be established. While there were some recommendations on where serology testing may be useful, further research is required to better understand the immune response against SARS-CoV-2 infection. This study evaluates the serologic response to SARS-CoV-2 infection using different commercial assays across different patient populations.

**Methods:** Eleven hospital and community laboratories across Canada contributed serology results for a total of 1,962 samples from 1,678 individuals collected from inpatients, outpatients and long-term care (LTC) residents. The samples were collected in 2020 prior to vaccine availability. Anti-SARS-CoV-2 antibodies were measured using four qualitative assays from Abbott, Roche, Ortho, and Siemens. Not all samples were tested on all assays. Antibody response over time and between patient populations were examined. **Results:** Differences in antibody response were observed between outpatients and LTC residents versus inpatients when measured using the Abbott anti-nucleocapsid IgG assay. Sustained antibody levels over time for inpatients,

outpatients and LTC residents was observed up to 16 weeks post-diagnosis. A small subset of inpatients was monitored over a longer period and had detectable antibodies up to ten months post-diagnosis, measured by all four assays. Inpatients had higher antibody response when measured with the Abbott assay compared to outpatients and LTC residents. The Roche anti-nucleocapsid total antibody assay showed inpatients and community patients had comparable antibody response to approximately eight weeks.

**Conclusion:** Antibody profiles varied across different patient populations and did not always display parallel changes among assays. Antibodies were detected for up to 10 months post-diagnosis among inpatients. Differences in antibody kinetics between inpatients, outpatients, and LTC residents warrant assay performance validation for specific patient populations.

### A-088

#### Evaluation of a Urine and CSF Pyrogallol Red Total Protein Assay for Use on the Binding Site Optilite Analyser

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**Background:** Quantification of total protein in cerebrospinal fluid (CSF) is a useful marker for disorders that cause increased permeability of the blood/brain barrier or synthesis of immunoglobulins in the central nervous system. Increased levels in urine can indicate decreased protein reabsorption or increased glomerular filtration in the kidney; as well as being a marker for post-renal proteinuria which can be caused by infections, bleeding, or malignant diseases of the urinary tract. Here we describe the evaluation of a pyrogallol red dye binding total protein assay for measurement of urine and CSF samples using the Binding Site's Optilite<sup>®</sup> analyser. The instrument is a random-access turbidimetric analyser, capable of a throughput of up to 120 tests per hour and on-board sample dilutions up to 1/10,000. Single-use cuvettes are employed to promote precision, which are automatically loaded and disposed of. Standardised assay calibration is upheld through traceability to the NIST-SRM927 reference standard. The assay utilises a single point calibration curve, with a standard measuring range of 40-1306.8mg/L and sensitivity of 40mg/L. High samples are automatically re-measured at a dilution of 1/11, with an upper limit of 7128mg/L. **Method and Results:** The colorimetric limit was determined to be >9000mg/L through extension of the calibration curve with dilutions of the NIST-SRM927 standard. Linearity studies have been performed using urine spiked with normal human serum (EP06-ED2) and synthetic CSF spiked with human serum (EP06-A), both of which demonstrated a linear range of at least 36mg/L - 1438mg/L. Precision studies were performed (EP05-A3), by testing 4 urine and 2 CSF levels on a single analyser over 20 days, across three kit lots over 5 days, and across three instruments over 5 days. All samples reported ≤1.7% within run CV, ≤2.0% between-lot CV, ≤4.2% between-instrument CV, and ≤4.1% total CV. External quality assessment (EQA) testing has been performed to assess accuracy for urinary and CSF total protein measurement using the CAP, UK NEQAS, INSTAND, and RfB (CSF only) urine chemistry and CSF protein schemes. Good agreement was observed when Optilite<sup>®</sup> data was analysed by Passing-Bablok regression comparing to the EQA assigned values;  $y=1.066x-19.55$  (n=80). Interference testing was performed (EP07-A3) at two urine concentrations, challenging with 14 interfering substances, including magnesium chloride (400mg/dL), urea (25g/L), urobilinogen (45mg/L) and uric acid (300mg/dL). Testing was also performed at two CSF concentrations challenging with 5 interfering substances including ascorbic acid (200mg/L) and acetaminophen (3mg/dL). All results were <10% different when compared to equivalent negative controls. **Conclusion:** This total protein assay for use on the Optilite<sup>®</sup> provides a reliable and precise method for quantifying total protein in both urine and CSF sample matrices and correlates well with existing methods.

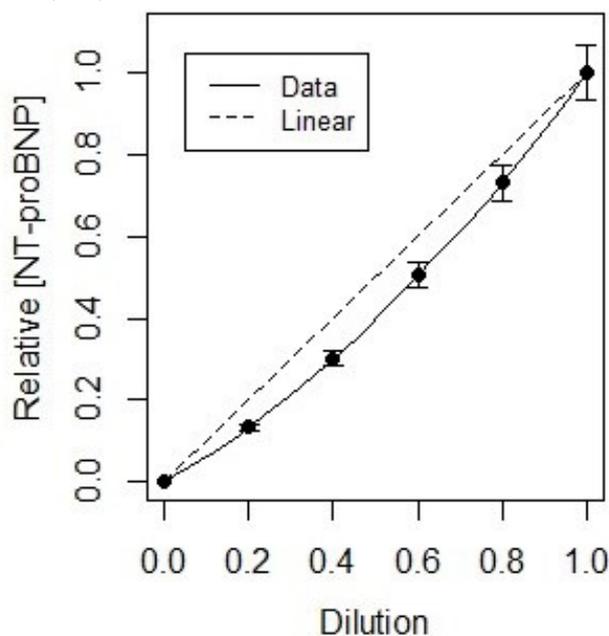
### A-089

#### CAP linearity surveys for NT-proBNP: nonlinear results for the Roche STAT assay

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**Background:** CAP proffers a calibration verification/linearity survey for NT-proBNP. Our survey results for Roche NT-proBNP STAT have occasionally been flagged as nonlinear while passing calibration verification. To investigate, we analyzed the pattern of the dilution curve, as reported for the survey peer group, for comparison to results expected for linear dilution/response curves. **Methods:** Peer group summary data from the CAP LN-30B 2021 survey were used (measured result vs. dilution factor). The data comprised 6 data points of evenly-spaced dilutions (1.0, 0.8, 0.6, 0.4,

0.2, 0). A primary characteristic of linearity is that the slope of the data should be constant. To estimate slopes, we calculated the value of  $\Delta(\text{result})/\Delta(\text{dilution})$  for adjacent data points (5 data intervals across 6 data points). **Results:** The primary data underestimate the theoretical linear (target) curve at all points other than the endpoints (dilutions 0 and 1) (see Figure). Deviations from linearity were up to 22% at 1:5 dilution. The slope of response curve decreased progressively with dilution. Normalized slopes ranged from 1.35 to 0.66 across dilutions, rather than the expected constant value of 1.0. Estimated slopes ( $y$ ) were essentially linear with dilution ( $x$ ):  $y = 0.8353x + 0.5807$ ;  $r^2 = 0.994$ . Importantly, results parallel those from preceding CAP surveys. These findings are inconsistent with linearity. Results from Vitros, EXL and VISTA did not display this nonlinear response in the same survey report, nor did our results using Roche's linearity set. **Conclusions:** In a recent CAP NT-proBNP linearity survey, results for the Roche NT-proBNP STAT assay were patently non-linear, in contrast to results for other NT-proBNP assays. Calibration for the Roche assay does not appear to accurately reflect actual concentration vs. response curves across the presumed "linear range" (5-35,000 pg/mL). However, for concentrations <25,000 pg/mL, errors of measurements are nonetheless within acceptable limits as specified by CAP ( $\pm 25\%$ ).



#### A-090

##### Development and Assessment of a New Assay for B-hydroxybutyrate on the HORIBA Yumizen C1200 Chemistry Analyzer.

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**Background:** Ketosis results from the increase of ketone bodies in blood due to a decreased use of carbohydrates for energy metabolism. Severe cases of ketosis can result in ketoacidosis, a serious, life-threatening condition. Diabetes mellitus and alcohol abuse are the most common causes of ketoacidosis in adults. According to the most recent Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus, specific measurement of  $\beta$ -hydroxybutyrate can be used for the diagnosis and monitoring of diabetic ketoacidosis. We have developed a test method for the quantification of  $\beta$ -hydroxybutyrate and applied it to the Yumizen C1200 analyzer.

**Methods:** This  $\beta$ -hydroxybutyrate method utilizes  $\beta$ -hydroxybutyrate dehydrogenase for the enzymatic, quantitative analysis of  $\beta$ -hydroxybutyrate.  $\beta$ -hydroxybutyrate in the sample, in the presence of NAD and  $\beta$ -hydroxybutyrate dehydrogenase, is converted to acetoacetate and NADH at a pH of 8.5. The generation of NADH causes an increase in absorbance measured at 340nm. The increase in absorbance at 340nm is proportional to the concentration of  $\beta$ -hydroxybutyrate in the sample. Analysis of performance on the Yumizen C1200 was based on modified versions of applicable CLSI protocols. Accuracy was assessed by comparing results from the Yumizen C1200 to those of a commercially available product performed on the Hitachi 717 analyzer. Within Run and Within-laboratory imprecision were determined by running three levels of control material. Within Run imprecision was determined using 20 replicates in

a single day. Within-laboratory imprecision was determined by running the controls in duplicate across 12 shifts. Linearity was assessed by running a series of diluted samples in triplicate and performing regression analysis. Interference from hemolysis, triglycerides, and bilirubin were determined using a cutoff of 10% from expected value for the presence of interference.

**Results:** The comparison study yielded  $r^2=0.998$  and a regression equation of  $y = 0.968x - 0.05$ . Within Run imprecision showed CVs of 2.7%, 2.3% and 2.1% on samples of 0.181 mM, 1.02 mM, and 4.04 mM, respectively. Within-laboratory imprecision showed CVs of 8.8%, 5.4 % and 5.3% on samples of 0.177 mM, 1.00 mM, and 3.96 mM, respectively. The assay demonstrated linear response from 0.0 to 4.2 mM with an  $r^2$  of 0.994. Minimal interference (<10%) was noted for hemoglobin up to 500 mg/dL, triglycerides up to 1000 mg/dL, and bilirubin up to 80 mg/dL.

**Conclusion:** Analytical results achieved on the HORIBA Yumizen C1200 indicate that this assay for  $\beta$ -hydroxybutyrate provides comparable results to a commonly used commercial assay. Within Run and Within-laboratory imprecision results for the reagent and analyzer combination indicate acceptable reproducibility. The assay provides a robust linear range and demonstrates resistance to the most common endogenous interferences. Based on these results, this  $\beta$ -hydroxybutyrate product, when performed on the Yumizen C1200 analyzer, is suitable for clinical use.

#### A-091

##### Surprising Findings Using Continuous Temperature Monitoring

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**Background:** To assess compliance with regulatory accreditation criteria, the ability of refrigerators and freezers distributed throughout a large academic medical center to maintain acceptable temperature ranges for storage of laboratory materials was assessed using a continuous temperature monitoring system. **Methods:** Temperature data was evaluated for seven refrigerators and seven freezers over 214 days of operation. Temperature recordings were obtained using a continuous temperature monitoring system (Viewpoint, Mesa Labs), and temperatures were recorded every fifteen minutes. Acceptable ranges for refrigerators and freezers were established based on requirements of materials stored within the equipment. Temperature means, standard deviations and coefficients of variation were calculated for each device over the 214-day period. **Results:** The ability of equipment to maintain the acceptable temperature range varied substantially by manufacturer and equipment type. Short-term temperature excursions were more common than expected, and were consistent with routine compressor cycling. Refrigerator/freezer combination units demonstrated the largest amount of temperature variation. High-performance equipment and large walk-in refrigerators had the lowest amount of temperature variation.  $-20^\circ\text{C}$  freezers had significant variation and temperatures were often warmer than  $-20^\circ\text{C}$ , even after optimization of temperature settings. Corrective actions include relocation of critical materials to equipment with acceptable variation and replacement of inadequate equipment. Preliminary analysis indicates that the majority of daily temperature excursions were less than 30 minutes in duration, and reflected routine compressor cycling. **Conclusion:** Continuous temperature monitoring data highlighted significant variation associated with multiple laboratory refrigerators and freezers, resulting in brief temperature excursions. Combination units had the poorest performance. Laboratories should implement continuous temperature monitoring or recording of minimum and maximum temperatures to verify that acceptable temperatures are consistently maintained. Laboratorians should closely evaluate equipment manufacturer's temperature range specifications and equipment quality before implementation. Future investigations include evaluation of brief temperature excursion effects on reagent and control material stability.

Equipment/Manufacturer/Type	Acceptable Range (°C)	214 Day Min/Max (°C)	Diff Max-Min (°C)	214 Day Min/Max (%CV)	#Days with Excursions (Low Range/High Range)
Cardinal/Combination Unit (Refrigerator)	1 to 8	1.3/ 11.0	9.7	18.0/ 26.7	0/72
General Electric/Mini Refrigerator	2 to 8	4.6/ 7.3	2.7	6.0/ 5.9	0/0
Cardinal/Combination Unit (Refrigerator)	1 to 8	-0.3/ 9.2	9.5	50.4/ 23.4	16/14
Thermo/Sliding Door Freezer	2 to 8	3.2/ 4.5	1.3	2.7/ 5.0	0/0
Kendro/Sliding Door Freezer	2 to 8	2.2/ 10.2	8.0	8.6/ 2.0	0/214
Gloekler/Walk-In Refrigerator	2 to 8	4.2/ 6.6	2.3	1.8/ 4.5	0/0
Elliott-Williams/Walk-In Refrigerator	2 to 8	2.3/ 5.8	3.5	10.8/ 3.3	0/0
Harris/Ultra Low Freezer	-90 to -60	-75.9/ -64.4	11.5	0.7/ 1.2	0/0
Thermo/Ultra Low Freezer	-69 to -50	-78.9/ -57.5	21.4	3.3/ 2.4	0/0
Cardinal/Combination Unit Freezer	-35 to -10	-27.5/ -5.3	22.2	7.8/ 16.4	0/11
Thermo /Single Door Freezer	-30 to -15	-21.3/ -0.7	20.6	2.7/ 12.0	0/30
Marvel/Single Door Freezer	-30 to -10	-26.9/ -10.2	16.7	4.6/ 10.6	0/2
Gloekler/Walk-In Freezer	-40 to -18	-21.9/ -18.5	3.4	0.5/ 1.0	0/0
Elliott-Williams/Walk-In Freezer	-40 to -20	-26.7/ -11.2	15.4	3.5/ 7.2	0/214

**A-092**

**Implementation and Assessment of a SmartZone Alert to Notify Clinicians of Critical Hyperbilirubinemia in Preterm Infants Less Than 35 Weeks Gestation**

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**Background:** Neonatal jaundice, also known as hyperbilirubinemia in term and pre-term infants, is treated with phototherapy when bilirubin results exceed gestational age- and age-specific medical decision levels (MDL) to prevent kernicterus and bilirubin-induced neurological damage. During phototherapy, unconjugated bilirubin is converted to water-soluble isomers that are excreted in the urine. Presently, the electronic medical record (EMR) at Children’s Hospital Los Angeles (CHLA) cannot use gestational age to stratify reference ranges thereby the associated flags and alerts would not be triggered, leading to delays in reviewing bilirubin results and placing phototherapy orders. The aim of this project is to replace the current manual assessment process for phototherapy with a newly designed alert to notify clinicians of elevated bilirubin results for preterm infants (<35 weeks gestation) and to minimize prolonged delays in placing phototherapy orders. We hypothesized that the SmartZone alert will encourage consistent and timely consideration of phototherapy.

**Method:** A SmartZone alert, built with Cerner command language and Discern Expert rules, evaluates total or neonatal bilirubin results for patients in the Newborn and Infant Critical Care Unit (NICCU), calculates the patient’s age using the recorded gestational age and birth time, and utilizes the following rules: 1) bilirubin result must exceed the pre-defined gestational age-specific MDLs, and 2) calculated gestational age is <35 weeks. Additionally, the SmartZone alert will not be triggered if there is an active phototherapy order. Once triggered, the alert will be visible for four hours to all caregivers who view the patient’s EMR. We compared the duration of time between verified bilirubin results and phototherapy orders before and after the implementation of the SmartZone alert.

**Result:** The SmartZone alert was implemented on 01/11/2022. Between 1/1/2021 - 1/11/2022, 95 preterm infants in the NICCU had neonatal and total bilirubin results, in which 19 met the criteria for the alert to be triggered, and 17 had phototherapy orders. Further analysis revealed that 12 orders were placed <24 hours, four orders were placed 24 - 72 hours, and one order was placed >72 hours after bilirubin results

were verified. Between 1/12/2022 – 2/13/2022, 13 newly admitted preterm infants in the NICCU had neonatal and total bilirubin results, in which one triggered the alert, and phototherapy was ordered within 15 hours.

**Conclusion:** A SmartZone alert was implemented to encourage consistent and timely consideration of phototherapy for preterm infants <35 weeks with critical hyperbilirubinemia. The alert is functioning as intended. This alert will be continuously monitored to further optimize its functionality and utilization.

**A-093**

**Implementation of High-sensitivity Troponin Improve ED Discharge Time at Multiple Facilities in a Hospital System in Central Pennsylvania**

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**Background:** High sensitivity troponin (hs-cTn) allows for earlier detection of troponin elevations. Therefore, it enables clinicians to more accurately identify patients with myocardial infarction, as well as rule out patients who do not have myocardial infarction. However, it was not clear how the use of troponin would improve patient throughput at the real life busy emergency departments (EDs).

**Methods:** We evaluated the impact of hs-cTn implementation at two of our busiest hospitals (300-600 beds) in the health system. hs-cTn was initially set up with the 0-3 hour diagnostic protocol in September 2017. In September 2018, a 0-1-3 hour diagnostic protocol was introduced in the hope to improve discharge time at the emergency rooms. Discharge time (as defined as triage end to disposition time) was analyzed in three phases - (1) January to August 2017: pre-hs-cTn implementation; (2) September 2017 to August 2018: 0-3 hour hs-cTn protocol; (3) September 2018 to October 2019: 0-1-3 hour hs-cTn protocol.

**Results:** A total of 15382 encounters in the chest pain patient group was analyzed. The median of discharge time for the chest pain patients with hs-cTn testing was reduced from 256 min to 205 min; and from 219 min to 196 min at the two busy EDs with the implementation of 0-1-3 hour hs-cTn protocol, and no significant change with the 0-3 hr hs-cTn protocol. A control group (non-chest pain patient group) of 158,814 encounters was also analyzed and show insignificant change of discharge time.

	Hospital 1				Hospital 2			
	Control		Chest pain patients with hs-cTn testing		Control		Chest pain patients with hs-cTn testing	
	N	Median ED discharge time	N	Median ED discharge time	N	Median ED discharge time	N	Median ED discharge time
Baseline – pre-hs-cTn phase	17134	147 min	1478	256 min	22467	124 min	2227	219 min
Phase1 (0-3 hour hs-cTn protocol)	25014	145 min	2244	251 min	35366	125 min	3653	210 min
Phase 2 (0-1-3 hour hs-cTn Protocol)	24330	140 min	2200	205 min	34503	136 min	3580	196 min

**Conclusion:** Implementation of 0-1-3 hr hs-cTn can improve throughput at busy EDs.

**A-094**

**Elevated serum uric acid level as a cost-reliable biomarker of tumor progression in patients with Non-Small Cell lung cancer**

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**Background:** The measurement of human serum uric acid level is a routine clinical chemistry test, performed in the blood samples of oncology department patients. Secondary hyperuricemia has known as an indicator of chronic inflammation, associated with malignancy and a dismal prognosis. This research has been conducted to clarify the prognostic effect of the increased uric acid level in cancer patients and verify the hypothesis of a strong prediction link between elevated uric acid level and recurrence or metastases in patients with Non-Small Cell Lung Cancer (NSCLC) receiving anticancer therapy.

**Methods:** The observational study fulfilled data of patients with localized primary or secondary tumor foci in the lungs in the 3 years amid overall 44 patients included. Data were collected from patients with NSCLC receiving first and second lines of treatment. Of the selected groups of patients, the first group received immunotherapy, the second group underwent targeted therapy and the third group received a combination of targeted therapy plus chemotherapy. Enzymatic color test for the quantitative determination of uric acid in human serum on Beckman Coulter AU-680 analyzer has been performed on 398 samples. The established reference intervals for women are 2.6-6.0 mg/dl and 3.5-7.2 mg/dl for men. Tumor response was assessed according to RECIST 1.1 criteria based on computed tomography data. **Results:** Of the study population, 13 (29.5%) were female and 31 (70.5%) were male patients. Among 44 patients included, 8 subjects were on targeted therapy, 14 received a combination of targeted therapy plus chemotherapy and 22 patients received immunotherapy. A significant increase of uric acid above the reference level was revealed in 36.7% of the samples and a total of 54% of the patients (95% confidence interval: 4.22-7.39). No difference in gender or age has been found in the following outcomes. Patients with auspicious treatment termination did not have registered episodes of elevated levels of uric acid in the 3-year follow-up observations (p = .011). Meanwhile, the increased level of serum uric acid was recorded in patients with exacerbation in the next cycle of chemotherapy or later on (p = .025).

**Conclusion:** As a result of the data processed, has been discovered a correlation between an increased level of uric acid and the severity of chronic inflammation associated with a malignant process. To conclude, regular assessment of serum uric acid levels is an available basic procedure, which can be easily implemented in routine follow-ups, and may serve as a sign for earlier imaging tests.

### A-095

#### The Role of RNase L in Metabolic Syndrome

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**Background:** Metabolic syndrome (MetS) is a set of risk factors that increase the likelihood of developing cardiovascular disease, type 2 diabetes, and nonalcoholic fatty liver disease. One of the major causes in the development of MetS is due to dysfunction of insulin signaling, resulting in persistent hyperglycemia. Binding of insulin to its receptor initiates a signaling cascade, leading to the translocation of GLUT4, a glucose transporter that facilitates uptake of glucose predominantly by muscle and adipose cells. However, the molecular mechanism underlying insulin resistance is largely unknown. RNase L is an interferon inducible enzyme that plays a critical role in degrading viral and cellular RNA for host-defense immunity. Recently, we made interesting observations that RNase L deficiency attenuated the activation of insulin signaling. **Methods:** RNase L in human liver SK-Hep-1 cells were knocked out by using the CRISPR/Cas9 method. Primary mouse embryonic fibroblasts (MEFs) were generated from RNase L knockout and wild type mice. The cells were treated with 100 ng/ml insulin for various times. Activation of the insulin signaling was assessed by analyzing the protein levels and phosphorylation status of the downstream components in the pathway by using western blot analyses. Glucose uptake was measured by using a fluorescent glucose analog. **Results:** The phosphorylation status of insulin receptor-β (IR-β) and its downstream component IRS1 was significantly reduced in RNase L deficient SK-Hep-1 cells. Similar results were obtained in MEFs. The phosphorylation of the IRS activates a signal transduction cascade that leads to the activation of other kinases, such as the PI3K/AKT/mTOR signaling pathway, that mediate the intracellular effects of insulin. Upon the insulin stimulation, the activation of AKT was markedly inhibited in RNase L deficient both types of cells. Most interestingly, lack of RNase L decreased the uptake of glucose and translocation of GLUT4. **Conclusion:** Our study suggests that RNase L plays a role in glucose metabolism by affecting insulin sensitivity. These findings suggest that RNase L may be a novel target in drug design and a predictive marker for MetS.

### A-096

#### Anti-Covid Antibody Generation After Vaccination and Natural Infection Over Time

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**Background:** covid vaccination strategies remain an effective modality to combat covid infection and mRNA mediated candidates, among others, have been approved for dispensation. However, the relationship of anti-covid antibody generation in the setting of natural infection post vaccination remain of interest. **Methods:** a healthy

(PCR and antigen negative) volunteer received the Pfizer mRNA vaccine in two doses separated by 21 days as recommended by the manufacturer. Blood samples were collected prior to immunization and every 10-25 days through day 380. Natural (Omicron) covid infection (nucleocapsid antigen positive) occurred at day 332 and the presence of IgG anti-SARS CoV-2 antibodies were assessed (Beckman DXI 800. Reactivity: IgG ≥10 arbitrary units [AU]/mL). Some samples were drawn as both serum and plasma aliquots for comparison. Results: No detectable antibodies were present in pre-vaccination samples. IgG anti-covid antibody levels, post vaccination series, were at their highest at Day 30 (76 IU/ml) and nadired at day 318 (1.8 AU/mL). Interestingly, post covid infection (day 332) IgG anti-covid antibody levels increased to 160 AU and 212 AU at day 360 and 380 respectively. Antibody levels assessed in serum and plasma were comparable. **Conclusions:** mRNA vaccination promoted initial IgG anti-covid antibody production which nadired over time. Natural infection promoted a robust memory response that exceeded the maximum anti-covid IgG antibody responses obtained from the initial vaccination series. These data demonstrate the importance to assess antibody production in those infected with natural infection post vaccination facilitating robust memory responses as a measure of augmented protection against this disease.

### A-100

#### Indirect Reference Intervals of Serum Calcium in Pakistani Children & Adolescents - A Comparison between the KOSMIC, Bhattacharya and Hoffman Methods

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**Background:** Reference intervals (RIs) are methodology, population and age specific. However, the ethical and practical challenges restrict the establishment of pediatric serum Calcium (Ca) RIs using conventional approaches advocates the use of indirect data mining-based algorithms. This study was carried out to estimate the RIs of serum Ca using three indirect methods. **Methods:** A data mining of serum Ca results ranging from birth till 18 years of age from 2013-2018 was done. Three subgroups on the basis of age from birth to 1 year, 2 to 4 years and 5 to 18 years were assessed as defined previously by Tahmasebi et al in the CALIPER cohort based on the Siemens Advia 1800 analyzer. The German study group's pre-validated indirect algorithm 'KOSMIC', Bhattacharya and Hoffman methods were utilized for the analysis. **Results:** A total of non-duplicate 40914 serum Ca tests were retrieved over a period of 6 years, including 38.7 % (n=15830) aged birth to 1 year, 16.3% (n=6641) 2 to 4 years, and 45.2 % (n=18443) 5 to 18 years respectively. The three methods revealed comparable performance with the direct RIs reported by Tahmasebi et al in the CALIPER cohort as shown in Table 01.

Age groups	Tahmasebi, Houtman, et al. (mg/dl)	Our Study (KOSMIC) (mg/dl)	Our Study (Hoffman) (mg/dl)	Our Study (Bhattacharya) (mg/dl)	Absolute % difference KOSMIC & Tahmasebi et al		Absolute % difference Hoffman & Tahmasebi et al		Absolute % difference Bhattacharya & Tahmasebi et al	
					LRI	URI	LRI	URI	LRI	URI
Birth to 1 year	9.5-11.5	8.5-11.2	8.3-10.6	8.1-11.3	-11.1	-2.6	-13.5	-8.1	-15.9	-1.8
2-4 years	9.5-10.8	8.6-10.6	8.7-10.2	8.5-10.6	-9.9	-1.9	-8.8	-5.7	-11.1	-1.9
5-18 years	9.1-10.2	8.5-10.5	8.7-10.1	8.4-10.5	-6.8	-2.9	-4.5	-1.0	-8.0	2.9

**Conclusions:** The study advocated the use of alternative validated indirect RI establishment tools, for generation of population specific RIs specially for resource constraint set up to aid clinical decision making. RI predicted by three indirect methods showed a variable degree of biases. However, it could be attributable to the population related differences.

### A-101

#### Is the Prolactin Value above which the Hook Effect may be induced in the Roche Elecsys Prolactin Assay consistent with the Package Insert?

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**Background:** In the diagnostic approach of hyperprolactinemia, the hook effect can be a potential confounding problem that deserves special attention. It is characterized by the finding of falsely low levels of serum prolactin (PRL) when two site immunoassays are used in diagnosis in patients with very high PRL levels. The hook effect should be excluded in patients with pituitary macroadenoma and PRL levels < 250 ng/

mL. Generally, the serum PRL values above which the hook effect may be induced are given in the package inserts of the immunoassay kits, and it depends on each particular assay. The Roche Elecsys Prolactin II package insert says that no hook effect was observed at prolactin concentration up to 12,690 ng/mL. The objective of this study was to evaluate if the hook effect in PRL measurements in Elecsys Prolactin II is consistent with the value described in the package insert, 12,690 ng/mL. **Methods:** We analyzed blood samples requesting serum PRL levels from patients admitted to a private reference clinical laboratory in Brazil, from 01/01/2016 to 12/31/2021. Anonymized data on laboratory tests was available from a database of the local Laboratory Information System. All the patients included in our study had PRL levels above 1,000 ng/mL (ECLIA, Roche). We evaluated the first result obtained by the equipment for each patient and verified if they were above 470 ng/mL, indicating dilution, not experiencing hook effect. **Results:** 701 patients were evaluated, 370 (53%) men, 331 (47%) women; mean age 39 (14 to 88) yrs. They were divided in six groups according PRL levels: 1,000 to 2,000 ng/mL – 316 (45,1%); 2,001 to 3,000 ng/mL – 124 (17,7%) ; 3,001 to 4,000 ng/mL – 72 (10,3%); 4,001 to 5,000 ng/mL – 167 (23,8%); 5,001 to 10,000 ng/mL – 17 (2,4%) and 10,001 to 16,968 ng/mL – 5 (0,7%) patients. All the patients had the first result above 470 ng/mL, with serial dilutions until the final result. **Conclusion:** The search for the hook effect is recommended for patients who have pituitary macroadenomas and apparently normal or mildly elevated prolactin levels. In this study we did not find hook effect in patients with prolactin levels up to 12,690 ng/mL, confirming what is reported in the Elecsys Prolactin II package insert.

**A-102**

**Pubertal development and growth in children with primary dyslipidemia**

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**BACKGROUND:** Despite several studies in the literature about dyslipidemia in children, there is a lack of knowledge regarding growth and sexual development in these patients. This study aims to describe the pattern of pubertal development and growth in children with primary dyslipidemia followed at a pediatric dyslipidemia referral center. **METHODOLOGY:** This is a cross-sectional and descriptive study. Children with primary dyslipidemia followed up between 2007 and 2021 were evaluated. These criterias were analyzed: 1. Height: assessed through the z score (normal height: z ± 2.0). 2. Body composition: assessed through the body mass index (obesity: < 5 yr with z score > +3.0 and ≥ 5 yr with z score > +2.0). 3. Staging pubertal: assessed by the Tanner stage, with the onset of puberty being normal in girls between 8 and 13 years old and, in boys, between 9 and 14 years old. **RESULTS:** Fourteen children were evaluated (7 with diagnosis of familial hypercholesterolemia and 7 with familial hyperchylomicronemia syndrome). Clinical-demographic data, presence of mutations, body mass index, stature and pubertal development pattern are described in table 1. The evaluation of growth parameters in this group did not identify cases of short stature attributed to the diagnosis of primary dyslipidemia. Two siblings (numbers 1 and 3) had short stature explained by the low parental target height and unrelated to primary dyslipidemia. The number 11 child presented a growth deceleration, probably related to recurrent episodes of pancreatitis. The number 5 child had a tall stature which can be explained by the condition of severe obesity also unrelated to primary dyslipidemia. No cases of precocious or delayed puberty were identified. **CONCLUSION:** In our study, primary dyslipidemia seems to have no impact on the potential for growth and pubertal development in the group of children in this case.

TABLE 1. Clinical and genetic characteristics of patients with primary dyslipidemia

PATIENT NUMBER	DIAGNOSIS	GENETIC MUTATION	AGE (YEARS) AT FIRST TEST	AGE (YEARS) AT LAST TEST	HEIGHT (Z SCORE) AT FIRST TEST	HEIGHT (Z SCORE) AT LAST TEST	BMZ (Z SCORE) AT FIRST TEST	BMZ (Z SCORE) AT LAST TEST	TANNER STAGE AT LAST TEST
174	PH	LDLR	1.01	10.01	1.21	1.11	-0.19	-0.75	1
174	PH	LDLR	1.08	8.05	1.10	1.14	-0.14	-0.50	1
174	PH	LDLR	4	10.08	2.41	2.11	-0.48	-0.60	1
147	PH	LDLR	6.71	8.1	0.44	-0.08	-0.1	-1.11	1
147	PH	LDLR	1.08	8.01	-1.01	-1.16	-1.06	-1.11	1
654	PH	Arsley mutation	8.21	8.21	-1.28	-1.28	-1.11	-1.11	1
104	PH	Arsley mutation	10.18	11.1	0.11	0.1	-0.08	-0.49	2
851	FDL	QPSBP1	0.08	0.18	0.78	0.78	-1.11	-1.11	1
81	FDL	LPL	7	10.18	-0.1	1.08	-1.48	-0.60	1
107	FDL	QPSBP1	1.01	1.71	1.11	1.14	-0.01	-0.08	1
117	FDL	Hmgcr	4.01	1.71	0.18	0.18	-0.08	-0.11	1
1034	FDL	Hmgcr	7	8.08	0.01	0.01	-0.48	-1.14	1
117	FDL	LPL	1.08	10.17	1.11	1.11	-0.08	-1.1	1
117	FDL	LPL	11	11.08	0.08	0.08	-0.11	-0.4	1

PH: familial hypercholesterolemia  
 FLDL: familial hypertriglyceridemia  
 QPSBP1: familial hypercholesterolemia  
 LPL: familial hypertriglyceridemia syndrome

**A-104**

**Impact of COVID-19 pandemic on serum vitamin D level**

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**Background:** The effects of vitamin D is unique among the vitamins. The combination of synthesis via UV action upon skin and its ability to act on specific target tissues make its classification as essential. The implementation of social distancing and home confinement after COVID-19 outbreak may elevate the risk of vitamin D deficiency. This study aimed to compare vitamin D level among overall ages from 2018 to 2019 (pre-pandemic period), and from 2020 to 2021 (during pandemic period).

**Methods:** This study included 363.158 results of vitamin D levels from individuals among overall ages who underwent health checks during 2018-2019 (N=172.180) .pre-pandemic - PP period; and those evaluated from 2020 to 2021, during pandemic - DP period (N=190.978). Total Vitamin D levels were measured using a chemiluminescent assay (Siemens®). R software was used for statistical analysis and p value < 0.05 was considered significant.

**Results:** The mean of serum vitamin D level was lower in DP period (26.90 ng/dL) than PP period (29.61 ng/dL, p<0.001). The mean in women PP (29.38 ng/dL) was lower than men PP (30.35 ng/dL, p<0.001). During the pandemic period, no difference was observed between women and men (p=0.902), but their vitamin D levels (26.89 and 26.91 ng/dL, respectively) were lower than values observed during PP period according to the gender (p<0.001).

**Conclusion:** The results showed that changes in lifestyle during the COVID-19 pandemic affected somehow the vitamin D levels among overall ages and gender. In PP period, women vitamin D levels were lower than men, but during pandemic period, no difference was reported between genders. Our data suggest a relationship between changes in social behavior, COVID-19 infection and vitamin D levels. However, the role of home confinement on vitamin D deficiency should be further evaluated.

**A-105**

**Sensitivity Gain and Immune Window Reduction with Automated Assay Improvement for SARSCoV2**

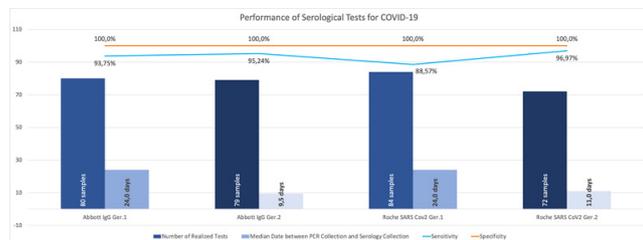
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**Background:** Since the emergence of the SARS-CoV-2 pandemic, there has been a need to diagnose the infection faster and better. Assays have emerged with improvements and new antigenic epitopes. We present our analyzes of technical validations of the serologies for COVID-19 of two different diagnostic sets in their two versions, in a period of 1 year, with the antigenic targets described in the Methods of this study, carried out by the Clinical Laboratory of the Hospital I in São Paulo, Brazil, since the beginning of the pandemic. Our objectives were to evaluate the differences in sensitivity of these diagnostic kits for the versions by manufacturer and to estimate the median of positivity between the PCR and serology collection dates.

**Methods:**The biological sample used was from the laboratory serum library. Negative samples before the pandemic and positive samples with a time interval between PCR and a known serum sample, shown in the graph, as well as the number of the realized tests. The methods used were Abbott SARS-CoV-2 IgG (1st Generation) automated chemiluminescence immunoassay (CMIA), for the qualitative determination of IgG antibodies against the nucleocapsid (N) protein of SARS-CoV-2; Abbott SARS-CoV-2 IgG (2nd Generation), for the quantitative determination of IgG antibodies against the receptor-binding domain (RBD) of the Spike (S) protein, in Architect (i2000SR). Quantitative test results were presented in BAU/mL; Automated Electrochemiluminescence Immunoassay (ECLIA) Roche SARS-CoV-2 Total (1st Generation), which uses the recombinant protein Nucleocapsid (N) in a sandwich technique for the qualitative detection of antibodies (including IgG) against SARS-CoV-2 and Roche SARS-CoV (2nd Generation), which uses the recombinant protein S-RBD in a sandwich technique for the quantitative detection of antibodies (including IgG) against SARS-CoV-2, on the Cobas e801 (Elecsys).

**Results:**figure

**Conclusion:**The comparison between the first- and second-generation assays showed an improvement in the sensitivity of the tests.



## A-106

### Configuration and Validation of 7 Open Channel Assays on the Roche cobas c503 Analyzer

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**Background:** Automation of assays in the clinical laboratory can be helpful to increase testing throughput, save time, and improve performance by eliminating variability and human error. Although a large portion of tests in a modern laboratory are already automated, there are many specialized assays that have yet to be adopted by major instrument manufacturers for their respective automated analyzers. There are, however, several clinical chemistry analyzers that have open channel (OC) or closed development channel configurations that allow use of third-party application parameters and reagents. The objective of this study was to evaluate the performance characteristics of 7 OC assays (topiramate, levetiracetam, lamotrigine, angiotensin converting enzyme, bile acids, urine citrate, and aldolase) configured on a Roche cobas c503 analyzer. **Methods:** Assay parameters were obtained from reagent manufacturers where available (ARK Diagnostics, Buhlmann Diagnostics Corp, Diazyme Laboratories). For assays that did not have c503 parameters available, assay type, sampling volume, reagent volume, incubation time, and photometric wavelengths were established based on parameters used on Roche cobas c502 and c702 analyzers and incorporated according to c503 instrument specifications. All OC assays were configured using the Roche cobas development channel file creator (version: 3.1.0.021). Modifications were incorporated, as needed, based on current laboratory procedures. Method comparison studies were conducted by running residual human serum or urine specimens (n=33-40) on validated c502 or c702 analyzers and comparing results to the c503. Linearity was assessed by using low and high concentration patient specimens to create percent splits (e.g. 0%, 25%, 50%, 75%, and 100%) spanning the reportable range for each assay. Intra-assay and inter-assay precision were evaluated by running 10 replicates of QC material in a single day or 20 replicates of QC material over 5 days, respectively. Limit of detection (LOD) was assessed by analyzing instrument responses for 10 replicates of water and 3 replicates of a low concentration specimen. Specimen carryover was evaluated by running high (H) and low (L) concentration specimens in a sequence (H, L, L, H, L, L, H, L, L, H, L, L) and calculating three standard deviation (SD) error limits of the 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, and 12<sup>th</sup> L specimens. The onboard dilution capability of each assay was evaluated by comparing manual dilution to the following instrument onboard dilution factors (dilution factor; assays): (x11; topiramate, levetiracetam, lamotrigine); (x3; angiotensin converting enzyme, urine citrate); (x1.5; bile acids); (x5; aldolase). **Results:** Method comparison for all assays showed acceptable correlation (r: 0.99 to 1.00; Slope: 0.96 to 1.07; Bias: -4.0% to 7.4%). Linearity was verified for all assays (%Recovery: 95.7% to 115.0%). All assays showed adequate intra-assay ( $\leq 4.0$  total %CV) and inter-assay precision ( $\leq 2.9$  total %CV). LOD calculated based on instrument responses were all below reagent manufacturer claims and lower reportable range of each assay. No carryover was observed. Percent differences between manual dilution to instrument onboard dilution results were acceptable (Average: -2.3 %Difference; Range: -10.4 to 4.8 %Difference). **Conclusion:** The 7 OC assays configured on the Roche cobas c503 analyzer demonstrated robust performance and were found to be acceptable for use.

## A-107

### Chymase truncation of apolipoprotein E in high-density lipoprotein is further promoted under myeloperoxidase oxidation

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**Background:** Apolipoprotein E (apoE), a 34-kDa glycoprotein, has antiatherogenic properties as part of high-density lipoprotein (HDL). In atherosclerotic artery walls, mast cells, inflammatory cells, are activated to secrete chymase. Chymase can truncate various HDL proteins in atherosclerotic lesions. The detection of truncated apolipoproteins can be a valuable biomarker of atherosclerosis progression. Previously, we have reported that C-terminally truncated apoA-I was found in human serum. On the other hand, other enzymes also coexist in the lesions. One of the prevalent enzymes is myeloperoxidase (MPO), which makes HDL dysfunctional by oxidation. We have also found that MPO-oxidation increased the apoA-I sensitivity to chymase. In this study, we focused on apoE containing HDL and investigated apoE fragmentation by chymase and modification of MPO. **Methods:** Blood samples were obtained from healthy volunteers. HDL (1.063 < d < 1.210 g/mL) was isolated from pooled human plasma by ultracentrifugation. HDL (2.0 mg protein/mL) was incubated with 20 nmol/L MPO, 0.1 mmol/L H<sub>2</sub>O<sub>2</sub>, 0.2 mmol/L diethylenetriamine pentaacetic acid and 0.4 mmol/L L-tyrosine in 20 mmol/L Tris-HCl (pH 7.4) containing 1 mmol/L EDTA-2K at 37°C for 2 hours. The treated HDL (1.0 mg protein/mL) was then incubated with 1 unit/mL chymase in 20 mmol/L Tris-HCl (pH 7.4) containing 1 mmol/L EDTA-2K and 150 mmol/L NaCl at 37°C for 24 hours. At the end of the incubation, the reaction was stopped by heat treatment at 100°C for 5 minutes. The identification of truncated apoE was analyzed by SDS-PAGE followed by western blotting and nanoscale liquid chromatograph-tandem mass spectrometer (nano LC-MS/MS). **Results:** ApoE was truncated into three types of fragments (approximately 20, 18, and 15-kDa) by chymase. The fragments of 20 and 18-kDa contained peptides on the N-terminal side of apoE, which was detected by nano LC-MS/MS analysis. The 15-kDa fragment contained peptides on the C-terminal side of apoE. In MPO-treated HDL, three apoE complexes were detected at over 40-kDa. Truncated apoE fragments were also generated by sequential treatment with MPO and chymase. Six types of fragmented apoE (5-kDa to 20-kDa) were detected, and all apoE complexes became almost undetectable under the sequential treatment. On the other hand, four types of truncated apoA-I (26, 24, 14.5, and 12-kDa) were confirmed, and no fragment of apoA-II could be identified under the sequential treatment. **Conclusion:** We have revealed that the truncation of apoE by chymase was further promoted under MPO oxidation. We speculated that apoE has more cleavage sites than apoA-I and apoA-II. In order to detect and measure these fragments in atherosclerotic lesions and plasma for predicting a risk of cardiovascular disease, we need to confirm the actual cleavage sites of apoE.

## A-108

### Body fluid source method validation on the Roche cobas pro ISE analytical unit

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**Background:** Most commercial FDA-cleared clinical laboratory assays are not validated by diagnostic manufacturers for non-serum, non-plasma, and non-urine specimens.<sup>1</sup> Yet, it is not uncommon for providers to request electrolyte analysis of body fluids. To meet this clinical need laboratories must perform studies to validate the performance of these assays in additional specimen matrices. The objective of this study was to evaluate cerebral spinal fluid (CSF), drain, fecal, pancreatic, pericardial, peritoneal, and pleural fluids for matrix interference using sodium, potassium, and chloride on the Roche cobas pro ion-selective electrode (ISE) analytical unit. **Methods:** Using an Institutional Review Board (IRB) approved protocol, residual clinical body fluid specimens were obtained, de-identified, and stored frozen (-20°C) until experiments were performed. Method comparisons were completed using a minimum of 20 specimens for each analyte/fluid type combination to evaluate fluid matrix accuracy. These samples were analyzed on the cobas pro ISE and compared to the cobas 8000 previously validated for body fluids.<sup>1</sup> Mixed dilution linearity studies were performed to provide support for accuracy and exclude matrix effect interference.<sup>2</sup> For most linearity studies, a high concentration serum, control, calibrator, or other linearity material was spiked into body fluid (less than 10% total volume) to obtain a specimen value within 20% of the upper end of the AMR. ISE diluent was used in some studies to achieve a specimen result within 20% of the low end of the AMR. Once body fluid

samples with high and low concentrations were prepared, dilutions were performed to acquire a minimum of 5 levels of material spanning the AMR that were analyzed (0%, 25%, 50%, 75% and 100% of the high pool). Method comparison and linearity results were analyzed using EP Evaluator 12. All other applicable studies (precision, sensitivity, carry-over, etc.) were previously performed on the cobas pro using serum samples.

**Results:** Method comparison studies between the two cobas systems were acceptable, with slopes ranging from 0.941-1.052 and average percent biases within  $\pm 15\%$  for each analyte in all seven body fluids. Repeat analysis was performed on some of the fluid types following additional conditioning of the cobas pro ISE which contributed to more accurate comparisons between the two methods. Additional conditioning of the ISE analytic unit involves running pooled serum to reapply the protein that is stripped following the wash rack. Conditioning is performed prior to calibrating the ISE analytic unit. Linearity studies were within the expected results of 85-115% recovery for each electrolyte on all seven body fluids. **Conclusion:** The findings after performing electrolyte testing on all seven fluid types using the cobas pro ISE provide substantial evidence for the exclusion of systemic matrix interference using this system. Method comparisons results improved once additional ISE conditioning was performed on the cobas pro ISE. <sup>1</sup>Owen et al. ClinBiochem 2015 <sup>2</sup>Block et al. AACC Press 2015

## A-109

### Accuracy of the new creatinine based equation to estimate glomerular filtration rate without race in Korea

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**Background:** The creatinine-based estimated glomerular filtration rate (eGFR) equation recommended by the 2012 Kidney Disease: Improving Global Outcomes guidelines is the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, which uses creatinine concentration, gender, age, and race variables to calculate eGFR. However, the race in eGFR equation is not a biological construct. Thus, recently, a new creatinine-based CKD-EPI equation without race, namely 2021 CKD-EPI, was introduced. To date, no evaluation studies for the performance of the new equation have been reported in Asian populations. We aim to assess the performance of the 2021 CKD-EPI equation in a Korean population.

**Methods:** A total of 1,899 Korean patients aged 18 years old and older who underwent chromium-51-ethylenediamine tetraacetic acid (<sup>51</sup>Cr-EDTA) GFR measurements (mGFR) were enrolled in this study. There were 756 (39.8%) females. The serum creatinine concentration was measured by a kinetic Jaffe method using an isotope dilution mass spectrometry traceable calibrator. The bias, root mean square error (RMSE), P30, and percent agreement between eGFR and mGFR categories of two creatinine-based eGFR equations (2009 CKD-EPI vs. 2021 CKD-EPI) were compared. P30 represents the proportion of eGFR within 30% of mGFR. Both eGFR and mGFR categories are classified into more than 90, 60 to 89, 45 to 59, 30 to 44, 15 to 29, and less than 15 mL/min/1.73m<sup>2</sup>.

**Results:** The mean bias (mL/min/1.73m<sup>2</sup>) of the 2021 CKD-EPI was significantly larger than that of the 2009 CKD-EPI equation (4.42 vs. 1.01, P<0.001). In sub-analyses according to gender (male and female) and age ( $\geq 70$  and <70 yrs), all of the bias of the 2021 CKD-EPI was significantly greater than that of the 2009 CKD-EPI as follows: male (n=1,143), 2.68 vs. -0.96, P<0.001; female (n=756), 7.06 vs. 3.97, P<0.001;  $\geq 70$  yrs (n=413), 6.7 vs. 3.3, P<0.001; <70 yrs (n=1,486), 3.8 vs. 0.4, P<0.001. The RMSE was 16.9 for 2021 CKD-EPI and 16.5 for 2009 CKD-EPI. P30 of the 2021 CKD-EPI (76.1%) was lower than that of the 2009 CKD-EPI (78.6%), but there was no statistical significance (P=0.066). Percent agreement between eGFR and mGFR categories is 62.2% for 2021 CKD-EPI, and 64.9% for 2009 CKD-EPI.

**Conclusion:** With our results, the new 2021 CKD-EPI equation has an increased bias in the Korean population. More discussion will be needed when applying the 2021 CKD-EPI equation without a race variable to Asians.

## A-110

### Effects of serum amyloid A on the structure and antioxidant ability of low-density lipoprotein

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**Background:** Serum amyloid A (SAA) levels increase during acute and chronic inflammation and are mainly associated with high-density lipoprotein (HDL). Our previous report showed that SAA affected HDL's composition and surface charge by displacing apolipoprotein A-I (apoA-I) and SAA enhanced its antioxidant ability. Although most reports indicate that SAA binds primarily to HDL, we have some data suggesting that the amount of SAA in apoB-containing lipoprotein fractions is increased in inflammatory patients with lower levels of HDL. In addition, several studies have reported a complex referred to as SAA-LDL associated with components of the metabolic syndrome, remnant-like particle cholesterol, smoking status, lifestyle interventions, and statin therapy. These studies suggest that SAA-LDL is a risk factor for cardiovascular disease. However, little is known about the influence of the distribution of SAA on apoB-containing lipoprotein. The purpose of this study was to determine whether SAA-LDL affects its structure and function. We investigated the effect of SAA on the distribution, surface charge, and antioxidant ability of LDL using recombinant human SAA (rhSAA).

**Methods:** Blood samples were collected from healthy volunteers. LDL (1.006 < d < 1.063 g/mL) was obtained from pooled human plasma by ultracentrifugation. Reconstituted SAA-LDL was prepared by incubating rhSAA with LDL isolated from plasma of healthy subjects. LDL (1.0 mg protein/mL) was incubated with 10, 200, and 400 mg/L rhSAA in PBS (pH 7.4) at 37°C for 3 hours. The SAA-LDL structure for analyzing the surface charge was characterized by agarose gel electrophoresis followed by western blotting. The particle size was analyzed by non-denaturing gel electrophoresis followed by western blotting. The antioxidant ability was estimated by the effect of SAA on LDL oxidation, and 50  $\mu$ g protein/mL LDL was incubated with 1.0  $\mu$ M copper sulfate. LDL oxidation was monitored as conjugated dienes formed at 234 nm in 5 min intervals. The antioxidant ability of SAA-LDL was determined as prolongation of the lag time, and a relative decrease of the maximum velocity (V<sub>max</sub>) compared to that of LDL.

**Results:** The electrophoretic mobility of LDL was slightly increased in SAA-LDL compared to intact LDL. The electrophoretic mobility increased with the concentration of SAA treatment. In this experiment, SAA was distributed in the same mobility position as apoB. LDL particle size was estimated by non-denaturing gel electrophoresis. Various LDL particle sizes were also observed in SAA-LDL. SAA was also distributed at the same particle size as apoB and located at the smaller particle size. The lag times (mean  $\pm$  SD) of SAA-LDL (10, 200, and 400 mg/L rhSAA) were 56.72  $\pm$  15.94, 84.56  $\pm$  15.62, and 119.99  $\pm$  13.75 min whereas that of LDL was 56.61  $\pm$  16.69 min. The relative V<sub>max</sub> of SAA-LDL (10, 200, and 400 mg/L rhSAA) were 0.99  $\pm$  0.09, 0.69  $\pm$  0.05, and 0.52  $\pm$  0.02.

**Conclusion:** This study showed that SAA affected the surface charge of LDL and increased the antioxidant ability of LDL. In our view of antioxidant capacity, SAA may play a role in preventing atherosclerosis as an essential component of antioxidants. (497 words)

## A-111

### The effect of sampling procedure and substrate type for activity assay on PON1 assessment

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**Background:** Paraoxonase 1 (PON1) related to high-density lipoprotein (HDL) is one of important enzymes contributing to antioxidation. HDL plucks lipid hydroperoxides from oxidized low-density lipoprotein to hydrolyze the hydroperoxides by PON1. Accordingly, PON1 is attracting attention as a clinical parameter, and its relationship with various diseases has been investigated for a long time. However, there is an inconsistency in the methods for sample preparation: even though PON1 is a calcium-dependent enzyme, EDTA plasma was used as a specimen in some papers. In addition, different types of PON1 activities (paraoxonase, arylesterase and lactonase) can be evaluated depending on the used substrate type, but it is unclear which activity is more suitable for PON1 assessment. As a result, some papers reported that PON1 activity was lower in atherosclerosis, whereas other papers reported no difference

among them. Differences in sample collection procedure and measured type of activities might be the cause of these results. Therefore, in this study, we investigated the difference in various PON1 activities by procedure for sample preparation.

**Methods and Results:** First, the effect of calcium addition on PON1 activity was investigated. When calcium was added to EDTA plasma from healthy volunteers by dialysis immediately after sample collection, plasma PON1 activities (paraoxonase, arylesterase and lactonase) were similar to those in serum, which suggests that the inactivation of plasma PON1 could be recovered by immediate calcium addition, and plasma could be applied to PON1 analysis. Next, we investigated the difference of PON1 activities and protein level by various HDL isolation procedures. HDL and lipoprotein depleted plasma (LDP) were isolated from serum (treated with/without EDTA-2K in the middle of ultracentrifugation steps for prevention of cooper oxidation) or plasma (treated with calcium by dialysis before ultracentrifugation). All samples were retreated with calcium by dialysis. PON1 protein levels in HDL and LDP were analyzed by electrophoresis followed by western blot analysis. As a result, despite the fact that PON1 activities were still remained in calcium-added plasma as described above, its activities in HDL isolated from plasma were about 70% lower than those in HDL from serum. HDL isolated from serum with EDTA-2K also showed lower PON activities. The cause of low PON1 activities may be reduction of PON1 mass. Most of PON1 was not bound to HDL isolated from serum. PON1 activities were proportional to the amount of PON1 in HDL fraction but some of PON1 protein were not bound to HDL. Moreover, the activities and amount of PON1 were not related for LDP. The tendency differed depending on the three types of PON1 activities. Paraoxonase and arylesterase activities of HDL were higher or similar than those of LDP, while homocysteine-thiolactonase activity was lower.

**Conclusion:** Plasma could be applied to PON1 analysis by immediate calcium addition, but its binding to HDL might be weakened by EDTA. Although some clinical studies have reported that lactase activity was a new risk factor for atherosclerosis, lactase activity might not be appropriate to be measured. It is important to select sample procedure and substrate selection for PON1 assessment.

## A-112

### Evaluation of an Improved Sysmex Troponin T Assay Including Effects of Renal Dysfunction

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**Background:** Recently Sysmex introduced a new Troponin T (TnT) assay. TnT is elevated in renal dysfunction. We studied the assay performance and the influence of renal dysfunction in a Singaporean cohort. **Methods:** The Sysmex TnT, measured on its HISCL-5000 automated immunoassay analyzer, is a 1-step sandwich assay where mouse anti-TnT coated magnetic particles (MP) reacts with sample TnT. Thereafter, alkaline phosphatase-labelled monoclonal mouse anti-TnT binds to MP-bound TnT. Following removal of unbound MP, a chemiluminescent substrate (chloro-dioxetane-phenyl-phosphate) is added and ALP generates a luminescent signal that is proportional to the sample TnT concentration. Assay performance was determined as per CLSI guidelines. Healthy ambulatory subjects (n=789; M=376, F=413) aged 18-94 (mean,SD: 50.4±18.5) with eGFR (CKD-EPI) >60mL/min, previously tested on the Roche hsTnT (Cobas e801), were studied to derive the 99<sup>th</sup> percentile (99P) upper reference limit (URL) for the Sysmex TnT; samples with abnormal eGFR (n=303) were also tested. All statistical analyses were performed on PRISM v9.0 (GraphPad Inc., USA). **Results:** LOB, LOD and LOQ were 1.3, 1.9 and 1.8 ng/L respectively. Inter-assay (n=20) precision (CV%) were 5.1 @ 17.3ng/L, 2.5 @ 106ng/L and 2.2 @3253ng/L. There was close agreement between the Sysmex TnT and the Roche hsTnT values. Passing-Bablok regression analyses revealed: r<sup>2</sup>=0.96; Sysmex = 0.9455Roche - 2.23. For healthy subjects the proportion of detectable TnT above LOD was - 59.4% (overall), 51.8% (F) and 67.8% (M). The 99P URL was: overall 14.1, F 13.4, and M 14.3 ng/L respectively and the CV at the 99P was 4.4%. These affirm that the Sysmex TnT is a high sensitivity assay. We observed a significant stepwise increase in median Sysmex TnT values beyond CKD stage 3 (see Table). **Conclusion:** This Sysmex TnT assay has high sensitivity performance and compares closely with the Roche hsTnT. With renal dysfunction there are more elevated TnT samples.

**Table. Distribution of Elevated Troponin values by CKD stage**

CKD Stage	Grade 3a	Grade 3b	Grade 4	Grade 5
eGFR mL/min	45-59	30-44	15-29	<15
Median TnT ng/L	14.3	34.2	55.5	102.2
TnT elevation (n)	58/110	62/85	31/41	65/67
TnT elevation %	52.7	72.9	75.6	97.0

## A-113

### Utility of alert tools in the laboratory for the diagnosis of severe hypercholesterolemia

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#### Background:

Hypercholesterolemia encompasses disorders of lipid metabolism that are manifested by abnormally high concentrations of lipoproteins in the blood. From an etiological point of view, they are classified as Primary or Genetic (the most frequent is the familial hypercholesterolemia, FH) and Secondary (due to pathologies, drugs and/or environmental factors). The clinical importance of these pathologies is because their association with the development of atherosclerosis and clinical complications. Among the different types of lipoproteins, LDL-cholesterol is the one with the highest atherogenic power, and its elevation in plasma has the highest risk of cardiovascular complications.

#### Methods:

Since May 2021, the following algorithm/expert rule has been implemented in our laboratory with the aim of screening cases of severe hypercholesterolemia, including FH. When LDL-cholesterol is higher than 250 mg/dl, the SERVOLAB laboratory computer system automatically generates the following comment to the test: "patient with severe dyslipidemia, it is recommended to contact by telephone (addressed to the requesting doctor, mainly from primary care) with the specialised vascular unit of the hospital for diagnostic orientation of FH or to assess secondary causes of dyslipidemia".

**Results:** During the year 2021, 95 comments have been activated for samples with LDL-cholesterol concentrations above 250 mg/dl, with a maximum value of 486 mg/dl. Then, we checked whether all these patients had actually been referred to the specialised vascular risk unit, by contacting the corresponding specialists by e-mail. The percentage of fulfillment of the screening objective was 24% of the patients: we have proved by consulting the corresponding medical histories that only 23 of them are being seen by the specialised vascular risk unit. 13 of these patients (54%) have been diagnosed with FH. For the majority of the remaining patients, it was found that although they were not referred to the specialised unit, they were treated with statins by their Primary Care doctor when high levels of LDL-cholesterol were found.

#### Conclusion:

With this warning system, 23 severe hypercholesterolemia cases have been diagnosed and treated prematurely. The consequences of not doing it could have triggered a higher percentage of cardiovascular events. Likewise, these pathologies would not have been diagnosed if they had not been referred to a specialised vascular risk unit. Furthermore, they would have been treated as different entities from those recommended in the guidelines, and possibly with less aggressive treatment than required. The advance warning system also allows early treatment of dyslipidemia by Primary Care in cases not referred to the specialised vascular risk unit.

## A-118

**Comparison Between AM and PM Testosterone Levels During Validation of Testosterone Reference Intervals**

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**Objective:** The average serum testosterone levels are different between genders, among different age groups in both genders, and is believed to have a diurnal fluctuation, with a peak in the morning and a decrease throughout the rest of the day. These characteristics create a challenge when establishing reference intervals. Ideally the reference intervals should be stratified by gender, age, and time of day. Currently in our laboratory, age and gender-specific reference ranges are in use. In order to stratify the intervals further by time of day, we studied the difference between AM and PM total serum testosterone levels for both men and women.

**Methods:** Historical data of total serum testosterone levels were obtained from the electronic health record system. Testosterone levels were separate into an AM and a PM group for each gender. In each group, testosterone values were transformed into natural log values and outliers excluded. A total of 424 female and 1262 male patients were included in the study. An indirect Hoffmann method was used to estimate the reference intervals for each group, and two sample T-test was used to evaluate statistical significance ( $p < 0.05$ ) between the AM and PM values.

**Results:** The reference intervals obtained are 29 to 67 ng/dL for the female (AM) group, and 26 to 64 ng/dL for female (PM) group, with  $p = 0.00034$ . The reference intervals are 209 to 684 ng/dL for the male (AM) group, and 199 to 687 ng/dL for the male (PM) group, with  $p = 0.34$ .

**Discussion:** There was a statistically significant difference between the AM and PM groups for female patients to support the establishment of separate reference intervals, but there was no difference for male patients. To further investigate the lack of significant diurnal differences among testosterone levels in male, two analyses will be performed in the next step: 1) For males, considering that age is a compounding factor, age-matched subgroups will be analyzed to account for possible confounding factor, and that possibility that diurnal rhythm is potentially testosterone level-dependent.

## A-119

**Circulating platelet-activating factor acetylhydrolase activity increases in rats with non-alcoholic steatohepatitis with fibrosis induced by high-fat/high-cholesterol diet**

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**Background:** Platelet-activating factor acetylhydrolase (PAF-AH) is an enzyme that inactivates platelet-activating factor (PAF) and oxidized phospholipids by hydrolyzing the sn-2 ester bond. Plasma PAF-AH is known as lipoprotein-associated phospholipase A<sub>2</sub> and binds to LDL and HDL. An experimental animal study has demonstrated that plasma PAF-AH activity increased in rats with thioacetamide-induced liver fibrosis and cirrhosis. However, there are few reports on the association of plasma PAF-AH activities with non-alcoholic fatty liver disease. In this study, we determined serum PAF-AH activities in rats with non-alcoholic steatohepatitis (NASH) induced by high-fat/high-cholesterol diet (HFC), and investigated the relationship between the enzyme activities and hepatic mRNA expression of fibrosis-associated genes.

**Methods:** Male Sprague-Dawley rats (8 weeks) were purchased from Charles River Laboratories and randomly divided into 4 groups ( $n = 5/\text{group}$ ): control group (MF diet from Oriental Yeast), high-fat group (HF; 68% MF, 30% palm oil, and 2% cholic acid), high-cholesterol group (HC; 95.5% MF, 2.5% cholesterol, and 2% cholic acid), and HFC group (65.5% MF, 30% palm oil, 2.5% cholesterol, and 2% cholic acid). After 10 weeks of feeding, blood and liver tissue specimens were collected under anesthesia. Serum PAF-AH activity was determined by PAF Acetylhydrolase Assay kit (Cayman).

**Results:** Serum PAF-AH activity was significantly higher in the HFC group (16.6-fold,  $P < 0.001$ ) than the control group, but not elevated in the HC (1.8-fold) and HF (1.4-fold) groups. The HFC group displayed a significant increase in hepatic cholesterol (40.4-fold,  $P < 0.001$ ), compared with the control group, and serum ALT and AST levels significantly elevated. Masson's trichrome staining showed significant fibrosis in the liver tissue from the HFC group, consistent with previous studies that demonstrated experimental NASH model rats. The HC group also displayed a significant increase in hepatic cholesterol (20.1-fold,  $P < 0.001$ ), compared with the control group, but there was no significant difference in serum ALT and AST. The HF group showed no significant lipid accumulation in the liver. Hepatic mRNA levels of collagen were

significantly increased in the HFC group (18.1-fold,  $P < 0.001$ ), compared with the control group, but not in the HC (1.5-fold) and HF (0.6-fold) groups. Serum PAF-AH activities significantly correlated with collagen mRNA levels ( $r = 0.915$ ,  $P < 0.001$ ), indicating the involvement of the activation of hepatic stellate cells.

**Conclusion:** The present observations suggest that serum PAF-AH activity may be a useful biomarker for liver fibrosis in diet-induced NASH model rats.

## A-121

**Investigation of Assays for Cholesterol Content of Erythrocytes Membrane**

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**Background:**

Serum cholesterol concentrations in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) have been widely measured to evaluate dyslipidemia. On the other hand, erythrocytes also contain the almost same amount of cholesterol as serum. Previously, we reported that incubation of erythrocytes with plasma promoted a transfer of large amounts of cholesterol from erythrocytes to HDL, while cholesterol in LDL was transferred to erythrocytes. These results indicate that erythrocytes are also involved in the cholesterol dynamics. In addition, since most of the cholesterol in erythrocytes are distributed on the membrane, cholesterol content of erythrocyte membrane (CEM) has been measured as an index of erythrocytes-related cholesterol. In fact, it was reported that CEM was positively correlated with the severity of coronary artery disease. However, an accurate method for measuring CEM has not yet been established. In this study, we investigated the method for measuring CEM more accurately, and the relationship of CEM with serum lipids concentration.

**Methods and Results:**

Whole blood samples were obtained from healthy volunteers or residual samples at clinical laboratory in TMDU hospital. Washed erythrocytes prepared from these samples were hemolyzed with a hypotonic solution. The erythrocyte membranes were collected from the hemolysate by repeating washing and centrifugation. First, we compared two methods: one is previous method measuring cholesterol levels by enzymatic assay after extracting lipids from the erythrocyte membrane by the Folch's method, and the other is current method measuring cholesterol levels in the erythrocyte membrane directly without extracting lipids. The CEM (mean  $\pm$  SD, three individuals) was higher in the direct method ( $227.4 \pm 12.6 \mu\text{g}/\text{mg}$  protein) than those in the extraction method ( $111.7 \pm 22.5 \mu\text{g}/\text{mg}$  protein). Then, to investigate the storage stability of the samples, whole blood samples were incubated at 4°C, room temperature or 37°C for 8 or 24 hours, and CEM was measured by the direct method. In all conditions, CEM tended to decrease according to incubation time, and the percentages of CEM in the samples incubated for 24 hours at 4°C, room temperature, and 37°C, were  $95.5 \pm 3.0\%$ ,  $93.3 \pm 2.1\%$ , and  $83.3 \pm 2.0\%$ , respectively, compared to those before incubation. Finally, the CEM of patient sample was measured by the direct method and compared with the serum concentrations of total cholesterol (TC), LDL-cholesterol, and HDL-cholesterol. There was no significant correlation between CEM and TC, LDL-cholesterol or HDL-cholesterol. In addition, some samples showed a large deviation from the correlation.

**Conclusion:**

The extraction method showed a low recovery rate, which suggests that direct method could be more suitable in terms of accuracy and simplicity of procedure. The transfer of cholesterol between lipoproteins and erythrocytes during incubation is thought to be a cause of CEM reduction, but it can be prevented by storing at 4°C. In addition, since CEM does not reflect serum cholesterol levels in some patient samples, CEM measurement might provide a new information for dyslipidemia. In the future, further studies on clinical significance are needed to investigate the relationship with the volume and number of erythrocytes.

## A-122

**Monthly Averages of Lipid Profile Results over a Three-Year Period in a Community Hospital**

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**Background:** Lipid profile testing is widely used to estimate ASCVD (atherosclerotic cardiovascular disease) risk and primarily includes total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein chole-

terol (LDLC). LDLC is essential for stratifying patients into different management groups with regard to statin dosing, and assessing the efficacy of and compliance with the therapeutic plan. LDLC can be either measured or calculated. This study is to determine the long-term variability of lipid profile tests, by examining the monthly averages of TC, TG, HDLC, directly measured LDLC (DL DLC), and calculated LDLC (CL DLC) with different equations using consecutive patient results.

**Methods:** The lipid profile results reported from Jun 1<sup>st</sup>, 2018 to Jul 31<sup>st</sup>, 2021 were extracted from the Laboratory Information System of United Hospital Center, a member of West Virginia University Health System. All the tests were consecutively measured on Beckman Coulter AU 5800. About 2.6% to 2.8% of samples have TG  $\geq$ 400 mg/dL over the years, and are excluded from this study. The CL DLCF, CL DLCM and CL DLCS were calculated by Friedewald equation, Martin/Hopkins equation and Sampson Equation, respectively. The non-HDLC was calculated as TC - HDLC, and MVL DLC was calculated as TC - HDLC - DL DLC. The monthly averages and medians were calculated for all parameters. The percent agreement between CL DLC and DL DLC was evaluated by the total allowable error of 12% set by the National Cholesterol Education Program (NCEP).

**Results:** The numbers of lipid profiles are relatively stable varying from 2819 to 4371 each month except March and April 2020 when COVID-19 pandemic started. The distribution of TG and DL DLC levels are stable in the dataset. The TC average fluctuated between 167.8 and 178.2 mg/dL, with a range of 10.4 mg/dL or 6.0% of grand mean. The HDLC average fluctuated between 46.1 and 49.4 mg/dL, with a range of 3.3 mg/dL or 6.9% of grand mean. The TG average fluctuated between 138.9 and 148.2 mg/dL, with a range of 9.4 mg/dL or 6.6% of grand mean. The DL DLC average fluctuated between 98.5 and 114.5 mg/dL, with a range of 16.1 mg/dL or 15.2% of grand mean. The CL DLCF average changed between 91.6 and 101.1 with a range of 9.5 mg/dL or 9.7% of grand mean. The CL DLCS average changed between 94.9 and 104.4 with a range of 9.5 mg/dL or 9.4% of grand mean. The CL DLCM average changed between 96.1 and 105.2 with a range of 9.2 mg/dL or 9.0% of grand mean. When compared with DL DLC, the percent agreement of calculated LDLC results varied from 41% to 73% for Friedewald equation, 52% to 83% for Sampson equation, and 55% to 88% for Martin/Hopkins equation.

**Conclusion:** TC, TG and HDLC assays, but none of LDLC methods, showed variability less than or equal to the two-fold of allowable bias set by NCEP. All three calculated LDLC equations showed smaller variability than DL DLC. The Martin/Hopkins equation had the smallest variability and best agreement with DL DLC over three years.

## A-124

### Performance characteristics of two immunoassays for interleukin-6 (IL-6) and comparison in a pediatric population

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**Background:** Interleukin-6 (IL-6) is an acute phase response pro-inflammatory cytokine. Utilization of IL-6 testing in pediatric populations is useful to assess sepsis severity and in the diagnosis of Multi-System Inflammatory syndrome associated with COVID-19. Our objective was to evaluate the performance characteristics of the research use only (RUO) MesoScale Discovery (MSD) IL-6 cytokine assay on the MSD SQ120 platform and the Beckman DXI600 IL-6 assay, one of 3 IL-6 assays with Emergency Use Authorization (EUA). We also investigated the comparability of the assays in a pediatric patient population. **Methods:** We used residual EDTA plasma and serum samples. The MSD and Beckman assays were validated by performing the following experiments: AMR/Linearity, inter and intra-assay precision, accuracy by method comparison with a previously validated assay (n=20, in-house RUO for Beckman and reference laboratory for the MSD assay), and reference interval. Furthermore, we compared pediatric specimens (n=65, age range: 8 months-21 years) between the MSD and Beckman assays. **Results:** The Beckman assay was linear in the range of 2.0-1528.0 pg/mL, and had a total imprecision of 2.4% and 2.0% at the concentrations 7.87 and 721.2 pg/mL, respectively. The MSD assay was linear in the range of 0.6-721.2 pg/mL and, had a total imprecision of 6.7% and 4.5% at the concentrations 7.8 and 279.6 pg/mL, respectively. The Beckman EUA assay compared well to the previous RUO assay (%bias: -4.29%, slope: 0.977 and R: 0.9980). The MSD assay showed acceptable comparability with the same assay in a reference laboratory (%bias: -0.74%, slope: 0.943 and R: 0.9909). The reference intervals of <6.0 pg/mL and <2.8 pg/mL were verified for the Beckman and MSD assays, respectively. Comparison of results was poor across the 2 assays in 65 pediatric samples tested. The range of concentrations were <2.0-2334.5 pg/mL in the Beckman assay and <0.6-1082.3 pg/mL in the MSD assay. The results in the MSD assay were consistently lower with a mean bias of -83.64 pg/mL (-59.96%, range -52.2- to -80.0%) and slope 0.459 (Deming regression) with a strong linear relationship across the methods

(R=0.9973). **Conclusions:** The MSD and Beckman IL-6 assays have acceptable performance characteristics for clinical use. The automated workflow of the Beckman DxI600 offers time and cost savings. The concentrations across the assays in pediatric patients were over 2-fold different in average, highlighting the lack of standardization across IL-6 assays represented in the literature and clinical laboratories. Clinicians should use caution when interpreting values from differing methodologies as values may vary significantly.

## Laboratory Management and Leadership

### A-125

#### Improving Specimen Handling in Central Processing Laboratory Using Lean Concepts

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#### Background:

The Central Processing Laboratory (CPL) is responsible for processing and handling of all specimens intended for the Clinical and Anatomic Pathology departments at Evanston Hospital. These specimens arrive at the Evanston hospital from 5 affiliated hospitals, numerous patient service centers that serve the medical group offices, immediate care centers and independent physician offices. This amounts to the handling of an average of 10,000 tests per day. Errors that occur because specimens are improperly processed or misplaced in CPL result in testing delays and possibly the need for patients to return for recollection. Recollection of specimens lead to delayed diagnosis and treatment along with decrease in satisfaction scores for the lab and the organization.

**Objective:** To decrease the amount of errors contributed to mishandling and misplacement of samples by 30%.

**Methods:** A thorough review of lab services issues (LSI) data was performed to identify problem areas for improvement. Spaghetti diagrams in the specimen handling area were constructed to determine the current workflow. A brain storming session using Fishbone Diagram identified three areas for improvement: (1) refrigerators and freezers required reorganization to improve specimen sorting prior to testing; (2) shift-to-shift communication log documentation was incomplete; and (3) some workstations required staff to simultaneously switch between 5-6 tasks at any given moment, reducing the efficiency and productivity, and leading to mishandling of specimens. A detailed step-by-step plan was drawn up and implemented over a three months period. Firstly, three refrigerators and 1 freezer were reorganized by labeling shelves, refreshing the labels on the specimen racks and creating bins/racks with labels for sendout body fluids and urines. A discard period was assigned to the sendout body fluids and urines to allow for more storage space. Secondly, a new shift-to-shift communication log was designed to capture endorsement from every workstation and acknowledgement from the incoming shift. Thirdly, workstation workload was reassigned to cover workstations that were too busy. Lastly, programmed excel spreadsheets were used to capture specimen receipt to automate documentation.

#### Results:

The average number of mishandled specimens per month decreased from 20 to 9 by improving the organization of storage spaces and providing additional support on high volume workstations. Shift-to-shift communication log documentation improved from 77% to 92%.

#### Conclusion:

The number of errors generated by the central processing team has been reduced by 55% thereby reducing delayed results and number of patients needing to return for recollection.

## A-126

### Examining healthcare cost of lab as a risk management metric

Z. C. Brooks. *AWESome Numbers Inc., Worthington, ON, Canada*

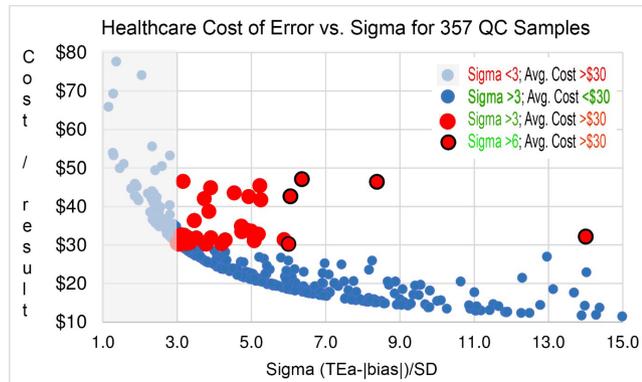
#### Background:

CLSI EP23A<sup>1</sup> defines risk as the “combination of the probability and severity of harm.” The number of “incorrect results - with a failure of measurement that exceeds a limit based on medical utility” drives the probability of harm. The severity of harm from lab error is reflected by the healthcare costs from unnecessary follow-up proce-

dures, or delayed or incorrect diagnosis or treatment reflect. The relationship between size and cost of error was established in 2004 for calcium tests by examining 89,000 Mayo Clinic charts to healthcare expenses incurred compared to initial calcium tests.<sup>ii</sup>

#### Method:

The author examined 357 routine QC samples collected for various posters available on ResearchGate<sup>iii</sup>. Sigma was calculated in Excel as  $[(TEa-bias)/SD]$ . The average cost of error was calculated by CatalystQC software<sup>iv</sup> from AWEsome Numbers Inc.



#### Results:

18% failed an acceptable risk standard of 3 sigma and had a cost of error above \$30. 69% passed the 3 sigma standard and had a cost of error below \$30. 11% passed the 3 sigma standard and had a cost of error greater than \$30. 2% of QC samples had a sigma above 6 with a cost of error above \$30.

**Conclusion:** This study reveals that there is no significant correlation between sigma and healthcare cost of error.

Endnotes<sup>i</sup> CLSI EP23A . Laboratory quality control based on risk management, Wayne PA: Clinical & Laboratory Standards Institute, 2011.<sup>ii</sup> National Institute of Standards and Technology. Planning Report 04.1. The impact of calibration error in medical decision making. <https://www.nist.gov/system/files/documents/director/planning/report04-1.pdf><sup>iii</sup> ResearchGate Zoe Brooks Publications <https://www.researchgate.net/profile/Zoe-Brooks-3><sup>iv</sup> [1] CatalystQC software AWEsome Numbers Inc. <https://awesome-numbers.com/>

## A-127

### Interactive tools for training and continuing medical education: the future of medical education applied here to the new concept of interactive scientific poster.

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#### Background:

In laboratory management, training and learning are essential for continuing medical education of medical and technical teams. The essential component of training is critical thinking, and active learning could enhance it. Interactive assessment systems that allow interactive participation of medical staff have emerged. The offer of audience response systems (ARS) accessible via personal electronic devices such as smartphones, tablets or computers has increased in recent years. We aimed to first evaluate interactive pedagogical tools (IPT) during real school lectures and during distance lessons on medical students. Secondly, we aimed to evaluate the use of interactive tools on posters at the AACC Chicago 2022 conference.

**Methods:** The interactive classroom was tested in medical laboratory courses at the University of Lille, Faculty of Pharmacy. 60 medical students will have attended at least one session using the IPT. After the lectures, an online questionnaire with 9 questions was submitted to the students about their interest in each system. The questions measured the students' perception on a scale of 1 to 10. To evaluate the interactive poster, we will submit questions to our reader via a QR code in our AACC session.

**Results:** Overall, the mean scores are higher for ARS (Wooclap, Socrative and Votar) compared to traditional lessons ( $p < 0.05$ ). The most appreciated items are learning, understanding and participation during class. The most appreciated points are learning, understanding and participation during class. Medical students also reported that they liked the tool because of its ease of use via smartphone. Results of evaluation of our interactive poster will be directly generate during AACC session scanning QR code on our poster.

**Conclusion:** This study shows the importance of having direct interaction and feedback to support learning and learner engagement, and not to carry out exclusively distance learning without direct contact and feedback. Thus, especially in the current context, we encourage medical teachers and trainers to use such tools to maintain direct interaction with students and learners - which is essential in pedagogy - and to ensure qualitative educational continuity. These systems are currently being developed for the training of students in laboratory medicine, but also for the continuing education of specialists in laboratory medicine and also for use in scientific congresses through conferences and poster sessions.

## A-128

### Stick to the Norm: Managing a Large-Scale Reference Interval Verification Study

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**Background:** Reference intervals (RIs) provide context and meaning to laboratory results, and are essential in guiding patient care. Herein we describe the process of verifying RIs for a variety of analytes (n=83), across multiple disciplines, in a reference lab setting. **Methods:** As part of a multi-department redesign, several next-generation automated chemistry and immunoassay analyzers were acquired. RI verification was included with assay validation studies. Selection of RIs to verify involved package insert review, comparison to current RIs, literature review, and consultation with practicing clinicians. Due to the diversity of analytes, a thorough list of inclusion/exclusion criteria were selected (e.g. fasting, no prescription medications, no history of disease). An Institutional Review Board-approved protocol was used, including a questionnaire to obtain demographic information. Participants were solicited by email, which described the inclusion/exclusion criteria. The number of donors (n=60) met the minimum number of participants recommended by Clinical & Laboratory Standards Institute (CLSI). Collection tubes and aliquots/volume needed for current and future studies were considered. Aliquots were stored at -80°C until analysis. On the day of testing, specimens were thawed, mixed, centrifuged, and analyzed, according to manufacturer's instructions. Data was analyzed using EP Evaluator 12. RI verification studies were performed after the assays were determined to exhibit acceptable performance characteristics (method comparison, linearity, precision, sensitivity, and carryover). Several analytes had partitions (sex-specific and/or decades of life), which were evaluated accordingly. Exclusions were applied for donors that exhibited patterns consistent with liver dysfunction or iron deficiency anemia; and only excluded for those affected analytes (not the entire dataset). Acceptability criteria for RI verification followed CLSI EP28-A3c guidelines, where passing criteria was 90% within the proposed RI. After the initial phase of studies, it was realized that an additional set of donors (n=67) were needed for partitions and in some cases, to establish the RI. Additional donor specimens were obtained following the original processes and criteria. Not described are the information technology efforts to incorporate and validate RI changes in laboratory information systems, and notify clients, both significant efforts. **Results:** Most RIs verified (82%) without the need for further intervention. Occasionally, appropriateness of the initial RI chosen was investigated, and sometimes a different RI was used. For some analytes, use of medical decision limits was suitable, even when the RI did not verify (e.g. prostate-specific antigen, cholesterol). Carbon dioxide proved difficult, due to the known instability, impacted by sample handling for a large-scale study. A few analytes did not verify; however, the RI was not adjusted, based upon the well-established nature of the RIs and their inclusion in reflex testing and algorithms (e.g. testosterone, sex-hormone binding globulin, dehydroepiandrosterone-sulfate). Complications arose for analytes where partitions involved menstrual status, since it was based upon self-reported first day of last menstrual period (e.g. follicle stimulating and luteinizing hormones). **Conclusions:** When undertaking an extensive RI study, thorough planning is imperative. Not all challenges are predictable, but considerations such as adequate number of participants, use of medical decision limits, and sample stability are recommended.

## A-130

**Proficiency Test Management and Continuous Improvement in a multisite Laboratory**

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**Background:** Quality management (QM) is intrinsically related to patient safety (PS) once one concerning situation for PS, according to the World Health Organization, is diagnostic errors. QM in the clinical laboratory “can be defined as accuracy, reliability and timeliness of reported test results” (WHO, 2011). Regarding to accuracy, one of the most powerful quality tools for clinical laboratories is the proficiency test (PT). PT compares laboratory’s results to its peer group or a reference method, therefore providing an actual information regarding the laboratory’s analytical performance. Since most of the PT’s evaluation are based on peer group information, a good performance is not only desirable for each laboratory, but it is healthy for the whole program. Implementing and maintaining a high-quality culture in a single laboratory is a challenge. Multisite laboratories present a higher degree of difficulty due to geographical distance and local culture influence. In this study, we share an experience of continuous improvement in the PT process of a multisite laboratory in Brazil.

**Methods:** Since 2019 modifications on how to manage the PT process in a large (over 70 million tests/year) clinical laboratory in São Paulo (Brazil) have been implemented using the Plan-Do-Check-Act (PDCA) methodology. Every PDCA cycle included data analysis by the quality team with the managers and laboratory director. Modifications implemented, in chronological order, were: put together a team with both quality and technical background to support and monitor the process; establish a review step for all inadequate results’ investigation; provide training and continuous education for the laboratory staff at all sites; documented procedure review; development of online tool to register the investigations and reviews; implementation and improvement of other quality processes, such as internal quality control monitoring. Individual sites and laboratory overall performance in the ControlLab PT program, a Brazilian national program, was monitored since 2018 through the adequate results index which is calculated by de number of tests/analytes with more than 80% of adequate results divided by the total tests/analytes evaluated multiplied by 100.

**Results:** A total of 47 sites were monitored. Two of them are a medium-sized laboratory and the main large laboratory, which performs about 42 million tests/year and has over 500 tests evaluated by the program. The other sites are small laboratories located within the hospitals through 8 different states. 34 sites have gradually improved their indicator since the modifications started. In 2018, the average percentage of all sites was 94%, with 7 sites performing 100% of adequacy. In 2021, the average percentage was 98.6%, with 29 sites performing 100% and more 16 sites above 95%.

**Conclusion:** Improving OS in the clinical laboratory requires a quality culture maintenance sustained by continuous improvement, personnel engagement, discipline and monitoring process. In multisite laboratories, integrate different sites towards a common goal, feature ways to promote experience interchange e built a trusting environment where the error is faced as a learning tool, not punishment, are fundamental steps to quality management and therefore to patient safety.

## A-132

**Sigma Metric equation proposed by James O. Westgard is wrong**

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**Background:**

Over the past several years, a few papers have criticized calculation of Sigma Metric (SM) using the equation adapted by James O. Westgard as ‘SM = (TEa - |Bias|)/SD’. The main argument is that bias shouldn’t be included in the SM equation and they attribute their claim to industrial approach e.g. citing ref. (1). So they claim that according to industrial approach the correct equation is: ‘SM = TL/SD’ (2) (where TL is tolerance limit).

**Methods:**

We are going to look at the industrial definition of SM in ref. (1) and show that Westgard equation for SM is a correct adaption of industrial approach.

**Results:**

An example from industrial field is “Subtract the average you just calculated from the nearest specification limit. Divide the result by the sigma (SD) you just calculated to get the number of sigma that fit. This number indicates how well the process runs when it is in control” (1). In Six Sigma terminology, “This number” is called Sigma

Metric. Noting “Subtract average from the nearest specification limit”, it is simply obvious that bias is taken into consideration in industrial approach. Lower specification limits (LSL) and upper specification limit (USL) are, respectively, calculated from subtracting and adding allowable deviation (AD) to target value (TV): LSL = TV - AD USL = TV + AD Assuming USL is the nearest SL to the performance average (i.e. a positive bias is present), SM according to the abovementioned instruction will be calculated as Eq. (1): SM = (USL - Avg.)/SD Eq. (1) Given bias is the difference between average and target value (i.e. Bias = Average - TV), replacing USL and Avg. in Eq. (1) with equivalent terms will give Eq. (2): USL - Avg. = (TV + AD) - (TV + Bias) = AD - Bias Therefore: SM = (AD - Bias)/SD Eq. (2) In laboratory medicine, allowable deviation is traditionally called allowable total errors, TEa, therefore replacing AD with TEa will give Eq. (3): SM = (TEa - Bias)/SD Eq. (3) To have a general equation for both negative and positive biases: SM = (TEa - Bias)/SD

**Conclusion:**

The equation adapted by J. O. Westgard is completely compatible with the industrial approach, and therefore is completely correct.

## A-133

**Point of Care Testing Boot Camp for Year III Medical Students- from Bench to Bed side**

S. Ahmed, L. Jafri, H. Majid, S. Shakeel. *Aga Khan University, Karachi, Pakistan*

**Background:** Point of care testing (POCT) has been integrated into the healthcare system, creating a paradigm shift and offering faster results using portable, easy-to-use devices that can lead to improved patient outcomes. Formal teaching of laboratory medicine, particularly POCT is a relatively neglected component of the medical school curriculum. An early practical based understanding of POCT is essential for medical students before transitioning into patient care. To develop, implement, and evaluate a 2 days POCT boot camp for Year III medical students

**Methods:** The experience was devised and directed by three Chemical Pathology faculty members and a laboratory scientist assisted by a team of teaching assistants (TAs). The boot camp was spread over 2 days and ran three hours each day with the Year II class split into two groups. The program included presentations on utility of POCT, in-depth overview of POCT operations at AKU, supervised hands-on individual performance on ROCHE Inform II Acu check glucometer in a simulated environment, competency assessment and sign off, case-based discussion on utility of POCT in diabetes management, and quick Assessment of data interpretation skills. A knowledge quiz via Kahoot was administered at the beginning and end of the experience and scores were compared statistically. Online evaluation and feedback were undertaken based on 10 questions using Likert’s scale.

**Results:** All the students 80 (100%) achieved a score of 95% or more on the competency assessment. An average of 45% correct responses were recorded on the pre test, whereas 92% correct responses were obtained re-quiz; the improvement was found to be statistically significant (P < 0.05). Feedback of 4 or greater on Likert’s scale was recorded on 7 questions by 100% respondents

**Conclusion:**

Although the assessment can only measure a small amount of knowledge recently acquired, the improvement observed by the medical students devoting a short period was encouraging. This medical student boot camp format allowed teaching of a POCT principles in 2 days to an entire medical school class.

## A-137

**Specimens dispatch in a large laboratory area: adapting autonomous mobile robot technology**

W. Ng, J. Ong, S. Loo, J. Su, C. Yeo. *Singapore General Hospital, Singapore, Singapore*

**Background:** In our 24/7 clinical chemistry and hematology laboratories, round-the-clock services to the entire hospital network (emergency, intensive care units, wards and outpatient clinics), involving specimen receipt, handling and distribution falls on the hands of the healthcare attendants/associates. The centralized specimen distribution role of Client & Specimen Management faces manpower constraints amid annual increases of specimen workload. The reception area sited between clinical chemistry and hematology laboratory areas requires staff to walk 22 - 45m to distribute specimens in this 1200 sq.m facility. Industry 4.0 provided the impetus to upskill and to mitigate an ageing workforce in light of recruitment and retention difficulties exacerbated in this pandemic condition as we introduced mechanized intelligent computer-controlled

autonomous robots into the laboratory. **Methods:** A quality improvement study identified replacement of manual specimen transport with an autonomous mobile robot (AMR). Upon securing funds for procurement and upon open tender, the selected robot was put to the test. The AMR's performance of 9 months (4 months familiarization, 5 months routine use), was studied with data captured in the robot's computer system. **Results:** With scheduled milk runs in 2 modes - day and night (15min, 30min intervals), the 152 trips daily translate to 21,584m travelled each day (657km/month or 7878km/year) saving an effort equivalent to 1.0 FTE. The milk runs/trips took up 26.1% for AMR utilization with wait time taking up 19.07%, charging time 54.08% and 0.76% on errors (monthly averages). There were 5 error types logged (>30 error flags in library) with the top 2 (obstacle detected, avoidance timeout) deemed operation-related. No system faults nor charging errors occurred during the 9 months' period. With a savings of \$45,864 (SGD) annually, the return on investment (ROI) could be attained in 2.5 years. Post-implementation surveys recorded better appreciation by HCAs and lab staff of the robotic technology compared to pre-implementation sentiments (78% vs 55%). **Conclusion:** Performance aspects of the AMR achieved its objectives, with 1.0 FTE savings for manual dispatches within the laboratory area and with favorable ROI and performance, deployment of such AMRs could only increase.

### A-138

#### An Automated System for Clinical Laboratory Quality Control Review and Management of Assay Performance

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**Background:** Quality control (QC) in the medical laboratory is one of the critical measures to ensure the quality and accuracy of patient results. Quality control samples of known concentrations are included in every assay setup. In addition, various statistical parameters are used to verify the accuracy of reported results and detect deficiencies or errors so that they can be corrected before releasing patient results. However, monitoring quality control parameters can be complicated in high-volume assays, particularly when multiple analytes or instruments are involved. Difficulties are compounded by the scarcity of comprehensive off-the-shelf QC management systems capable of managing highly complex platforms such as LC-MS/MS with single and multiple analytes across different instruments. As a result, QC review processes are often time-consuming and lack automated features for assessing and tracking assay performance. The lack of automation for the QC review process can impact laboratory productivity, the accuracy of QC data entered, assay troubleshooting capability, audit preparedness, responsiveness to physician inquiries, and overall client satisfaction. We developed a modular, customizable software application that integrates, processes, and displays data across multiple instruments. Here, we describe the features of the software. **Methods and Results:** We built a customizable, cloud-based software application using internal resources that automates the QC review process and monitor various aspects of assay performance. This automated QC and assay management program, named LURA for the initials of the primary developers, includes modules for complex mass spectrometry assays, chemistry analyzer assays, and ELISA and fluorescence-based plate reader assays. LURA provides the following functionalities: automated collection and analysis of processed assay data, Levey-Jennings chart data integration across multiple instruments, graphical visualization of assay data with customizable date ranges, consolidation and centralization of instrument data, and calculation of positivity rates and medians for monitoring of overall assay performance and reference interval evaluation. At the same time, the program can generate automated email notifications for pending QC lot expiration, reminders for weekly/monthly supervisor/Director reviews, and send alerts about abnormal patient results to genetics counselors or Directors to enable timely communication of critical results to providers. LURA can also track tests not performed (TNP) and provides a comprehensive QC audit trail. **Conclusion:** This program can improve overall assay quality in the clinical laboratory and can save significant FTE time by automating time-consuming data entry steps and eliminating clerical errors. The ability to be nimble and to adapt to new assay requirements is especially crucial for large laboratories with a comprehensive and varied test menu that incorporates multiple assay platforms and can be lacking in some commercially available software. One of the benefits of our software is the ease with which we can update the program capabilities and customize them to meet our specific and changing needs.

## Laboratory Stewardship and Patient Safety

### A-139

#### Impact of Multidisciplinary End-To-End Total Laboratory Automation Solution on Diagnostics Delivery at an Apex Tertiary Care Hospital Setup in India

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**Background:** Total laboratory automation (TLA) combines full automatization of preanalytics, analytics, and postanalytics, such that specimens are processed, tested, and stored with minimal user intervention. It increases laboratory efficiency by reducing error, increasing productivity in terms of sample processing capacity, offers cost and space savings and safer working conditions for the staff due to minimal sample handling. In May 2020, AIIMS Delhi inaugurated TLA enabled Laboratory on campus with full-fledged operations by Sept 2020. The aim of this study was to assess the impact of TLA on diagnostics delivery. **Methods:** Impact of TLA was assessed under four heads: Increased Capacity, Turn-around-time (TAT), Lab Efficiency and Benefits to patients. Pre-TLA data was curated from Annual reports (FY 19-20) and Post-TLA data from our LIS (FY 20-21, FY 21-22). Post-TLA, test volumes are available monthly and due to digitalization of preanalytical, analytical and post analytical processes tracking of multiple aspects of laboratory workflow was possible. For analysis, two representative days were chosen from two comparable months (Sept 2020 and Nov 2021). Data downloaded from our Middleware for 17-Sept-2020 and 18-Nov-2021, was evaluated for TAT. The intra-lab TAT was defined as time between Sample scan at Receiving counter to report release of last parameter to LIS. Lab efficiency was assessed in terms of Space and Manpower; benefits to patients were assessed using subjective tools like distance to walk, number of phlebotomies done and waiting time. Microsoft Excel 2018 was used for analysis. Objective comparisons with pre TLA data was not always possible as similar records are non-existent. **Results:** Pre-TLA, laboratory services offered a Directory of Services (DoS) with 86 unique parameters scattered amongst 4 major labs. Post-TLA, it was increased to 123 (43% increase) across Biochemistry, Immunoassay, Infectious serology, Hematology and Coagulation studies, at one location. Monthly load of lab tests for Clinical Chemistry, Immunoassay and Serology increased from 267452 to 622280 (132.7% increase) between Sept 2020 and Nov 2021. The median TAT was 157mins in Sept 2020 v/s 174mins in Nov 2021: this, despite an increase in test volume. 90th percentile TAT in Sept 2020 was 260mins v/s 287 minutes in Nov 2021. In pre-TLA times, 35 lab staff catered to a comparable workload v/s 18 in current times. The number of reported tests per sq.m per day has increased from 150.4 considering 1 year (April'19-March'20) to 221.5 (47% increase) considering year (Oct'20- Sept'21). Blood sampling was done earlier at 3 collection centers distributed within the campus requiring patients to walk approx 500-750m, spending approx 3hrs in phlebotomy queues and getting bled thrice on an average. Post TLA, walk around has decreased to <200m, waiting time reduced to <1 hour, blood collection pricks reduced to 1, and blood volume collected reduced to 5-7mL from previous collection of 10-12mL. **Conclusion:** Overall, delivery of diagnostics has improved in terms of lab quality indicators, TAT, DOS. It has brought in space and manpower economy and improved benefits to patients even with increased sample turnover.

### A-140

#### Classification of Kidney Stone Composition using an Artificial Intelligence model: Twelve Month Prospective Study Demonstrates Improvements in Quality and Patient Safety

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**Background:** Kidney stones are painful, costly, and highly prevalent throughout the world. Effective treatment and prevention of future kidney stones relies on an accurate characterization of kidney stone constituents which is manually accomplished using Fourier Transform Infrared Spectroscopy (FTIR). However, the standard clinical FTIR analytic process is laborious, inefficient, and potentially error prone. These errors include spectra misinterpretation, typographical errors, and reporting errors even with secondary review. Each of these error types can be potentially attenuated with the appropriate application of artificial intelligence (AI). Our group created a suite of novel, internally developed AI algorithms that could quantitatively characterize FTIR kidney stone spectra and be used to detect potentially erroneous stone composition

results reported in the laboratory information system (LIS). These AI algorithms were used prospectively during a 12-month study period to determine if AI could detect misclassified stone constituents in the LIS thus improving quality within the laboratory and ultimately enhancing patient safety.

**Methods:** For algorithm development, 70,000 kidney stone spectra from previously reported kidney stones were used for AI training while another set of 16,491 kidney stone spectra were used for algorithm validation. The overall concordance rate of the algorithm during validation was 95.2%. For 12 months, the AI algorithms then analyzed FTIR spectra that were recently reviewed and reported in the LIS by a clinical laboratory technologist as part of clinical practice. The AI-enabled program generated a list of stone spectra where there was discordance between what the technologist reported in the LIS and what the AI program interpreted the spectra to be. Dissimilar spectra interpretations were reviewed by a qualified technologist to determine if an incorrect result was reported to the patient care team through the LIS. If an incorrect result was detected, a remedy was entered into the laboratory event management system (LEMS) and the care team was contacted about the revised report. The number of incorrect results reported in the LIS was compared to the number of kidney stone laboratory events one year prior to the study period to assess how many additional incorrect kidney stones results were identified and corrected with the incorporation of AI into our clinical laboratory practice.

**Results:** Within the 12-month study period, 81,517 kidney stone spectra were reviewed by AI. Overall clinical concordance between the technologist and AI was 90.0% (73,388/81,517). The kidney stone relative revision rate during the AI implementation period was nearly 8 times higher than the control period (RR 7.9, 95% CI: 4.1 to 15.2). This included 62 incorrect kidney stone constituent interpretations that were detected by AI. These incorrect stone constituent results may have not been identified without the use of AI.

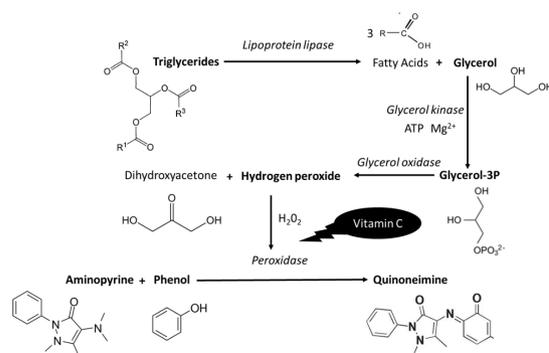
**Conclusion:** This study demonstrated that an AI program that reviews human spectra interpretations can result in a significant increase in the detection of erroneous spectra results in the LIS. This can reduce errors within the clinical laboratory, improve patient care and safety, and potentially reduce healthcare costs associated with erroneous laboratory results.

## A-141

### Hide and seek with triglycerides: a vitaminized unusual suspect

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**Context:** A patient was admitted in the Intensive Care Unit (ICU) for septic shock. Laboratory investigations revealed in a surprising way undetectable triglycerides (< 0.09g/L) without other notable abnormalities. Conventional interferences such as analytical bubbles, clogging and improper storage conditions have been excluded. Hence, interference was suspected and we aimed here to investigate it. **Methods:** Triglycerides were measured by colorimetric enzymatic method, using the Trinder reaction, on Cobas 8000® C502 (Mannheim, Germany). In order to overcome suspected interference, successive dilutions (1/10, 1/50, 1/100) were performed. **Results:** Despite successive dilutions, stable triglycerides concentration could not be obtained and reaction curve remained disturb. Exogenous interference was next suspected and furthermore investigations revealed that patient received several intravenous injections of ascorbic acid (estimate at 6g) that could disturb triglycerides assay. **Discussion:** The last dilution was not enough to obtain a fix triglycerides concentration. A higher dilution could be done but uncertainty dosage was also impact by dilutions. Beyond 1/100, uncertainty was too important to return a reliable result. It was shown that acid ascorbic interferes with Trinder reaction by several mechanisms including depletion of peroxide (H<sub>2</sub>O<sub>2</sub>), interfering with dye formation. Moreover, this mechanism is known to interfere with cholesterol and glucose assays, also using Trinder reaction. Actually, the use of ascorbic acid intravenous injection increases, especially in COVID-19 context. Hence this interference requires great attention from laboratory staff to detect it.



## A-142

### Implementation of POCT COVID-19 Testing Using a LumiraDx SARS-CoV-2 Testing Platform in Patient and Employee Testing Environments

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**Background:** Throughout the global Coronavirus disease 2019 (COVID-19) pandemic, patient care adjustments have been continually addressed at the Johns Hopkins Hospital (JHH). One example of this evolution and expansion is in the use of the LumiraDx-SARS-CoV-2 Antigen test. In January 2021, two projects justified the need for rapid SARS-CoV-2 tests: first, excluding COVID-19 in children presenting to clinic with one symptom; second, general anesthesia surgical patients unable to obtain a test within the approved pre-surgery timeframe. In the fall of 2021, Johns Hopkins Occupational Health (OH) piloted a program designed to provide rapid SARS-CoV-2 testing for symptomatic employees. Following pilot program success, this use case expanded to include a total of six collection/testing sites. Finally, in early 2022 during the Omicron surge, two outpatient clinics justified the need for rapid SARS-CoV-2 testing. In each of these use cases, the LumiraDx-SARS-CoV-2 Antigen test was identified as the best fit based on time-to-results, ease of use, and availability of resources.

**Methods:** The LumiraDx-SARS-CoV-2 Antigen test requires collection of a nasal swab specimen, and generates results within 12 minutes of test start. In each use case, individuals from varying clinical backgrounds completed extensive training by a Point-of-Care Coordinator and/or an approved trainer prior to independently collecting and testing patient specimens. Wearing HEIC-approved personal protective equipment (PPE), testing personnel completed specimen collection and testing in accordance with the manufacturer's specifications. A result interface is not currently utilized, necessitating documentation of all test results on paper patient result logs, which is then used to document results in the Electronic Medical Record. Quality oversight activities performed by the JHH Point-of-Care Testing Office include monitoring of positivity rates, audits of all logs (QC, patient result, and maintenance), and swipe testing to check for the presence of SARS-CoV-2 antigen on the testing surfaces and LumiraDx instruments.

**Results:** The rapid turnaround time of the LumiraDx-SARS-CoV-2 Antigen test facilitates desired outcomes in each of the identified use cases. Availability of this test for patients without a SARS-CoV-2 test result within the designated pre-surgical window prevents delays in OR schedules. In this use case, the positivity rate has been negligible across the past 13 months (average 0.8%; 2/263 total tests). In the OH project, expediting employee return to work processes was identified as the primary mission. This became especially pertinent during the Omicron surge, when high positivity rates (December 2021 average: 29.1%)(January 2022 average: 29.6%) severely impacted operations. The adult outpatient clinics' implementation of POCT SARS-CoV-2 testing during the Omicron surge (January 2022: 19.6%, 18.2% positivity) allowed for rapid adjustment of patient care plans given limited resources.

**Conclusion:** In each of the four use cases, the LumiraDx-SARS-CoV-2 Antigen test provided a viable, easy-to-use POCT solution at the Johns Hopkins Hospital. Extensive use of the instrument has led to improvements in result turnaround times, return to work processes, utilization of scarce resources, and in the quality and safety of patient care. However, robust quality oversight remains key in ensuring success during project implementation and long-term oversight.

## A-143

**Shining Renewed Light on a Tried-and-True Test: The Critical Role of Osmolality Testing in Improving Health and Economic Outcomes in Patients with Hyponatremia**

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**Background:** Hyponatremia, defined as a serum sodium concentration of  $\leq 135$  mEq/L, is the most common electrolyte disorder encountered in clinical practice, occurring in up to 30% of hospitalized patients. Our research aimed to investigate (1) the impact of hyponatremia on patient outcomes and resource utilization, (2) the impact of hyponatremia correction on patient outcomes and resource utilization, and (3) utilization of osmolality testing to determine the etiology of hyponatremia and ensure appropriate patient management.

**Methods:** We conducted a thorough systematic review of the PubMed database. Our search criteria included English-language peer-reviewed articles published from 1967 to 2022 by institutions located nationally and internationally. In total, we analyzed over 60 publications including prospective and retrospective research studies, clinical practice guidelines, expert panel recommendations, and consensus statements.

**Results:** Our research revealed a scientific consensus in the literature. Several key findings included (1) the association of hyponatremia with poor patient outcomes and high resource utilization, (2) the association of hyponatremia correction with improved patient outcomes and decreased resource utilization, (3) underutilization of osmolality testing in determining the etiology of hyponatremia (ordered in as little as 23% of patients), and (4) the association of ordering osmolality with improved patient outcomes. Further, we observed evidence of a gap in education on hyponatremia management.

**Conclusion:** In summary, hyponatremia is an important medical and economic problem. Osmolality testing is used to determine the etiology of hyponatremia, yet the test is underutilized in clinical practice. Education on the value of measuring osmolality, in addition to protocolizing the test into clinical pathways, may help drive appropriate utilization and, in turn, improve patient care and reduce excess resource utilization.

## A-144

**Evaluation of thyroid stimulating hormone receptor antibody test utilization patterns from a national reference laboratory**

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**Background:** Graves' disease is the leading cause of thyrotoxicosis and may be identified and monitored by measuring thyroid-stimulating hormone receptor auto-antibodies (TRAbs). TRAbs include: (1) thyroid stimulating antibodies (TSI), that stimulate the receptor and are a direct cause of Graves' disease, (2) thyroid blocking antibodies (TBAb), which inhibit TSH-receptor binding, and (3) neutral antibodies that neither stimulate nor block the receptor. Currently there are several laboratory assays to measure TRAb, which can be divided into two groups. First are assays that detect TRAb in sera but cannot differentiate stimulating from non-stimulating TRAbs. Second are assays that specifically detect stimulating antibodies (TSI). TSIs are traditionally measured using a cell-based bioassay (TSI-BA). More recently, a quantitative immunoassay specific for TSI has become available (TSI-IA). Current clinical guidelines pre-date the TSI-IA and do not specifically address what testing methodology should be used. General recommendations suggest measurement of TRAb to determine the etiology of hyperthyroidism, but do not specify the use of one test over another, which may lead to inappropriate test utilization. This study investigated the ordering patterns of TRAb and TSI, specifically when both were ordered for the same patient in the same encounter. **Methods:** Test ordering patterns for >200,000 orders for TRAb and TSI (TSI-BA and TSI-IA) in our laboratory were examined. The frequency and qualitative agreement of simultaneous TRAb/TSI-BA and TRAb/TSI-IA orders were evaluated. We also evaluated 374 encounters with simultaneous orders for FT4, TRAb, and TSI-BA or TSI-IA. **Results:** We observed a total of 101,445 orders for TRAb and/or TSI-BA, of which there was 12,673 simultaneous orders for TRAb and TSI-BA (30.9% of all TRAb orders and 21% of all TSI-BA). Similarly, a total of 117,770 orders for TRAb and/or TSI-IA were examined, of which there were 17,447 simultaneous orders for TRAb and TSI-IA (36.8% of all TRAb orders and 24.8% of all TSI-IA). Qualitative comparison using manufacturer-defined cutoffs showed discordant clinical interpretation between TRAb and TSI-BA in 1,590 simultaneous orders, or 12.5%. The discordance between TRAb and TSI-IA was lower at 1,149 simultaneous orders, or 6.6%. We also evaluated 153 results with simultaneous TRAb, TSI-BA, and FT4 orders. Twenty-one (14%) had discordant results between TRAb and TSI-BA (8 TRAb+/TSI-BA- and 13 TRAb-/TSI-BA+). Two results in the TRAb+/TSI-BA- group had elevated FT4 while 5 in the TRAb-/TSI-BA+ group had

elevated FT4. Likewise, 221 results with simultaneous TRAb, TSI-IA, and FT4 orders were examined. Seventeen (8%) had discordant results between TRAb and TSI-IA (4 TRAb+/TSI-IA- and 13 TRAb-/TSI-IA+). Five results in the TRAb-/TSI-IA+ group had elevated FT4, however, no elevations in FT4 were observed in the TRAb+/TSI-IA- group. **Conclusions:** Taken together, these data support overall clinical agreement between TRAb and TSI assays, with improved agreement between TRAb and TSI-IA compared to TSI-BA. The observed simultaneous orders for both TRAb and TSI therefore suggests a high frequency of redundant ordering patterns in the assessment of autoimmune-mediated hyperthyroidism. Use of a single test to assess TRAb would be appropriate in most scenarios and may lead to considerable cost savings.

## A-145

**Automatic Signature Algorithm for Laboratory Exams in Hospital Units: Implementation and Evaluation of Effectiveness**

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**Background:** The release of laboratory tests is still a process dependent on specialized human resources, which has long been sharing space with technology in order to optimize and increase quality and patient care. The development of an algorithm that positively contributes to automatic process, brings improvements such as: turnaroundtime (TAT) reduction, guarantee of results double-checked by intelligence, and others that generate positive impacts for the patient, thus medical decision-making can be faster and more assertive. For this, it's necessary to insert checkpoints based on specific criteria adopted by the laboratory or from the literature. These checkpoints are inserted in an interfacing program (Middleware) and in a Laboratory Information System (LIS). The rules are checked in two stages according to the premises established, the first being the analysis, verification and release, and the second, their signature and availability to the patient. Results that aren't validated by the established rules, must be reviewed. In this scenario, a feasibility study was proposed for the implementation of an algorithm for automatic signature of laboratory tests in hospital units in the city of São Paulo, Brazil.

**Methods:** A bibliographic research was performed to define criteria and premises about checkpoints used in the algorithm. First checkpoint (middleware), delta-check rule will be inserted, whose definition was applied to the RCV (reference change value) formula, which considers the coefficient of variation analysis (CVa), intra-individual coefficient of variation (CVi) and significance level. The CVa was calculated from the midpoint of the internal quality control, the CVi was obtained through the EuBIVAS database (European Biological Variation Study), using the level of significance Z of 95%. The variation index will be applied to patients who have previous results in a period of up to three days. For those who don't have previous results, a second checkpoint, determined by the reference intervals applied in the tests, will be considered. The chosen analytes were blood urea nitrogen (BUN) and creatinine, due to their frequency of request, analytical variability and clinical importance in hospital procedures. The algorithm was simulated in a real database of three hospitals, whose different profiles allowed validation on different scenarios. Chosen analytes results over a six-month period were analyzed and validated.

**Results:** In the initial simulation, the data predicts an average of 60% (n=8,030) the total of exams automatically signed, which corresponds to a reduction of 12% (6 minutes) in the average time of availability of the result to the patient/medical team. Considering hospital emergency scenario, its clear that reduction on results releasing time for patients and medical team is very importante to faster and, consequently, better clinical outcome. With the promising results of the algorithm applied in the database it's being implemented in the aforementioned hospitais, whose rules will be tested in a monitored and safe environment so that there're not impacts on patients.

**Conclusion:** Due to the arguments presented, the process of implementing an automatic signature algorithm proves to be an efficient tool for standardizing release criteria and reduction on releasing time.

## A-146

**Study of Immunohistochemical Markers for Breast Cancer in Search of an Indicator for Immunotherapeutic Treatment**

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**Background:** Cancer immunotherapy has recently emerged as a leading-edge antitumor strategy. In general, it can be divided into three branches: passive immunotherapy

(eg, trastuzumab), active immunotherapy (eg, checkpoint inhibitors), and adoptive immunotherapy (eg, CAR-T cells). Immunotherapy allows the immune system to take advantage of the body's own ability to recognize and attack cancer cells. Targeting unique biomarkers to tumor cells or immune checkpoints, along with their ligands, has revolutionized cancer immunotherapy. In this scenario, its importance turned to breast cancer. Through the analysis of estrogen receptor (ER), progesterone receptor (PR), C-erbB-2 oncoprotein, and Ki-67 antigen markers, it is possible to define one of the 4 molecular profiles of breast adenocarcinoma - types LUMINAL A, LUMINAL B, HER-2, or TRIPLE NEGATIVE BREAST CANCER (TNBC). This definition has predictive value, as it helps in choosing the most appropriate treatment. In this context, the authors of this study evaluated the correlation of immunohistochemistry biomarkers with WBC (White Blood Cells), age, and molecular subtypes (Luminal A, Luminal B, HER2+, and TNBC) both together and separately, hypothesizing an indicator for immunotherapeutic treatment. **Material and Methods:** This is an observational, descriptive study that covered anatomopathological exams analyzed between August and November 2021 in a large pathology laboratory in São Paulo - Brazil. A total of 143 breast biopsies were included in this study, which underwent anatomopathological and immunohistochemical examination and which had a leukogram. The variables analyzed were the age of the women, the histological diagnosis with Nottingham's grade, and their correlations with immunohistochemical markers (Ki-67, C-erbB-2, ER, and PR). Based on the reports, four subtypes were defined. **Results:** No positive correlations were found between C-erbB-2, estrogen, and progesterone markers with age, Nottingham classification and phenotypes in the sample studied. For these analyses, the Ki-67 marker was the one that stood out the most with a positive and moderate correlation for phenotypes (molecular subtypes) and age ( $r=0.67$ ). Regarding the molecular subtypes Luminal A, Luminal B, and TNBC, Ki-67 showed a weak correlation ( $r=0.4$ ). Considering the Nottingham classification 1, 2, and 3, Ki-67 presented a positive and moderate correlation for grade 3 ( $r=0.5$ ). The age variation again did not influence the results. There're no significant correlations between IHC finds with WBC results. **Conclusion:** Our data suggest that the Ki-67 cell proliferation marker was the one that most showed positive correlations between the classifications studied for breast cancer, however the suggestion to analyze in combination with other markers that express (AR and E-cadherin expression) as well as Ki-67 status might be useful prognostic markers in TNBC. Hence, these molecular determinants could play an interesting role to classify subgroups of TNBC.

**A-147**

**Designing Multi-Instrument QC Procedures to Reduce the Risk of Patient Harm From Erroneous Results**

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**Background:** Designing QC procedures for multiple identical instruments testing the same analyte has been an open topic for many years and formal guidance is lacking. We evaluated the impact of using a single fixed QC target and SD vs. individual instrument means and SDs for 5 instruments testing the same analytes. The single QC target is the mean of the 5 instruments and the SD is selected so that the false rejection rate of a 1:3s|2:2s|R4S QC rule on a single instrument matches the false rejection rate of the aggregated instruments.

**Methods:** Three months of QC data from 8 analytes were evaluated. For each approach, the maximum risk management index (RMI) and the average RMI were computed for each instrument for each analyte. For the fixed mean/SD and the false rejection rate (FRR) for each instrument for each analyte was also computed.

$$P_E = P_{EI} + P_{EO}$$

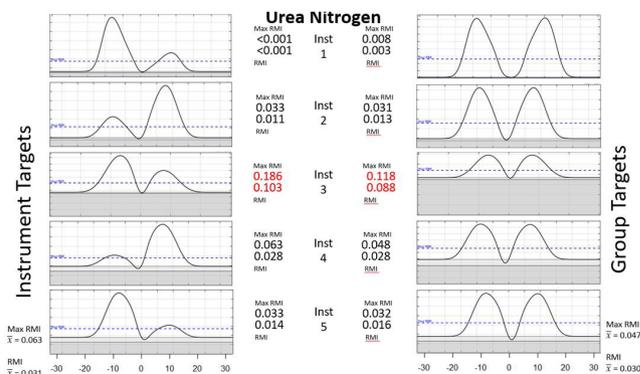
$$P_{PH} = P_E * P_{hu}$$

$$RMI = P_{PH} / \text{Acceptable } P_H$$

Where:

- $P_{EI}$  = probability of producing erroneous results while “in-control”
- $P_{EO}$  = probability of producing erroneous results while “out-of-control”
- $P_E$  = probability of producing erroneous results
- $P_{PH}$  = predicted probability of harm from erroneous results
- $P_{hu}$  = probability that an erroneous result will cause patient harm
- Acceptable  $P_H$  from the risk acceptability matrix

**Results:** Asymmetrical RMI curves have a greater max RMI; higher RMI=higher risk.



RMI plots for Urea Nitrogen from each instrument with the plots on the left using instrument targets (QC target = instrument mean), and the plots on the right is using group targets (QC target = group mean). Risk is managed when RMI ≤ 1. The lower the RMI, the lower the risk.

**Conclusion:** Using a single QC target/SD for managing multiple instruments testing the same analyte reduces the risk of patient harm from erroneous results as the maximum risk will always be less than using individual instrument means and SD's for QC. Using the same QC target and SD across all instruments testing the same analyte focuses attention on the instrument with the most bias and the worst imprecision as it will be the instrument most likely to reject on QC evaluation. This practice should also reduce the work to manage the QC program as there will be fewer QC means and SD's to manage.

Molecular Diagnostics

**A-148**

**Visby Medical Sexual Health Click test: A patient-centric Portable Palm-sized PCR Platform - The Next Generation in Molecular Diagnostics**

T. Abraham, A. Poh, R. Nambudiri, P. Dentinger, A. Lam, S. Roy, B. Cio-pyk, B. Andreyev, D. McSheery, V. Briones, A. Postlewaite, S. Lanning, D. Swenson, P. Cao, J. Albrecht, J. Hong, E. Biba, V. Pham, G. Loney, M. Meyer, G. Schoolnik, A. D. Zerdá. *Visby Medical, San Jose, CA*

**Background:** Traditionally the word ‘diagnostic’ invokes the image of a clinical laboratory with huge instruments and expensive microscopes tucked away in the basement of a hospital. Visby Medical was founded in 2012 with a vision of changing the norm and making diagnostics more accessible. Since then, Visby Medical has built a palm-sized, single-use, platform that combines sample preparation, nucleic acid amplification and the detection of amplified sequences into an integrated 40 - cycle PCR (polymerase chain reaction) device and delivers the results within 30 minutes. This versatile platform was first shown to detect DNA from the pathogens that cause three of the most common and curable sexually transmitted infections (STIs) - Chlamydia, Gonorrhea and Trichomoniasis. Together these pathogens resulted in close to 12.5 million infections in 2021. Although these infections are curable, most of the infections remain undetected particularly in women who often present without symptoms. When left untreated, these STIs can result in infertility, ectopic pregnancies, and premature deliveries in women. Typically, these infections are diagnosed using nucleic acid amplification tests (NAATs) in a laboratory, which usually takes several days to return results. Visby Medical’s Sexual Health Click test is a portable, palm-sized, PCR platform that enables on-site patient testing in a single visit. **Methods:** Performance characteristics of the test were evaluated based on clinical and analytical studies. Clinical studies were carried out at 14 clinical sites encompassing a wide range of CLIA-waived settings including sexual health clinics, university clinics, primary care clinics, and HIV clinics. A total of 1789 subjects were included in the performance evaluation, 48% of whom were asymptomatic. The study subjects were females 14 years and older who provided self-collected vaginal swabs using the Visby Medical Vaginal Collection Kit. Three additional swab samples were collected for each subject for comparator testing. The analytical studies conducted include limit of detection (LoD) determination, inclusivity analysis, cross reactivity, microbial interference, competitive interference, reproducibility studies and studies to determine interfering substances. **Results:** The Visby Medical Sexual Health Click Test had a positive percent agreement (PPA) of 97.4% (95% CI 93.5-99.0%) and negative percent agreement (NPA) of 97.8% (95% CI 96.9-98.4%) for *C. trachomatis* (n = 1774), PPA of 97.8% (95% CI 88.4-99.6%) and NPA of 99.1% (95% CI 98.5-99.4%) for *N. gonorrhoeae* (n = 1786), and a sensitivity of 99.3% (95% CI 96.0-99.9%) and a specificity of 96.7%

(95% CI 95.8-97.5%) for *T. vaginalis* (n = 1765). The panel of analytical studies conducted substantiated the robust clinical performance. Conclusion: The Visby Medical Sexual Health Click Test has now obtained FDA clearance and can be used in CLIA-waived settings including STI clinics, urgent care centers, and others. Results within 30 minutes can now inform data-driven treatment for the patient as well enable a more specific discussion between clinician and patient on STI transmission, long-term complications, prevention and even partner treatment. During the COVID 19 pandemic, Visby Medical used the same platform to build a diagnostic test for SARS-CoV-2 demonstrating the versatile nature of this miniature PCR platform.

### A-149

#### Clinical Evaluation of Aptiva CTD Essential for the Detection of Antibodies Associated With Systemic Lupus Erythematosus in a Spanish Cohort.

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**Background:** Detection of antinuclear antibodies (ANA), specifically anti-dsDNA, anti-Sm and anti-Ribo-P, are important in the diagnosis of systemic lupus erythematosus (SLE). The adoption of fully automated solid phase assays is increasing in clinical laboratories worldwide. This study aimed to evaluate the performance of Aptiva CTD Essential Reagent based on a novel fully automated particle-based multi-analyte technology (PMAT) with reference methods using a cohort of SLE patients from Spain.

**Methods:** Samples from a total of 292 SLE patients along with 1017 individuals with other diseases including celiac disease (n=75), rheumatoid arthritis (n=172), antiphospholipid syndrome (n=30), infectious disease (n=197) inflammatory bowel disease (n=50), Myositis (n=49), Sjögren's syndrome (n=101), systemic sclerosis (n=90), thyroidopathy (n=56) vasculitis (n=11), polymyalgia rheumatica/giant cell arteritis (n=64), other arthritis (n=47), apparently healthy (n=75) were tested for anti-dsDNA, anti-Sm, anti-Ribo-P antibodies, by the novel PMAT system utilizing the Aptiva CTD Essential Reagent (research use only, Inova Diagnostics, USA). Clinical performance was assessed for all analytes including sensitivity, specificity, odds ratios and area under the curve (AUC).

**Results:** The clinical evaluation focused on disease specific markers namely anti-dsDNA, anti-anti-Sm and anti-Ribo-P antibodies (see Table 1). Of the 292 SLE samples, 30.5% (n=89) were positive for anti-dsDNA only, 0.7% (n=2) were positive for anti-Sm only, and 1.7% (n=5) were positive for anti-Ribo-P only. In addition, 2.7% (n=8) were positive for all three analytes and 7.5% (n=22) were positive for a combination of two analytes.

Table 1 - Clinical Performance in SLE markers

Parameter	Aptiva dsDNA	Aptiva Sm	Aptiva Ribo-P	Aptiva dsDNA + Sm	Aptiva dsDNA + Ribo-P	Triple positives
Sensitivity (95% CI)	40.4 (34.9 – 46.1%)	6.2 (3.9 – 9.5%)	9.6 (6.7 – 13.5%)	5.1 (3.1 – 8.3%)	7.5 (5.0 – 11.1%)	2.7 (1.4 – 5.3%)
Specificity (95% CI)	95.9 (94.5 – 96.9%)	100.0 (99.6 – 100.0%)	98.7 (97.8 – 99.3%)	100.0 (99.6 – 100.0%)	99.8 (99.3 – 99.9%)	100.0 (99.6 – 100.0%)
Odds ratio (95% CI)	15.74 (10.70 – 23.16)	+∞ (17.32 – +∞)	8.19 (4.23 – 15.86)	+∞ (14.28 – +∞)	41.35 (10.70 – 159.62)	+∞ (7.42 – +∞)
Area under the curve	0.791 (0.759 – 0.823)	0.641 (0.610 – 0.672)	0.630 (0.598 – 0.663)	N/A	N/A	N/A

**Conclusion:** Anti-dsDNA, anti-Sm and anti-Ribo-P antibodies showed good clinical performance. As expected, dsDNA, Sm and Ribo-P monospecific samples highlight the need for all analytes in the diagnostic testing algorithm.

### A-150

#### Performance evaluation of the QuantStudio 5 Dx Real-Time PCR System and the QuantStudio 7 Pro Dx Real-Time PCR System

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**Background** In response to the COVID-19 pandemic, clinical diagnostic and hospital laboratories have ramped up PCR testing of *in vitro diagnostic* assays for the detection of SARS-CoV-2. To support customers in this space, Thermo Fisher Scientific released two new high-performance qPCR instruments: The Applied Biosystems QuantStudio 5 Dx and QuantStudio 7 Pro Dx. Both systems were designed with increased security and IVD compliance requirements in mind, and the automated QuantStudio 7 Pro Dx features interchangeable 96-well and 384-well block options for maximum flexibility and increased throughput. In this study, we demonstrate and compare the performance of these new Real-Time PCR Systems. **Methods** For each PCR system, we evaluated performance through precision (repeatability and reproducibility) studies and method comparison studies. Each system used a representative, clinically relevant assay for the detection of Influenza A and B in human clinical or contrived nasopharyngeal swab samples. The Lyra™ Influenza A+B Assay (Quidel Corporation) was used to evaluate the performance of the QuantStudio 5 Dx, while the Applied Biosystems™ TaqPath™ COVID19, Flu A, Flu B Combo Kit was used for the QuantStudio 7 Pro Dx. In the repeatability studies, 2 operators conducted 2 runs per instrument (1 QuantStudio 5 Dx instrument and 3 QuantStudio 7 Dx instruments) per day, for 12 non-consecutive days. The run-to-run call concordance was calculated for each instrument-to-user combination. For reproducibility, tests were performed at 3 laboratories by 2 operators on 3 instruments per day, for 5 non-consecutive days. Site-to-site call concordance was calculated to summarize the instrument-to-instrument and operator-to-operator reproducibility for all three sites. For method comparison, the QuantStudio 5 Dx was compared to the QuantStudio Dx (run on a single instrument), while the QuantStudio 7 Pro Dx system was compared to the QuantStudio 5 Dx system (run on three instruments). Positive percent agreement (PPA) and negative percent agreement (NPA) were calculated to demonstrate performance compared to a previously established PCR system. **Results** From the repeatability studies, call concordance was above 95% for all instrument-operator combinations for both systems. Overall site-to-site call concordance from reproducibility studies was 97.89% for the QuantStudio 5 Dx, and 98.67% for the QuantStudio 7 Pro Dx. Results from the method comparison study for the QuantStudio 5 Dx as compared to its predicate system, showed a PPA of 94.44% for the detection of Influenza A, and 98.86% for the detection of Influenza B. The NPA between the two systems was 100% for Influenza A, and 96.43% for Influenza B. Results for the QuantStudio 7 Pro Dx as compared to its predicate system showed a 100% PPA across three separate instruments, for both Influenza A and B. The NPA across three instruments was 99.07%, 99.07%, and 100% for the detection of Influenza A, and 100%, 98.85%, and 98.85% for the detection of Influenza B. **Conclusions** The QuantStudio 5 Dx and QuantStudio 7 Pro Dx Real-Time PCR Systems both display excellent precision, as well as equivalent performance to predicate IVD systems. Our innovative qPCR solutions enable customers to effectively respond to today's rapidly evolving testing challenges.

### A-151

#### Role Of Interleukin-6 In Tuberculosis Patients of India

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**Background:** Tuberculosis (TB) is probably the most seasoned illness of the humanity. Disease progression is dependent on the balance between the microorganism and the host defense systems. Course of the disease or subsequent death from this infection has been frequently connected with cytokine storm. Interleukin-6 (IL-6) plays a crucial role in the immune response to tuberculosis. It is a pleiotropic cytokine produced in response to inflammation and plays a vital role in cellular proliferation and death. Studies have demonstrated that IL-6 plays a role in potentiating immunity during early infection. The aim of the present study was to estimate circulating IL-6 and its gene expression levels in the peripheral blood of TB patients and healthy controls and to assess its diagnostic efficacy. **Methods:** 80 sputum positive TB patients and 80 apparently healthy controls were enrolled in the study taking into account the exclusion and inclusion criteria. A written informed consent was obtained from all the participants. 6mL venous blood was withdrawn in plain and EDTA vacutainers under strict aseptic conditions. Serum IL-6 levels were estimated using ADVIA Centaur Interleukin-6 assay, an automated direct chemiluminescent immunoassay. Total RNA was isolated from whole blood, converted to cDNA and gene expression of IL-6

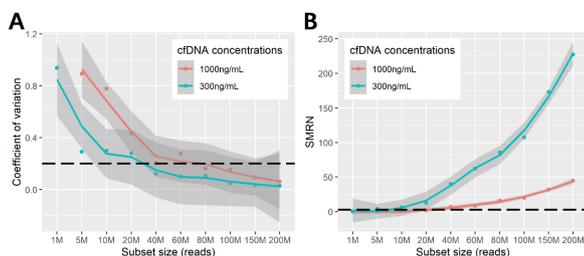
was done using Sybr green quantitative real time PCR technology. Statistical analysis was performed using SPSS. **Results:** The median (IQR) of serum IL-6 was significantly increased in TB patients compared to controls (18.2 (31.8) vs 0.9 (1.35) pg/mL);  $p < 0.0001$ ). Relative IL-6 expression was significantly upregulated with a fold change value of 5.46 in TB patients that positively correlated with its protein levels. On ROC analysis, IL-6 discriminated TB patients from healthy controls at 2.45pg/mL with a sensitivity and specificity of 92% and 86% respectively and an area under the curve of 0.957. **Conclusion:** Serum IL-6 levels and its expression were found to be significantly elevated in TB patients when compared to healthy controls. ROC shows good diagnostic sensitivity and specificity, which can be explored in future to monitor the progression of infection in TB patients as it is the major cytokine that plays a role in the clinical manifestations and pathological events during TB infection.

## A-152

### Evaluation of analytical sensitivity of metagenomic sequencing test for the bloodstream

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**Background:** Metagenomic testing is a powerful tool to identify broad-range pathogens in an unbiased manner. However, its technical assessments about the detection sensitivity and stability of microbial cell-free DNA (cfDNA) remain unknown for clinical applications to bloodstream infections. **Methods:** The performance features were established in two different human plasma cfDNA concentrations representing medium-human-plasma and high-human-plasma. Fragmented genomic DNA from *Klebsiella pneumoniae* was spiked into healthy human plasma to simulate medium- and high-human-plasma specimens. Both contrived samples containing identical LoD concentration of 1,000copies/mL *Klebsiella pneumoniae* were used for metagenomic sequencing to yield more than 200 million (M) reads. We randomly resampled sequencing reads from each sample to desired sub-dataset sizes of 1M, 5M, 10M, 20M, 40M, 60M, 80M, 100M, 150M, 200M reads and repeated 10 times. Downstream bioinformatic analysis was performed by PMSeq Datician analysis system, which enabled rapid, accurate and effortless identification of pathogens. Stringently mapped reads number (SMRN) was equivalent to the species-specific reads number. **Results:** The 74.2nd and 94.2nd percentiles of plasma cfDNA concentrations observed in totally 481 clinical blood samples were about 300ng/mL for medium-human-plasma and 1,000ng/mL for high-human-plasma, respectively. We calculated the coefficient of variations (CV) and 95th percentile of *Klebsiella pneumoniae* SMRN in two human background levels at various sequencing data sizes (Figure AB). With the increase of data size, CVs decreased correspondingly, which showed better stability. The minimum-sequencing-size for medium-human-plasma need at least 40M to reach CVs below dotted-line-marked 0.2, for high-human-plasma at least 80M. Microbial SMRN increased with sequencing data size. The threshold of test positive was at dotted-line-marked 3 SMRNs. The minimum-sequencing-size of high-human-plasma required at least 30M to detect positive microbial cfDNA. **Conclusion:** Raising sequencing data size to above 80M in any human plasma background brought about significant increase in the sensitivity and stability of microbial cfDNA detection in bloodstream.



## A-153

### Identification of SARS-CoV-2 Variants by RT-qPCR Genotyping as a more Affordable Option for Laboratories

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**Introduction:** Throughout 2021, several variants of SARS-CoV-2 emerged, grouped into variants of concern (VOC), of interest (VOI) and monitoring (VBM). Rapid dissemination generates the need for constant epidemiological surveillance and through NGS sequencing, this need is limited by several factors such as: costs, technology, time, among others. In this sense, it is extremely necessary and interesting to study techniques that allow the monitoring of these variants over time, quickly and at low costs. This study aims to compare RT-qPCR Genotyping and NGS Sequencing techniques and identify SARS-CoV-2 variants existing between June and November 2021 at the Molecular Biology sector of Laboratory Saude in Goiania, Brazil. **Methods:** The qPCR-Genotyping and NGS techniques were compared regarding: price, bench time, TAT (Turnaround time), machinery cost (implantation), need for specialized professionals, effectiveness for known variants and effectiveness for new variants. 832 genotyping was performed using the RT-qPCR Genotyping methodology to detect point mutations of the SARS-CoV-2 S protein through positive samples with known Cts (Cycle threshold) and below 35. The viral RNA extraction was performed using the MagaPure virus DNA/RNA purification KIT (BIGFISH, Hangzhou). PCR assays were performed using TaqMan™ SARS-CoV-2 Mutation Panel Assay (ThermoFisher, USA) targeting mutations in the S protein (N501Y, E484K, K417T, K417N, DEL69/70, P681H, T20N, L452R, P681R, T478K, E484Q and V1176F), capable of detecting 9 of the variants between VOC and VOI of the time: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Zeta (p.2), Epsilon (B.1.429), Theta (P.3), Eta (B.1.525), Kappa (B.1.617.1) and Delta (B.1.617.2). Reactions utilized positive control (1x10<sup>6</sup> copies/μL) (Twist Bioscience, USA). **Results:** Seven commercial characteristics of utility for epidemiological surveillance were evaluated between the RT-qPCR and NGS Genotyping methodologies, with the RT-qPCR methodology with 57.1% (4/7) of the evaluation preferences, with two requirements (need for specialized professionals and effectiveness for known variants) showed a technical tie in the evaluation 28.5% (2/7) and a requirement (effectiveness to identify new variants) 14.2% (1/7) was overcome by the NGS methodology. The 832 genotyping of SARS-CoV-2 variants yielded the following results: a) 4 different variants (Alpha, Gamma, Theta and Delta) were identified; b) Only 2 samples did not work, representing 0.2% (2/832) of the tests; c) Variants were identified at the following frequency: 0.1% (1/832) Alpha (B.1.1.7), 0.1% (1/832) Theta (P.3), 36.5% (304/832) Gamma (P.1) and 62.9% (524/832) Delta (B.1.617.2); d) Identified the transition of the Gamma variant in relation to the Delta variant. **Conclusion:** The RT-qPCR Genotyping methodology proved to be an excellent option for small and medium-sized laboratories, and it can be effective on identifying the variants of SARS-CoV-2 already known, both for epidemiological surveillance and for laboratory routine, with cost 4 times lower and with a response time (TAT) of 5h. Four out of nine variants described were identified and it was possible to follow the loss evolution of Gamma variant's dominance and Delta's rising in the capital of Goias State - Brazil.

## A-154

### SARS-CoV-2 ECLIA Antigen Test Performance Compared to RT-PCR Positive Samples from Central Brazil

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**Background:** Laboratory diagnosis of SARS-CoV-2 infection is one of the main healthcare system strategies for studying the disease epidemiology and for monitoring viral transmission. The purpose of this study was to evaluate the performance of an electrochemiluminescence immunoassay (ECLIA) to detect SARS-CoV-2 antigen in comparison to positive samples diagnosed by RT-PCR, the gold standard methodology for the viral diagnosis of COVID-19. **Methods:** One hundred RT-PCR SARS-CoV-2 positive nasopharyngeal swab specimens collected in saline solutions from clinically suspected patients who went to our laboratory facility were selected for the study. RNA was extracted using a magnetic-bead based extraction protocol as a manufacture's instruction, TANBead Maelstrom™ 4800 (Taiwan Advanced Nanotech). After extraction, the real-time RT-PCR for SARS-CoV-2 detection was performed

using a 2019-nCoV Nucleic Acid Detection Kit (PCR-fluorescence probing) (Bio-Germ Medical Biotechnology Co. Ltd.) The BioGerm kit detects the ORF1ab gene and the N gene. The result was determined as positive when the CT values of both the ORF1ab and N genes were  $\leq 38$ ; a negative result was determined when the CT values of both the ORF1ab and N genes were  $>38$ . When only one of the genes had a CT value  $\leq 38$ , a presumptive positive result was assumed but retesting was required; if the ORF1ab or N gene Ct value was  $\leq 38$  for the second time, the result was considered positive. All RT-PCR positive samples were tested for antigen detection using electrochemiluminescence immunoassay, (ECLIA) following manufacture's instruction, Elecsys® SARS-CoV-2 Antigen. This assay uses monoclonal antibodies directed against the SARS-CoV-2 N protein in a double-antibody sandwich assay format for the qualitative detection of SARS-CoV-2 in upper respiratory tract specimens. A reactive test result was determined when cutoff was 1.0 and a non-reactive test result was considered when cutoff was  $< 1.0$ . In order to compare the performance of the ECLIA antigen test with the SARS-CoV-2 RT-PCR test, the 100 RT-PCR positive samples were classified into 3 categories, according to CT values: CT  $\leq 25$  (33 samples), CT 25-30 (32 samples) e CT  $>30$  (35 samples) and the concordance between the tests was calculated. Chi square tests in SPSS v.26 were used for statistical analysis. **Results:** SARS-CoV-2 ECLIA antigen test and RT-PCR were 100% concordant in the 33 samples with CTs  $\leq 25$ . In the 25-30 CT range, 20/32 (62.5%) were concordant (p= and when CTs were  $>30$ , only 4/35 (11.4%) were concordant. **Conclusion:** Antigen testing through ECLIA methodology showed good correlation with the RT-PCR when SARS-CoV-2 viral shedding was high but when viral loads were lower, correlation dropped significantly. SARS-CoV-2 ECLIA antigen is a valuable viral diagnostic tool but its interpretation requires a careful clinical and epidemiological evaluation of the patient being tested. A molecular test such as the RT-PCR should be recommended when negative antigen tests are found in highly suspicious SARS-CoV-2 infected patients.

## A-155

### Incorporating spinal muscular atrophy screening by next-generation sequencing into a comprehensive multigene panel for newborn sequencing: a pilot evaluation

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**Background:** Newborn sequencing is a powerful tool for improving the future health-care of infants because of its potential to screening for a wide range of disorders with a single test. Spinal muscular atrophy (SMA) screening would be one of the tested conditions due to its high incidence rate (1 in 6000-10,000 live births) and disease severity (leading cause of infant death after cystic fibrosis). However, SMA screening are not routinely performed on NGS data of targeted sequencing because the disease-causing gene (SMN1) has a near identical paralog (SMN2) being difficult to determine if sequences are from SMN1 or SMN2 (they are discarded by bioinformatics pipelines). The coding region of SMN1 differs from SMN2 by a variation in exon 7 (c.840C>T), the c.840C nucleotide is a proxy for SMN1. The biallelic absence of SMN1:c.840C in qPCR-based SMA screening indicates the deletion of both SMN1 copies (or its conversion to SMN2), mutational events that causes 95-98% of SMA cases. Thus, we aim to validate an SMA screening using a NGS targeted sequencing mimicking the PCR-based SMA screening by testing for the biallelic absence of c.840C nucleotide unique to SMN1 gene.

**Methods:** DNA samples screened for SMA by qPCR (TaqMan SCID/SMA - Thermo-fisher) were enrolled in this validation (n=52, 46 with at least one SMN1 copy and 6 with homozygous loss of this gene). 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). Used panel includes more than 4700 genes, a newborn sequencing sub-panel of 388 genes is derived from the data and exon 7 of SMN1/2 gene is targeted. Read alignment were performed with Dragen Enrichment v.3.9.5 (Illumina). BCFtools mpileup command was used for calling the nucleotide at c.840 genomic position of SMN1/SMN2 directly from BAM file. SMN1/SMN2 genes only share sequences with each other allowing the setting of mapping quality to zero avoiding their exclusion during nucleotide calling. Results interpretation were: SMN1:c.840C coverage depth  $\geq 8X$  - at least one copy of SMN1, between 7-1X undetermined, 0X homozygous loss of SMN1. Samples with clinical exome sequencing coverage and depth  $<97.5\%$  20X were excluded. The total, positive and negative agreement between qPCR and NGS were computed along with their respective 95% confidence interval.

**Results:** Four samples presented sequencing coverage and depth  $<97.5\%$  20X and were excluded. Samples with at least one SMN1 copy by qPCR showed SMN1:c.840C coverage depth ranging from 15X to 53X with median of 26X. No NGS result was

classified as undetermined. Samples with homozygous loss of SMN1 by qPCR presented no nucleotide at SMN1:c.840C position. Total, positive and negative agreement between NGS and qPCR were 48/48 (100% 95%CI 93.1-100%), 6/6 (100% 95%CI 61-100%) and 42/42 (100%, 95%CI 92.3-100%), respectively.

**Conclusion:** There was complete agreement between qPCR and NGS in the detection of SMN1 biallelic loss using c.840C nucleotide as proxy. This result suggests that SMA can be screened from NGS targeted sequencing data.

## A-156

### Simultaneous Tumor Theranostics with Tetrahedral DNA-assisted Catalytic Hairpin Assembly

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#### Background:

Early detection of cancers has been demonstrated to be a highly effective practical component in reducing complication and fatality. MicroRNA is being thought as a potential diagnostic and therapeutical biomarker for early cancer detection owing to its close association with a variety of tumors. Although, numerous attempts have been tried, simultaneous tumor detection and efficient therapy has not yet been realized. Integrating miRNA detection with therapeutic modalities is crucial for monitoring cancer progression in response to therapy and establishing a synchronous theranostics platform for cancer prognosis and precision medicine.

#### Methods:

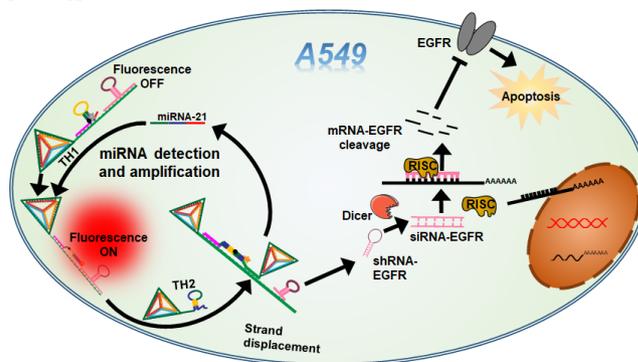
We deliberately designed a nano-system that contains two DNA tetrahedrons equipped with distinctive catalytic hairpin probe elements. The DNA tetrahedrons have featured rigid geometries for higher membrane penetration. One tetrahedron (TH1) contains fluorescence-labelled catalytic hairpin-1 and shRNA, and another tetrahedron (TH2) contains hairpin-2 that is responsible for the release of shRNA and the cyclic process of Catalytic hairpin assembly (CHA) for *in-situ* cancer theranostic. The proposed system was validated by *in-situ* molecular imaging of endogenous microRNA-21, EGFR protein inhibition and cell apoptosis. We evaluated the proposed approach feasibility by comparing its analytical results to conventional methods.

#### Results:

We constructed the nano-system using two DNA tetrahedrons. The presence of specific microRNAs triggers the CHA process which simultaneously release shRNA through strand displacement reaction, initiating RNAi-based post-translational gene silencing. Additionally, a target concentration-dependent fluorescence increase was observed within a linear from 100pM to 10nM. Under optimal operating parameters, we achieved a detection limit of 3.2 fM and up to 35% EGFR protein inhibition, resulting in 54.3% cell apoptosis.

#### Conclusion:

We described a sensitive, specific, and non-destructive approach for the simultaneous detection of microRNAs and EGFR post-transcriptional gene silencing *in-situ*. This method provides a novel strategy to investigate microRNA abundance and *on-site* cancer progression surveillance, facilitating subsequent clinical diagnostic and therapeutic applications.



## A-158

## Gut microbiota assessment in healthy adults from North America and Canada

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**Background:** A healthy microbiota profile constitutes the baseline from which deviations correlating to disease states are assessed. Understanding what constitutes a healthy microbiome and establishing a healthy reference microbiota is important for developing tools for microbiota analysis. Our objective was to characterize the gut microbiota composition of healthy adults in North America, and to assess similarities to healthy Europeans. **Methods:** 88 healthy volunteers aged 18-40 years from two locations (Emory University Hospital, USA and Calgary University, Canada) each provided 2 fecal samples (collected 1 week apart) that were analyzed with the GA-map® Dysbiosis Test Lx (Genetic Analysis, Norway). The GA-map® test utilizes the 16S rRNA gene variable regions V3-V9 to characterize gut bacterial profiles. The test detects 48 predefined bacterial markers that can distinguish the microbiota profile of dysbiotic IBS or IBD patients from healthy. Using a healthy reference microbiota, the GA-map® algorithm calculates a dysbiosis index (DI) with scores of normobiosis (DI=1-2), mild dysbiosis (DI = 3) or severe dysbiosis (DI = 4-5). **Results:** DI distribution of the samples from two sites are as shown in figure 1. A median DI of 2 was found at both week 1 and week 2. Overall, 74% of the subjects were non-dysbiotic, which correlates well to the European validation cohort with 84% non-dysbiotic (1). **Conclusion:** The results showed that the microbiota from healthy adults in North America is similar to the healthy reference profile established for the GA-map® test and validated in healthy EU adults. Variability between the two North American sites was small, and the week-to-week microbiota measurements within one subject showed a high degree of intra individual stability in the microbiota. Ref 1 Casén C, *et al.* (2015). *APT*; 42(1):71-83.

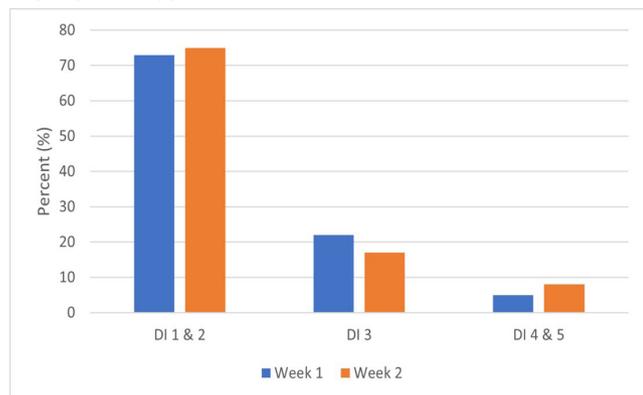


Figure 1. DI distribution (percentage) in fecal samples from healthy volunteers from North America, at week 1 (n=88) and 2 (n=88).

## A-159

## Comparison of Laboratory Developed Tests (LDT) on Molecular Multiplex Platforms used for Urinary Tract Infection Detection in Proficiency Testing Studies

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**Background:** Rapid identification of pathogens causing urinary tract infections (UTI) provides high value to health care providers, guiding decisions made regarding treatment. Many laboratories have implemented molecular technologies to identify the causative agents of UTI's and screen for resistance genes. CLIA '88 mandated proficiency testing for physician office laboratories beginning in 1994, which was an expansion of the requirement for hospital and reference laboratories instituted under CLIA '67. This study compares the accuracy of laboratory performance for LDT completed on molecular multiplex platforms in the detection of urinary pathogens from gold standard qualified samples in a novel proficiency program.

**Methods:** American Proficiency Institute, an independent proficiency testing provider, studied the recovery of laboratory developed molecular multiplex systems against simulated patient samples in the first test event of 2022. Participants were instructed to only report results for organisms and resistance genes their systems were capable

of testing for from a list of 28 bacterial species, 7 yeast species, and 27 resistance genes. Failure rates for participants were evaluated against an 80% participant consensus standard for bacterial identification, mycology identification and detection of resistance genes.

**Results:** Bacterial and fungal pathogen identification was reported by 101 total laboratories. Accuracy for correct identification of negative targets ranged between 76.0%-100%, while accuracy for correct identification of positive targets ranged between 70.7%-94.9%. Resistance gene detection was reported by 79 total laboratories who provided results for 27 resistance genes. Overall accuracy of resistance genes was less decisive, with consensus for positive targets ranging between 48.5%-80%.

**Conclusion:** Based on the results reported by participants for API's initial proficiency event, LDT Molecular Multiplex platforms for UTI detection are accurately identifying pathogens, however, they lack consistency and proper reporting of resistance genes. Most respondents reported resistance genes not appropriate for the organism recovered, potentially leading to inappropriate antimicrobial therapies being prescribed.

## A-160

## RNase L Contributes to the Development of Nonalcoholic Steatohepatitis (NASH) by Regulating the Expression of the Key Genes Involved in Lipid Metabolism, Immune Responses, and Fibrosis Signaling

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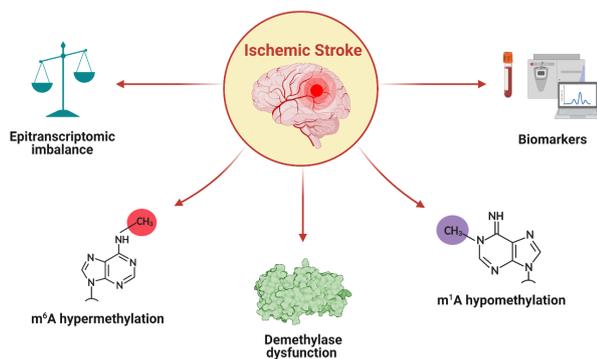
**Background:** Nonalcoholic fatty liver disease (NAFLD), intimately associated with metabolic risk factors, has become the most common chronic liver disease worldwide. Progression to nonalcoholic steatohepatitis (NASH) and even cirrhosis substantially increases disease morbidity and mortality. However, the molecular mechanism underlying the development of this disease remains to be elucidated. RNase L is well known for its role in innate immunity, and exerts potent effects on cellular gene expression to mediate diverse biologic activities. Interestingly, our preliminary data showed that RNase L mediated macrophage lipid metabolism through regulating the expression of scavenger receptors. Furthermore, deficiency of RNase L increased the expression of fatty acid synthase (FASN) in the liver of RNase L knockout mice, as revealed by LC-MS, qPCR and immune blot analysis. In this study, we investigated whether RNase L is involved in the pathogenesis of NAFLD by modulating immune response and/or lipid metabolism. **Methods:** Age-matched male wild type (WT) and RNase L knockout (RL-KO) mice (n=6-8) in the C57BL/6 background were fed with a High-Fat-High-Cholesterol Diet (HFHCD) either for 12 weeks or 22 weeks to induce NAFLD and NASH. The mice were weighed every week. After the termination of the experiments, the blood and liver tissues were collected. The samples were used to: 1) determine the development of steatosis/steatohepatitis by performing histological staining (H&E and Trichrome); 2) analyze the parameters associated with physiological changes, such as the development of obesity, dyslipidemia, and systemic inflammation by biochemistry tests and complete blood count (CBC); and 3) investigate the activation of the key cellular pathways related to lipid metabolism, inflammation signaling, fibrogenic signaling. Technical methods include ELISA, RT-PCR, and Western Blot analysis. **Results:** 1) Histological staining revealed that RL-KO mice showed significantly more and larger lipid droplets, although both types of mice developed simple hepatic steatosis after being fed with HFHCD for 12 weeks. Interestingly, the mice fed with HFHCD for 22 weeks developed NASH, and the liver tissues of WT mice exhibited substantially more severe NASH, evidenced by a larger degree of hepatocyte damage, lobular inflammation, and fibrosis. 2) Examination of the physiological changes showed that RL-KO mice gained more body weight, and lipid (cholesterol, triglyceride) and liver enzyme (ALT, AST) patterns were different between two genotypes. Besides, some parameters of CBC also showed differences between WT and RL-KO mice, among which the WBC counts were significantly elevated in WT mice. 3) Further investigation of molecular mechanism demonstrated that RNase L might be involved in the development of NAFLD through regulating the expression of the key genes in the lipid metabolism (FASN and HMGCR), several cytokines and chemokines (IL-1 $\beta$ , IL-10, TGF- $\beta$ , CCL2). **Conclusion:** These results suggest that RNase L is involved in lipid homeostasis, contributing to NAFLD development and progression through many aspects, including regulating the expression of the key genes in the lipid metabolic pathway, immune responses, and fibrogenic signaling. Our findings may provide a better understanding of the driving factors in disease progression from NAFL to NASH, and help develop diagnostic and prognostic biomarkers, and effective treatment for individuals with NASH.

## A-161

## Clinical utility of RNA chemical modifications as biomarkers for acute ischemic stroke

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**Background:** Cellular RNAs are pervasively tagged with diverse chemical moieties (~ 172) collectively defined as epitranscriptomic modifications. These modifications form an additional layer of post-transcriptional control, facilitating the fine-tuning of gene expression. Recent studies showed that epitranscriptomic modifications regulate multiple physiological (synaptic plasticity and neurogenesis) and pathological (tumorigenesis and nerve injury) processes of the brain. However, their role in the pathogenesis of ischemic stroke remains elusive. **Methods:** To estimate the gross abundance of epitranscriptomic modifications in the ischemic brain, we employed an *in vivo* experimental stroke model of transient middle cerebral artery occlusion (MCAO) and performed LC-MS/MS and immuno-dot blotting. We also quantified the expression of epitranscriptomic enzymes responsible for deposition and removal of these modifications in the ischemic brain by real-time PCR and western blotting. **Results:** The two common RNA methylation marks, N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) and N<sup>1</sup>-methyladenosine (m<sup>1</sup>A) are found to be highly sensitive to cerebral ischemia. In male mice subjected to transient MCAO, m<sup>6</sup>A levels increased and m<sup>1</sup>A levels decreased significantly in the peri-infarct cerebral cortex compared to sham control. Expression of m<sup>6</sup>A and m<sup>1</sup>A methylases remain unaltered following cerebral ischemia. Whereas the expression of the m<sup>6</sup>A demethylase decreased and m<sup>1</sup>A demethylase increased significantly in the peri-infarct cerebral cortex compared to sham control. **Conclusion:** Therefore, ischemia-induced downregulation of m<sup>6</sup>A demethylase potentially elevates m<sup>6</sup>A, while upregulation of m<sup>1</sup>A demethylase possibly diminishes m<sup>1</sup>A in the cortex. As m<sup>6</sup>A and m<sup>1</sup>A regulate vital mRNA processing steps, their modulation might drive the post-stroke brain damage. This is the first study to show that stroke robustly alters cerebral epitranscriptome, opening new avenues for molecular diagnostics.



## A-163

## Estimation of the prevalence of hypophosphatasia in the Spanish population

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**Background:** Hypophosphatasia (HPP) is an ultra-rare genetic disease caused by one or more loss-of-function mutations in the gene encoding tissue-specific alkaline phosphatase (TNSALP). HPP is considered an inborn error of metabolism with highly variable expression, but is usually implicated in a defect in bone and dental mineralization. García-Fontana C., *et al.* in 2019 estimated the prevalence of moderate HPP in the Spanish population at 1/3100, similar to that estimated by Mornet, *et al.* in the European population in 2021 (1/2430).

**Methods:** The blood tests of 64013 subjects with any determination of alkaline phosphatase (ALP) activity in the laboratory of the Hospital Universitario Clínico San Cecilio de Granada were prospectively reviewed. Subjects with ALP activity below the reference range were selected and those with secondary hypophosphatasemia

(coronary artery bypass surgery, celiac disease, hypothyroidism, Cushing's syndrome, massive transfusion, multiple myeloma, treatment with clofibrate, bone antiresorptive, glucocorticoids, chemotherapy, etc.) were excluded. Those subjects with persistently low ALP activity had their serum pyridoxal-5'-phosphate (PLP) concentrations measured and subsequent sequencing of the *ALPL* gene. Serum ALP activity was determined by absorption spectrophotometry on AU5800 analyzers (Beckman Coulter), using the method based on the recommendations of the International Federation of Clinical Biochemistry (IFCC). The ALP reference values stratified by age and sex used in the pediatric population were obtained from the CALIPER study, while for the adult population they were those recommended by the IFCC. The determination of PLP was performed by isocratic HPLC with fluorescence detector. The PLP reference values ranged from 3.6 to 18 ng/mL. For the genetic study, Sanger sequencing of the coding regions and exon-intron junctions of the *ALPL* gene was performed using the truncated sequence NM\_000478.4 as reference.

**Results:** Of the 64013 subjects with ALP determination, 765 subjects with decreased ALP activity were selected. Of these, 115 subjects had persistently decreased ALP activity. After study of the medical records of these subjects, 54 were ruled out for possible causes of secondary hypophosphatasemia. Of the resulting 61 subjects with suspected HPP, only 24 patients who had their ALP levels measured participated in the study. Of these, 17 adults and 3 children had PLP concentrations above the reference ranges. Of the 17 adult patients, 11 of them had previously described mutations in the *ALPL* gene: p.(Val128Met), p.(Ala443Thr), p.(Gly456Arg), p.(Trp186\*), p.(Arg136His), p.(Gly112Arg), p.(Ala443Val), c.(473-2A>G) (2 patients) and p.(Glu191Lys) (2 patients). In addition, 3 of the 17 adult patients had previously undescribed variants: p.(Pro292Leu), p.(His379Asn) and p.(Glu23\_Lys24del), the latter being a variant of uncertain significance. Of the 3 patients in the pediatric population, 2 of them had previously undescribed *ALPL* gene mutations: p.(Leu6Ser) and p.(Thr167del).

**Conclusion:** More than half of the study population with high suspicion of HPP (62%) was diagnosed with the disease. If we extrapolate these data to the current population of Spain and taking into account the proportion of patients with suspected HPP who did not participate in the study (60%), the estimated prevalence of moderate HPP in our study could almost double the estimates previously made in the Spanish population (1/1692 vs. 1/3100).

## A-164

## Prevalence of molecular and serological tests of the new coronavirus SARS-CoV-2 in the Clinical Laboratory Analysis in Cuiabá, MT, Brazil

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**BACKGROUND:** Coronavirus disease 2019, which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), became a pandemic in 2020, with a fatality rate of 2% and strong transmissibility, necessitating epidemiological investigations. This virus spreads swiftly by aerosols. As a result, due to general factors ranging from health concerns to the manner of life to which they are exposed, some demographic groups are more sensitive to being afflicted by the disease. Patients who fit the criteria for suspected cases should be tested for SARS-CoV-2 using nasal swab samples taken from the nasopharyngeal mucus. Reverse transcription-polymerase chain reaction (RT-PCR) detects the RNA, and a positive test validates the diagnosis of COVID-19. The aim of this investigation was to verify the prevalence the people who performed the molecular and serological tests for SARS-CoV2 in a clinical chemistry laboratory in Cuiabá, Brazil in Cuiabá to contribute to epidemiological surveillance of each citizen to decrease the transmissibility of SARS-CoV-2. **METHODS:** All samples taken from nasal swabs analysed by RT-PCR and serological (SOROVID-19) for severe acute respiratory syndrome coronavirus 2 and IgM/IgG from the population attended between April and December 2020 were used in this retrospective cross-sectional analysis. Data was tabulated and reported as absolute frequency and percentages. Prism software v.5.04 (GraphPad) was used to calculate the temporal trend and age distribution of the SARS-CoV2 cases found in the research laboratory. The Cuzik test was used to examine the time trend across. **RESULTS:** There were

23,631 RT-PCR for SARS-CoV2 assays performed throughout the analyzed period, 7,649 (32.37 %) tested positive, whereas 15,982 (66.31 %) did not identify viral RNA and 374 of the findings were unclear. The highest number of positive RT-PCR results were found in July (n=5,878), with 35.65% (n=2,096) of total cases. On the serological test SOROVID-19, a total of 8,884 tests were performed, with a peak of 1,169 (57.16%) positive tests for severe acute respiratory syndrome coronavirus 2 in July. Individuals between the ages of 20 and 59 performed the most exams in that month, accounting for 4,296 (56.2%) of the total number of cases and 3,353 (43.8%) of the men between April and December 2020. There were 5,778 tests conducted in July, with 2,102 (36.38 %) being confirmed positive for PCR COVID and 3,676 (63.62 %) being confirmed negative. Of the total cases in July, 3,468 (60.0%) were women and 2,310 (40.0%) were men, remaining in the same range of individuals aged 20–59 years. No temporal trend was observed with either SOROVID (p=0.561) or PCR (p=0.289). CONCLUSIONS: Molecular positivity and serological tests, both peaked in July 2020, were mostly present in women aged 20–59 years, characterizing Cuiabá as the epicentre of the Midwest region in this period due to the high rate of transmissibility of SARS-CoV-2.

**A-165**

**Potential Effect of the Accredited Vaccines on Body Protection Against SARS-CoV-2 Infection, in Iran.**

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**Background:**

Analyzing antibodies against spike-receptor binding domain (S-RBD) represented valuable tools to assess potential individual body protection against the current pandemic disease, COVID-19. There are still limited data available evaluating anti-RBD level in either affected or vaccinated individuals, particularly in Iran. In this study, we aimed to compare level of anti-RBD antibody in the Iranian patients and normal individuals before and after vaccination.

**Methods:**

A total of 168 individuals were classified in five different groups, including (A) those who were neither disease affected nor administrated vaccine, (B) those who were disease affected but not administrated vaccine, (D) previously affected individuals who were injected two vaccine shots against SARS-CoV-2 and (F) non-affected individuals who were administrated two vaccine shot(s). Peripheral blood sample was obtained from different groups and serum IgA/IgG anti-RBD antibody was analyzed using ELISA kit (Razi Institute, Iran). A cut-off was considered positive for anti-RBD level (≥1.15).

**Results:**

Data analyses showed anti-RBD level of ≥1.15 in only 21% of the group (A) individuals. This antibody level was detected in 78% of the group (B) individuals. Curiously, level of the male antibody was significantly higher than female in this group. Positive antibody anti-RBD level was observed in the all group (D) members, while it was positive in only 96% of the group (F) individuals. Further investigations demonstrated antibody level of ≥7 in 92% of the group (D), while this was only detected in 71% of the group (F) samples. Curiously, compared to the group (F) with 24%, only 17% of the group (D) members representing anti-RBD level of ≥7 were later infected by COVID-19. These findings suggested lower chance of infection by raising the level of anti-RBD.

**Conclusion:**

Analyses of anti-RBD antibody level suggested that potential of body protection against SARS-CoV-2 was significantly higher in affected male compared to the affected female. Additionally, less chance of COVID-19 infection in the previously infected individuals administrating accredited vaccines, in Iran, suggested that booster antibody level and subsequently higher chance of protection against SARS-CoV-2.

**A-166**

**Performance evaluation of a High Throughput automated RT-PCR platform for the detection of SARS-CoV-2**

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**Background:**

The COVID-19 pandemic has led to an unprecedented need for large scale diagnostic testing, especially during surges. Thermo Fisher’s Amplitude™ Platform is a high-throughput laboratory platform for the extraction and qualitative detection of nucleic acid by realtime PCR, capable of processing up to 8,000 samples in a 24-hour period. The aim of this study was to evaluate the clinical performance of the TaqPath™ COVID19 HT Kit on the Amplitude™ Platform for detection of SARS-CoV-2.

**Methods:**

The retrospective study was performed on 554 upper respiratory tract samples collected in the United Kingdom in December 2021. All samples were tested in parallel in a blinded and randomized manner using the TaqPath™ COVID19 HT Kit on the Amplitude™ Platform and the cobas® SARS-CoV-2 Assay on the Roche 6800 instrument. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were assessed.

**Results:**

Of 554 samples, 1 sample was excluded from the analysis due to repeated inconclusive or invalid results using either of the two methods. Of 152 samples positive by the cobas® assay, 151 samples showed a positive result using the TaqPath™ COVID19 HT Kit, resulting in the PPA of 99.34%. Of the 401 samples negative by the cobas® assay, 396 samples tested negative using the TaqPath™ COVID19 HT Kit resulting in a NPA of 98.75%. The one discordant sample that tested negative using the TaqPath™ COVID19 HT Kit yielded a presumptive positive result on the cobas® assay with only E-gene target positive (Ct=38.26). All the 5 samples which tested negative using the cobas® assay had low viral loads detected by the TaqPath™ COVID19 HT Kit (Ct>32).

**Conclusion:**The TaqPath™ COVID19 HT Kit is a highly accurate method for the detection of SARS-CoV-2. The Amplitude™ Platform is an ideal platform for high volume molecular testing laboratories for COVID-19 testing, for scalable testing requirements.

		cobas® SARS-CoV-2 Assay		
		Positive	Negative	Total
TaqPath™ COVID-19 HT Kit on Amplitude™ Platform	Positive	151	5	156
	Negative	1	396	397
	Total	152	401	553
Positive Percent Agreement (PPA)		99.34% [96.37% to 99.88%]		
Negative Percent Agreement (NPA)		98.75% [97.11% to 99.47%]		

**A-167**

**Direct RT-PCR of SARS-CoV-2 on Gargle Samples for a Safe and Open University Campus**

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**Background** To keep the campus free of COVID-19 transmission while allowing students and staff to safely resume their activities, Technical University Dortmund has developed a “no-COVID-19” strategy, which includes as one of their central pillars a high-frequency, high-quality testing strategy. Using routine tap-water for mouth gargles is the most convenient sample type, does not need special transport medium or swabs, deploys reusable tubes, and can be performed at the comfort of one’s home.

The TaqMan™ SARS-CoV-2 Fast PCR Combo Kit 2.0 is a direct RT-PCR assay that does not require RNA extraction, thereby enabling faster time to result (<2hours) as compared to standard extraction-based methods. In this study, we evaluated the performance of the direct RT-PCR testing method for stopping spread of COVID-19 in a University setting. **Methods** University employees, students and their family members provided self-collected samples by gargling tap water up to four times/week from October 2021 - February 2022. Samples were collected in pools of maximum 10 samples. Pooled samples were tested using the TaqMan™ SARS-CoV-2 Fast PCR Combo Kit 2.0. Results were published online on the same day without specific personal information. Positive pools were resolved by individual sample testing, results of which were available the next day, at the latest. If a screening result was positive, those individuals were referred for confirmatory COVID-19 diagnostic testing. In addition, participants were provided with free rapid antigen tests (RADT) and the results of the pooled PCR testing using gargles were compared against results of the RADT. **Results** In total 102 positive cases were detected over the 4-month period. No known false negative tests were recorded using the direct RT-PCR on pooled samples approach among the approximately 25,000 tested individuals. To evaluate false negative rate, our strategy was accompanied by a very extensive antigen testing strategy of university members. In total 80,000 RADT tests were performed in parallel to the 25,000 PCR tests. Only a single false positive test was detected, source of which was likely a human error which was resolved within 24h. In at least 12 cases, the positive cases were detected by RT-PCR while negative results were obtained on the same day using an antigen test. In addition, in 4 cases, false negatives of external PCR-diagnostic tests were discovered. The overall number of infections was about an order of magnitude less and the average SARS-CoV-2 positivity almost two order of magnitude lower than in the city of Dortmund (~ 0.4% vs ~ 30%). **Conclusion** The application of direct RT-PCR on pooled gargle samples using tap-water enables a fast, accurate and cost-effective screening strategy for identifying SARS-CoV-2 positive individuals and their family members, partners and friends. Such a testing approach when combined with high vaccination rates and clean air strategies helps prevent community transmission significantly and ensures a safe campus environment, which allows for in-person courses, study groups and exams despite high infection rates in the region.

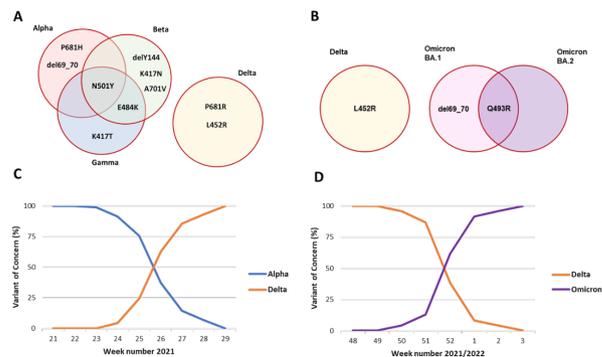
## A-168

### PCR-based Genotyping Assays as a Complement to Whole Genome Sequencing for Surveillance of SARS-CoV-2 Variants of Concern, including Omicron

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**Background** Surveillance testing plays an important role in tracking the spread of novel SARS-CoV-2 variants of concern (VOC) and enabling public health countermeasures. As each VOC carries a characteristic set of mutations, PCR-based genotyping assays can be used as a quick surveillance method. This study compared the performance of custom TaqMan SARS-CoV-2 mutation panels to whole genome sequencing (WGS) for identification of SARS-CoV-2 VOC, including Omicron. **Methods** The study was performed on randomly selected SARS-CoV-2 positive samples (Ct<32), collected in two time periods: May-July 2021 (N=331) and December 2021-January 2022 (N=309) in the Netherlands. Samples collected in the first period were analyzed with 10 genotyping assays covering Alpha, Beta, Gamma and Delta VOC (Figure 1A). Samples collected in the second period were tested using 3 genotyping assays to differentiate Delta and Omicron variants (Figure 1B). WGS was performed using the Ion AmpliSeq SARS-CoV-2 research panel on the Ion S5 instrument. **Results** Of 640 samples WGS identified 32.3% Alpha (N=207); 16.6% Omicron (N=106), 50.6% Delta (N=324); 0.3% Beta (N=2) and 1 case of C.36.3.1 variant. In one sample BA.2 Omicron variant was detected. Matching results were obtained using the TaqMan SARS-CoV-2 mutation panel in 99.8% (639 of 640) samples, demonstrating accuracy of genotyping assays in VOC detection. C.36.3.1 variant positive sample could not be assigned to a specific lineage by genotyping. Temporal analysis of our dataset illustrates rapid increase in Delta variant prevalence in June-July 2021 (Figure 1C), and Omicron variant spread in December 2021-January 2022 (Figure 1D). **Conclusions** Genotyping assays enable quick (<24h) and highly accurate detection of SARS-CoV-2 VOC with higher throughput and lower cost compared to WGS. This study shows that once defined, consensus VOC can be rapidly detected with specific accurate PCR assays while WGS remains the method of choice for detection of new variants.

Figure 1.



## A-169

### A Rapid Tool to Detect Vancomycin Resistance Genes in *Enterococcus faecium* Isolates

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**Background:** Multidrug-resistant bacteria cause high morbi-mortality and, consequently, high healthcare costs. *Enterococcus faecalis* and *Enterococcus faecium* resistant to glycopeptides, are *vanA* and *vanB* resistance genes carriers. It requires microbiological surveillance priority due to their clinical significance and to their high dissemination capacity. The objective of this retrospective study was to test the usefulness of PCR VIASURE Vancomycin resistance RT PCR detection kit to detect the resistance mechanism in order to accelerate the laboratory response time. **Methods:** This study was performed with clinical and/or epidemiological samples from patients from different Miguel Servet University Hospital (HUMS) clinical areas. A total of 28 *E. faecium* isolates were analysed. Isolates proceeded from urine samples (11), ascitic liquid (2), abdominal abscess (2), rectal smear (7), bronchial aspirate (1) ulcer (1), triple epidemiological smear (2) and blood (2) samples. Antibiotic susceptibility was performed by MicroScan WalkaAway plus System (Beckman Coulter) and MIC Test Strip (Liofilchem) according to EUCAST breakpoint tables. In the context of the existing collaboration between HUMS and CerTest Biotec, from November 2021 to February 2022, after the identification and characterization of *E. faecium*, the PCR “VIASURE Vancomycin resistance RT PCR detection kit” was performed. Acid nucleic extractions were performed by automated nucleic acid extraction instrument magLEAD®6cG PSS. Amplifications were performed at CFX96® IVD Real-Time PCR Detection System (Bio-Rad). Fluorogenic data was collected through the FAM (*vanA* gene), ROX (*vanB* gene) and HEX (Internal Control) channels. Furthermore, *vanA* gene detection was confirmed by sequencing in seven samples. **Results:** Among the 28 *E. faecium* isolates 21 resulted vancomycin and teicoplanin resistant and 7 vancomycin and teicoplanin susceptible. The *vanA* gene amplification was obtained in the 21 *E. faecium* isolates vancomycin and teicoplanin resistant. No signal was obtained for *vanB* gene channel (ROX). Regarding *E. faecium* vancomycin and teicoplanin susceptible specimens, no amplification was observed. All samples presented a correct amplification signal for the internal control. No discrepant results were obtained proving to be as specific as HUMS methods. **Conclusion:** These results demonstrate the usefulness of the “VIASURE Vancomycin resistance RT PCR detection kit” for the rapid detection of vancomycin resistance genes. This kit can be included as a rapid diagnostic test since the result can be obtained in 2 hours. Its freeze-dried format, in

addition to providing it with a long shelf life, allows transport and storage to be at room temperature. These characteristics and its easy handling allow its use in any Microbiology Laboratory.

## A-170

### Clinical Sensitivity of VIASURE Complete Automatized Workflow for the Molecular Diagnosis of Gastrointestinal Infections

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**Background:** Gastrointestinal infections are generally auto limited, requiring rehydration and electrolytes as only therapy to compensate the losses caused at intestinal level. Nevertheless, in some cases, it is necessary to know the etiological agent to provide a specific pharmacological treatment. Molecular diagnosis allows the sensible and specific detection of these pathogens. In this study, the clinical sensitivity of the complete VIASURE workflow for molecular diagnosis of gastrointestinal infections using de DNA/RNA Pathogens Extraction Kit within the new platform VIASURE VFlex has been evaluated. **Methods:** The study was performed using positive clinical gastrointestinal samples previously analyzed with qPCR by Hospital Clínico Universitario Lozano Blesa (Zaragoza). For analysis, 100 mg of fecal sample were resuspended in 900 ul of distilled water by vigorous mixing, and then centrifuged 3000 rpm 2 minutes. Samples were extracted in VFlex platform using DNA/RNA Pathogens Extraction Kit and dispensed in VIASURE Real Time PCR plates by the same platform. The experiment was performed in parallel with MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit with the platform KingFisher Flex (ThermoScientific) as reference method in order to evaluate sample degradation. The obtained purified nucleic acids were amplified with VIASURE Real Time PCR in the VLab Cycler. **Results:**

A total 180 faecal samples (20 positive to each analysed pathogen: *Salmonella* spp, *Campylobacter* spp, *Cryptosporidium*, *Giardia*, Adenovirus, Astrovirus, Rotavirus, Norovirus, *Clostridium*) previously characterized with PCR, were analysed with the two previously described kits and platforms (n=360) using VIASURE Real Time PCR products. Results obtained with the kit and platform under study were concordant with the reference method, obtaining sensitivity values >90% for all the analysed pathogens. **Conclusion:** With this evaluation it was possible to verify the adequate performance and usability of VFLEX platform for the extraction, purification and PCR plate dispensing, as well as the clinical sensitivity of DNA/RNA Pathogens Extraction Kit for gastrointestinal samples. It is important to remark that this new automatized solution only requires to introduce the samples, consumables and reagents in the platform and the removal of the PCR plate ready for amplification after the run, avoiding sample manipulation at any point of the process and allowing the analysis of a single sample with multiple PCR products intended for gastrointestinal infections diagnosis.

## A-171

### Comparison of Five Nucleic Acids Processing Methods for SARS-CoV-2 Molecular Diagnosis

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#### Background:

The SARS-CoV-2 pandemic has created an unprecedented need for rapid molecular diagnostic tools. During the pandemic, new quick nucleic acids (NA) processing methods emerged. These methods, based on the lysis and the release of the NA, simplify typical extraction methods maintaining the genetic material integrity and being compatible with downstream molecular applications. The aim of this study was to evaluate the utility and the clinical features of three quick NA processing methods and one automatic extractor in combination with VIASURE SARS-CoV-2 qPCR products for PCR assays.

#### Methods:

A total of 252 nasopharyngeal swabs samples in viral transport media, both positive (52) and negative (200) were evaluated. The initial diagnosis for positive samples was performed using the Cobas® SARS-CoV-2 test (Roche Molecular Systems), and for negative samples, the extraction was performed with MagDEA Dx SV kit, using the magLEAD® 12gC instrument and NA were analyzed using the CDC China and Atlanta SARS-CoV-2 detection protocols. Samples were processed in parallel with different NA processing methods (Table 1). After that, NA were analyzed with

VIASURE SARS-CoV-2 qPCR products (CerTest Biotec S.L.) using the CFX96™ Real-Time PCR Detection System (Bio-Rad). Results were compared with the initial characterization and taking the automatic extraction system as reference values.

#### Results:

The process conducted with the automatic extraction system obtained the best correlation with the initial characterization. Among the quick NA processing methods, the VIASURE Resp. viruses Quick Lysis Reagent was the most sensitivity 0.96 (0.86 - 0.99). Viral RNA Extraction Buffer (0.86 (0.73-0.94)) and Enzymatic DNA/RNA Extract Buffer (0.88 (0.76-0.95)) presented similar results.

#### Conclusion:

All NA processing methods resulted compatible with VIASURE SARS-CoV-2 qPCR products. In this study it was demonstrated that the VIASURE Resp. viruses Quick Lysis Reagent is a good tool in the rapid diagnostic workflow process of SARS-CoV-2 diagnosis.

Table 1.

Quick NA processing methods	Automatic extractor
VIASURE Resp. viruses Quick Lysis Reagent (CerTest Biotec S.L.)	MagDEA Dx SV kit, using the magLEAD® 12gC instrument (Precision System Science Co., Ltd)
Viral RNA Extraction Buffer (Merck KGaA)	
Enzymatic DNA/RNA Extract Buffer (Chai Inc)	

## A-172

### Contribution of Molecular Techniques for the Early Diagnosis of Patients With Haemocultures Positive to Gram-Negative Bacilli

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#### Background:

Sepsis is a time-dependent pathological state in which successful cases are directly related to the speed in which an adequate treatment is established. The early identification of the causing microorganism and the start of treatment in the first hours after onset can significantly reduce its mortality. However, the long time required by conventional microbiology methods presents an obstacle when choosing the optimal treatment. Therefore, alternative, faster, more sensitive and more specific techniques to detect these causing microorganisms and their possible antibiotic resistance mechanisms are in dire need. **Objective** To study a panel composed of 4 RT-PCR multiplex assays for the detection and differentiation of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter* spp., *Acinetobacter baumannii* and *Escherichia coli*, along with the beta-lactamases resistance genes *CTX*, *TEM*, *SHV*, *MCR-1*, *NDM*, *VIM*, *OXA-48*, *KPC* and *IMP*, in hemocultures of patients attended in a secondary hospital.

#### Methods:

413 hemocultures from 202 patients collected between March and May 2021 in the Hospital Royo Villanova (Zaragoza, Spain) previously identified using with Combo panels (Microscan®, Beckman Coulter) and/or MALDI-TOF) and with known antibiotic sensitivity [microdilution panels (Microscan®), diffusion disc method and, in cases of suspicion of carbapenems resistance or ESBL, with immunochromatography] were analysed. Due to their shared thermal protocol, the nucleic acids could be analysed using the four VIASURE assays concurrently: *Carbapenemase-producing Enterobacteriaceae*, *P. aeruginosa*, *K. pneumoniae* & *P. mirabilis*, *Enterobacter*, *A.baumannii* & *E. coli* and *CTX*, *TEM*, *SHV* & *mcr* and the results were compared with the ones obtained using routine diagnosis. The study was further rounded out comparing these results with the ones obtained with the following molecular assays that cover the span of the panel under study: ARM-D® Kit, TEM/SHV/GES Detected Variants y β-Lactamase Detected Variants (STRECK), Magicplex™ Sepsis ID 4, ID 6 and ID 7 Real-time Detection Tests (Seegene).

**Results:**

According to initial diagnosis, 124 haemocultures were positive to at least one of the bacteria under study and sensitive to third generation cephalosporines and carbapenems (85 *E. coli*, 18 *P. mirabilis*, 8 *K. pneumoniae*, 8 *E. aerogenes* and 5 *P. aeruginosa*). 37 of them were positive and resistant to third generation cephalosporines and had an ESBL profile but sensitive to carbapenems (24 *E. coli* and 13 *K. pneumoniae* isolates) and 263 were negative to all the targets under study. The combined use of the 4 VIASURE assays allowed to detect all the positive haemocultures included in the study. None of the carbapenems resistance genes was detected in the samples. *CTX* gene was only detected in haemocultures characterised as ESBL, while *SHV-1* gene was detected in 13 samples (all of them non-ESBL *K. pneumoniae*) and *TEM-1/TEM-2* genes were detected in 40 samples (31 *E. coli* and 9 *P. mirabilis*, all of them non-ESBL).

**Conclusion:**

The 4 VIASURE multiplex assay reported the results by genus and species and its multi-resistance identification faster than conventional methodology would. The implementation of its use would allow a prompt treatment choice, avoiding unnecessary use of broad-spectrum antibiotics.

**A-173****Detection and Differentiation of *Bordetella pertussis*, *parapertussis* and *holmesii* Using the Integrated Nucleic Acid Extraction and Amplification Platform BD MAX™**

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**Background:**

*Bordetella* genus is composed of eight species, four of which are known to infect humans: *B. pertussis*, *B. parapertussis*, *B. holmesii*, and *B. bronchiseptica*. Whooping cough or pertussis is a very contagious disease caused by *B. pertussis* that produces and inflammation of the respiratory airways. Parapertussis is a similar disease caused in this case by *B. parapertussis*, however it is less frequent and generally a less serious illness. *B. holmesii* has been isolated from patients with a subacute serious disease and *B. bronchiseptica* is usually restricted to animals, but it has been isolated from immunocompromised patients a few instances. Despite vaccination, whooping cough remains endemic in most areas of the world. Therefore, a reliable diagnosis is required to start an adequate treatment and contact prophylaxis, if needed. This is especially relevant in case of exposition of unvaccinated children, for whom this disease might be mortal. The objective of the present study was to evaluate the clinical performance of the new real time PCR product for the BD MAX™ platform in a lyophilised format directed to the detection and differentiation of *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella holmesii*.

**Methods:**

Clinical evaluation was performed over 288 respiratory samples: 254 nasopharyngeal samples previously characterised as 84 *Bordetella pertussis* and 150 *Bordetella* negative, and 54 nasopharyngeal simulated samples (spiked samples) (22 *Bordetella pertussis*, 12 *Bordetella parapertussis* and 20 *Bordetella holmesii*) used to complement the clinical evaluation due to the scarce availability of *B. parapertussis* and *B. holmesii* clinical samples. The clinical performance analysis of the molecular assay VIASURE *Bordetella* Real Time PCR Detection Kit for the BD MAX™ system was carried out comparing the reference values obtained with the initial characterization with the commercial kit SmartCycler *Bordetella pertussis/parapertussis* (Cepheid) and an in-house master mix of Eurofins targeting IS481 and IS1001 genes.

**Results:**

The obtained sensitivity and specificity values were 96,1% (95% CI, 0.903-0.989) and 100% (95% CI, 0.980-1), respectively, for *Bordetella pertussis*; 100% (95% CI, 0.735-1) and 100% (0.987-1) for *Bordetella parapertussis*; and 100% (95% CI, 0.832-1) and 100% (95% CI, 0.986-1) for *Bordetella holmesii*.

**Conclusion:**

With this evaluation it was possible to verify a correct correlation between the prototype under study and the reference molecular method employed for the clinical diagnosis of *Bordetella pertussis*, *B. parapertussis* and *B. holmesii*. It is noteworthy that BD MAX™, system is an automated platform that integrates the nucleic acid extraction and RT-PCR processes and reduces considerably the workflow time, allowing for an increase in the technicians' productivity. In addition, the kit is manufactured in a lyophilised and ready to use format, reducing even more the time and handling required in the laboratory, avoiding possible contaminations and allowing its transport and storage at room temperature.

**A-174****Detection of *Staphylococcus aureus* Bacteraemia and Other Methicillin-Sensitive and Methicillin-Resistant *Staphylococci***

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**Background:**

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are frequent in our environment (Spain, Europe). According to the EARS-Net surveillance program, the percentage of MRSA infections of invasive origin is around 25% nationwide. These infections are an important cause of morbidity and mortality as they are frequently related to a lack of effective empirical treatment coverage, which implies delay in the administration of suitable treatment in severe infections. Conventional methodologies used to detect MRSA are tedious and require 24-72 hours, whereas molecular assays offer faster detection speed, sensitivity and specificity. **Objective:** To analyse the clinical utility of a multiplex real-time PCR assay in bacteraemias diagnosis for detecting both methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS), and to evaluate the implementation of this molecular assay in the Microbiology Service for the early detection of these microorganisms in positive blood cultures.

**Methods:**

A comparative-prospective study was performed with 210 blood cultures from ICU patients of Hospital Royo Villanova (Zaragoza, Spain). Initial diagnosis of these samples was carried out by combining the blood culture results (using conventional techniques such as Combo 37 or microdilution panels with only sensitivity (p33) (Microscan®, Beckman), MALDI-TOF, disc-diffusion techniques, PBP2a, etc). DNAs extracted from blood cultures were analysed using VIASURE *Methicillin-resistant Staphylococcus aureus* Real Time Detection Kit (CerTest Biotec, Zaragoza) and the results were compared with those obtained in routine clinical diagnosis. The clinical sensitivity and specificity study was completed by comparing both methods with a molecular assay widely-used in clinical diagnosis of this microorganism: RIDA®GENE MRSA real-time PCR™ (R-biopharm, Germany).

**Results:**

According to conventional diagnosis, MRCoNS was isolated from 56 samples, 16 were MRSA, 33 were MSSA, 3 were coinfecting with MSSA and MRCoNS and 102 were negative to these microorganisms. The results obtained using VIASURE assay were: 14 samples positive for MRSA, 31 were MSSA, 41 MRCoNS, 3 with mixed isolation of MSSA and MRCoNS, and 121 samples negative to the targets in study. Discordant samples were analysed and resolved by using the RIDA molecular assay, and 3 incongruent results were actually obtained: 2 samples which VIASURE considered positive to MSSA and MRCoNS while both reference methods only detected MSSA, and 1 false detection of MRCoNS in a negative sample. The final sensitivity and specificity values obtained by VIASURE were 1 (0.95-1) and 0.99 (0.98-0.99), respectively, showing it to be a tool not only with a greater sensitivity but also with a higher ability to discern between *Staphylococcus aureus* and *Staphylococcus spp.* and both methicillin-resistant and methicillin-sensitive.

**Conclusion:**

With this evaluation it was possible to verify the good correlation of VIASURE assay's results with those of the reference molecular assay, proving to be an excellent tool for the early diagnosis of *S. aureus*, as well as for the differentiation of sensitive isolates from methicillin-resistant isolates in blood cultures.

**A-175****DNA Free Taq Polymerase to Eliminate False-Positives in *Escherichia coli* qPCR Sepsis Diagnosis**

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**Background:** *Taq* DNA polymerase (*Taq*) was first isolated from *Thermophilus aquaticus*, nowadays, is produced in *Escherichia coli* using genetic engineering. During the *Taq* purification process, DNA is not able to be eliminated, therefore polymerases are contaminated with *E. coli* DNA. *E. coli* is one of the main bacteremia causative microorganisms. Rapid microbiological diagnosis is crucial in sepsis for

providing quality healthcare. Real-time PCR (qPCR) technique, for *E. coli* detection, has been hindered by false positives, due to endogenous contamination of *Taq* with *E. coli* DNA. This research main objective was to develop a DNA decontamination method for *Taq* to obtain an *E. coli* real-time PCR kit with fewer false-positives results and higher sensitivity. **Methods:** A qPCR method was developed for *E. coli* detection, targeting the 16S rRNA gene. Furthermore, a decontamination protocol with 0.5U/μL DNase I (Roche) was applied to *Taq* and compared to ultrafiltration with 50KDa and 100KDa Amicon Ltd device (Millipore Corporation, Massachusetts, USA). Analytical sensitivity was evaluated by the establishment of the detection limit (LOD) using a non-treated and treated *Taq* polymerase. To determine, the LOD, synthetic dsDNA and spiked blood samples with *Escherichia coli* (BAA-2340) were used. An automated nucleic acid extraction system (magLEAD® 12gC) and Bio-Rad CFX96™ Real-Time PCR thermocycler were used. **Results:** Using a non-treated *Taq*, in an *E. coli* qPCR detection kit, 100% of reactions were contaminated, with a Ct value between 28 and 33 (*Taq* batch dependent). After DNase I decontamination, only residual contamination is observed in 20% of the reactions tested, with a Ct value >35. On the other hand, the ultrafiltration method only (without DNase I), neither to 50KDa nor 100KDa, does not eliminate DNA contaminant from *Taq*. *Taq* treated with DNase I, allow improving LOD of technique from  $2 \times 10^2$  to 10 copies/μL (0.005 CFU/μL). Other commercially available *Taq* also present *E. coli* DNA contamination but with a Ct >35. **Conclusions:** *Taq* decontamination method with DNase I significantly reduce *E. coli* false-positive amplifications and allow a LOD of 0.005cfu/μL. A DNA free *Taq* has great clinical importance to reduce false-positive results to give an accurate and reliable *E. coli* test in sepsis diagnosis.

## A-176

### Early Detection of MRSA in Epidemiologic Surveillance Samples From Hospitalised Patients

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#### Background:

*Staphylococcus aureus* infections are remarkable due to their high morbimortality. According to the EPINE report (Prevalence study of nosocomial infections in Spain) released in 2019, the prevalence of methicillin-resistance *S. aureus* was 32.12%. Antibiotic resistance is a cause of concern since it worsens the patient prognosis and it's associated to higher morbidity, mortality and cost of treatment. Therefore, in order to improve the patient's situation it would be necessary to have a sensitive and fast diagnosis, which is not always feasible by using conventional methodologies. In our healthcare area (Zaragoza, Spain, I sector) there is a high prevalence of MRSA, so it's essential to detect its presence as quickly as possible in clinical and epidemiological samples.

**Objective:** To study a new multiplex real-time PCR assay for the early detection of methicillin-resistant *S. aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS) directly in samples and to evaluate its contribution to the diagnosis and management of the patients.

#### Methods:

689 clinical samples of epithelial and soft tissues infections and epidemiological surveillance samples from patients hospitalised at Hospital Royo Villanova (Zaragoza) were analysed. They had been characterized by conventional methodology (culture, MALDI-TOF identification and/or Combo panels (Microscan®, Beckman Coulter) and sensitivity study of disk-diffusion or microdilution commercialized panels. The results were compared to those obtained with the molecular assay VIASURE Methicillin-resistant *Staphylococcus aureus* Real Time Detection Kit (CerTest Biotec, Spain). In addition, the clinical performance of the assay was analysed in comparison with a molecular assay used in clinical diagnosis (RIDA®GENE MRSA real-time PCR (R-biopharm, Germany)).

#### Results:

From the total of samples, conventional diagnosis reported the following results: 264 MRCoNS, 52 MRSA, 75 MSSA and 287 negative samples. The developed molecular assay deemed 48 MRSA, 54 MSSA, 347 MRCoNS, 48 coinfecting samples with MSSA and MRCoNS and 195 samples negative to the targets in study. When comparing these results to those obtained using conventional methods, discordant results were found, which were subsequently resolved with the reference molecular method.

After the molecular comparison, there were 6 samples with incongruent results. The sensitivity and specificity obtained were 0.99 (0.98-1) and 0.998 (0.995-0.999), respectively.

#### Conclusion:

A good correlation of the results obtained by the molecular assay and those of the reference methods was achieved. Therefore, it was proved that the test used is a good tool for the identification of *S. aureus* and the detection of resistance/susceptibility to methicillin, and also for the differentiation of methicillin-resistant *S. aureus* and other species of the genus which usually implies less importance and severity. Thus, VIASURE assay would allow much faster and safer detection of staphylococcal infections, and the detection of sensitivity or resistance to methicillin would allow the safe adjustment of the treatment, avoiding the overuse of antimicrobials such as daptomycin, linezolid or vancomycin.

## A-177

### Molecular Diagnosis of SARS-CoV-2: Nucleic Acid Extraction + PCR SetUP in the new VIASURE VLFLEX Platform

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#### Background:

In the last decade the use molecular diagnosis of human infections has notably increased due to the high sensitivity and specificity of the method, having a particular impact in the last two years in the epidemiologic control of SARS-CoV-2 pandemic context. Besides, the number of samples to be processed by diagnostic laboratories each day has increased as well, leading to a high demand of the automatization of DNA/RNA extraction and purification, PCR dispensation and result interpretation. In this study the clinical sensitivity and usability of the new VIASURE VLFLEX system for automatic sample processing of samples with suspicion of SARS-CoV-2 has been evaluated.

#### Methods:

The analysis of respiratory samples (nasopharyngeal swabs in biocomma® Virus Transport and Preservation Medium) of patients with suspicion of SARS-CoV-2 provided by Biobanco del Sistema de Salud de Aragón (BSSA) was carried out. These samples were previously characterised using the routine molecular method of the hospital of origin. Sample vials were directly introduced in the equipment with and without disposing of the swab inside of them. Samples were extracted with VIASURE DNA/RNA Pathogens Extraction Kit and dispensed into PCR plates for the amplification process using VIASURE VLFLEX platform. The same samples were processed in parallel using the KingFisher Flex (ThermoScientific) platform with the MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit. In both cases the sample volume was 200 μL, the elution volume was 100 μL and purified nucleic acids were amplified using the VIASURE SARS-CoV-2 Real Time PCR Detection Kit in the VIASURE VLab Cycler.

#### Results:

A total of 140 samples (40 positive and 100 negative) previously characterised by PCR were analysed. Firstly, the pipetting ability of the system was evaluated through its volume detection with swabs inside the vial. 50 negative samples were introduced with the swab inside, observing that the swab presence hindered an adequate pipetting performance in a high rate of the samples (10/50). Secondly, 39 out of the 40 positive samples were consistent with the two analysed methods and initial diagnosis, obtaining a single false negative result for the *ORF1ab* target gene when using VIASURE VLFLEX workflow. Lastly, VLFLEX results compared with initial diagnosis and reference method showed sensitivity values of 97.5% for *ORF1ab* target and 100% for *N* gene target.

#### Conclusion:

This evaluation was useful to verify the correct performance and usability of VIASURE VLFLEX platform for extraction, purification and dispensing of PCR plates, as well as the clinical sensitivity of the extraction kit prototype developed for respiratory samples. However, it is recommended to remove any object that may hinder the detection and pipetting of the sample. The use of an automated platform such as the one under study allows the decrease of sample handling for the technicians, improving biological safety and saving personal resources for the laboratory.

## A-178

## New Automated Solution for Simultaneous Molecular Diagnosis of Gastrointestinal, Respiratory and Sexual Infections

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**Background:**

The use of molecular diagnosis for infectious diseases has increased significantly during the last decade, thanks to the advantages in terms of sensitivity, specificity, precision and robustness that it offers if compared to traditional microbiological techniques. One of the main objectives of molecular diagnosis is to give a solution for the simultaneous detection of pathogens from different type of clinical samples, allowing to process a higher number of daily specimens in a single run. In this work the functionality of *DNA/RNA Pathogens Extraction kit* within the new platform VIASURE VFlex has been studied.

**Methods:**

Firstly, the capacity of the platform VFlex to process 96 randomized samples (12 positives and 84 negatives) without causing cross contamination, as well as the compatibility of the universal extraction control from VIASURE in different biological matrices: pharyngeal swab, saliva, urine and feces, was verified. Secondly, the capacity of *DNA/RNA Pathogens Extraction Kit* to extract and purify nucleic acids from respiratory, gastrointestinal and sexual clinical samples, as well as experimentally spiked samples was evaluated (Table 1). The obtained results were compared with the commercial kit and automated extraction platform of reference; MagDEA Dx SV Kit (MagLEAD® 12gC (Precision System Science Co.)). The purified nucleic acids were amplified in the VIASURE VLab Cycler.

**Results:**

There was no cross contamination during neither the extraction nor PCR setup. The universal control amplified always in the admitted ranged (Ct<30). The obtained results from processing clinical samples and experimentally spiked samples, with DNA/RNA Pathogens Extraction Kit within VFlex platform were comparable with the reference method MagDEA Dx SV Kit in terms of Ct and fluorescence.

**Conclusion:**

The Kit and the automated platform developed allowed for the extraction and purification of nucleic acids from clinical samples of respiratory gastrointestinal and sexual, and their dispensation in PCR kits in a single run.

Table 1. Samples, cultures, and VIASURE Real Time PCR Kits.

Pathogen	Samples origin	Matrix	VIASURE Real Time PCR Kit
Influenza A	ATCC VR-95PQ	Nasopharyngeal swab	ABC
		Saliva	ABR
Bocavirus	Hospital Can Ruti	Nasopharyngeal aspirate	AMB
<i>Pseudomonas aeruginosa</i>	ATCC 10145	Sputum	PSA
<i>Aspergillus flavus</i>	CECT 2949	Sputum	ASP
SARS-CoV-2	Biobanco IACS (SA21-05)	Nasopharyngeal swab in Biocomma inactivating media	NCO3
Papillomavirus	Hospital Universitario Miguel Servet	Endocervical and exocervical Swab Hologic®	HR1
<i>Salmonella spp.</i>	Hospital Clínico Universitario Lozano Blesa	Feces	SCS
<i>Campylobacter</i>			KGE
<i>Cryptosporidium</i>			ADV
<i>Giardia</i>			ATV
Adenovirus			
Astrovirus			

## A-179

## Saliva Samples for Quick Detection of SARS-CoV-2: Compatibility With Several Nucleic Acid Processing Methods

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**Background:**

The COVID-19 pandemic has highlighted the need for quick, robust, and efficient diagnostic procedures to identify the microorganisms that cause viral infections. Although the most common way to obtain a sample for the real-time PCR (qPCR) diagnosis of SARS-CoV-2 is by nasopharyngeal swab, this could cause discomfort to the patient, and it is sometimes more complicated to perform in children. Alternatively, saliva sample collection is easier, saves time and reduces aerosol production. However, this type of sample contains RNases and inhibitors that could affect the qPCR results. Therefore, a correct sample processing to obtain the nucleic acids is essential to generate a correct diagnosis.

**Methods:**

A comparative study of 40 saliva samples was performed. The samples were processed at the same time with an automatic extraction system (MagDEA Dx SV kit in magLEAD® 12gC instrument, PSS) and the quick processing methods: VIASURE Resp. viruses Quick Lysis Reagent (CerTest Biotec S.L.), Enzymatic DNA/RNA Extraction Buffer (Chai Inc), and Viral RNA Extraction Buffer (Merck KGaA), following the manufacturer's instructions. The nucleic acids were analyzed with the qPCR product VIASURE SARS-CoV-2 Real Time PCR Detection Kit (CerTest Biotec S.L.) in the CFX96™ Real-Time PCR Detection System (Bio-Rad). The results obtained with the automatic extraction system were considered as the reference value.

**Results:**

With the automatic extraction system, 10 positive samples (25%) and 30 negative samples were identified. VIASURE Resp. viruses Quick Lysis Reagent identified all positive samples (sensitivity 1 (0.66-1)). In the case of Enzymatic DNA/RNA Extraction Buffer and Viral RNA Extraction Buffer, 7 positive samples (sensitivity 0.70 (0.35-0.92)) and 4 positive samples (sensitivity 0.40 (0.14-0.73)) were identified, respectively.

**Conclusion:**

The quick processing method VIASURE Resp. viruses Quick Lysis Reagent obtained similar results to the automatic extraction method for saliva samples in combination with the qPCR VIASURE SARS-CoV-2 Real Time PCR Detection Kit, achieving a good sensitivity and specificity for the diagnosis of SARS-CoV-2.

## A-180

Study of SARS-CoV-2 *in silico* Analysis Mismatches, Can the Different Variants be Monitored Whilst Verifying the PCR Performance?

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**Background:**

During COVID-19 pandemic and due to the high mutation rate of SARS-CoV-2, different variants have emerged, each one characterized by a specific set of characteristic mutations. As a consequence, the PCR Diagnostic products that detect the virus targeting specific nucleotide sequences may be compromised. Therefore, *in silico* analyses are necessary to monitor their correct performance. In this work the connection between the evolution of variants over time and new mutations detected in the *in silico* analyses in the primers and probes regions of the two PCR product VIASURE SARS-CoV-2 and VIASURE SARS-CoV-2 (N1 + N2) Real Time PCR Detection Kit is established.

**Methods:**

The analysis was performed downloading the sequences from GISAID EpiCoV database entered between October 2020 and January 2022. The filters *complete*, *low coverage excluded* and *high coverage* were applied, limiting the analyses to sequences with <1% Ns, insertions or deletions previously identified by the submitter. Then, an in-house software based on Biopython libraries was used to perform alignments and detect mismatches and discrepancies between the design of the previously mentioned products and the target regions of the downloaded sequences. Afterwards, the obtained mismatches and mutations were reviewed according to previously established relevance criteria based on number of mismatches and location within the primers

and probes. Lastly, the potential compromised performance of the PCR products was considered and the evolution of most predominant variants characteristic mutations frequency over time was studied between October 2020 and January 2022.

#### Results:

The predicted correct performance of the kits was verified, and the characteristic mutations of Alpha, Delta and Omicron variants were identified, obtaining the following results: - Between October 2020 and July 2021 Alpha variant characteristic mutation was present in 53.11% of sequences, Delta variant characteristic mutation in 13.38% and Omicron variant characteristic mutation in <0.50%. - Between July 2021 and November 2021 Alpha variant characteristic mutation was present in 5.56% of sequences, Delta variant characteristic mutation in 84.12% and Omicron variant characteristic mutation in <0.50%. - Between November 2021 and January 2022 Alpha variant characteristic mutation was present in 1.00% of sequences, Delta variant characteristic mutation in 91.45% and Omicron variant characteristic mutation in <1%. These prevalence values are “skewed” by the high coverage filter, as it removes 98% omicron sequences due to the low sequence quality (high number of Ns). When using GISAID unfiltered sequences, ratios change to <1.00% for Alpha, 80.61% for Delta and 13.20% for Omicron.

#### Conclusion:

*In silico* analysis of specific mutations of SARS-CoV-2, in addition to aiding in the correct performance surveillance of molecular diagnostic products may help monitoring variant evolution. The disappearance of Alpha variant with the concurrent emergence of Delta variant, or the recent surfacing of Omicron may be reflected in this type of analyses, although it considerably depends on the number of sequences and the quality of sequencing performed.

## A-181

### The Most Desirable Scenario: Rapid Typing of Clinical Isolates for the Epidemiological Control of Influenza A Virus

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#### Background:

Rapid and accurate diagnosis of respiratory viruses can help in epidemiologic monitoring and implementing preventing measures. Influenza A virus is the major cause of large influenza epidemics and pandemics. Several diagnostic approaches have been used to detect influenza. Analysis based on polymerase chain reaction detects virus-specific genetic materials, rather than viral antigens or antibodies, and can identify different subtypes of influenza viruses. The objective of this study was to evaluate the performance of a real time PCR product for the detection and differentiation of Influenza A H1N1, H3N2, H5N1 and H7N9 in previously characterized Flu A virus samples.

#### Methods:

The clinical performance of VIASURE *Flu typing II* Real Time PCR Detection Kit was conducted with different studies, a clinical evaluation in Hospital Clínico Universitario Lozano Blesa (Zaragoza, Spain), external quality assessment (EQA) programs (21 programs) and purchased reference material/strains (NIBSC and ATCC) (24 + 46 strains).

#### Results:

A total of 119 throat clinical samples were analysed: 85 Flu A H1N1 and 34 Flu A H3N2 samples. In addition, 70 reference strains (including 6 H1N1, 20 H3N2, 18 H5N1 and 2 H7N9) were analysed and 225 spiked samples (including 39 H1N1, 51 H3N2, 11 H5N1, 6 H5N8, 5 H7N9 and 5 H7N7) from QCMD, UK NEQAS, INSTAND, RCPAQAP and CAP EQA programs. These samples included inactivated/activated Influenza strains viruses in transport medium, simulated nasopharyngeal swab or aspirate, and simulated throat swab. Sensitivity and specificity mean values for Flu A H1N1 were 0.97 (0.92-0.99) and 0.99 (0.97-1); for H3N2 were 0.98 (0.93-1) and 1 (0.98-1); for H5N1 were 1 (0.69-0.) and 1 (0.98-1); and for H7N9 were 1 (0.47-1) and 1 (0.98-1), respectively.

#### Conclusion:

The clinical performance of VIASURE *Flu typing II* Real time PCR Detection Kit was evaluated comparing it with a reference molecular assay. From the total samples analyzed, it was obtained 4 false negative values (3 Flu A H1N1 and 1 Flu A H3N2) and one Flu A H1N1 false positive value. Therefore, it could be concluded that this assay could be used for the detection of the strains H1N1, H3N2, H5N1 and H7N9 in reference material, strains and Influenza A positive clinical samples.

## A-182

### Bacterial Signatures for Type 2 Diabetes

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#### Background:

Gut microbiota is important for immunomodulation and overall health, and disruptions have been associated with various diseases, such as type 2 diabetes (T2D). The aim of this pilot study was to explore the potential differences in the gut bacterial profiles of pre-T2D/T2D patients and healthy subjects, and gut bacterial -derived signatures discriminating these groups.

#### Methods:

Fecal samples from 40 patients (22 pre-T2D, 18 T2D) and 38 healthy adults were analyzed with the GA-map® Technology Platform, described previously (1), however using a 131-plex research-only panel of bacterial markers (probes). The platform utilizes the 16S rRNA gene variable regions V3-V9 to characterize gut bacterial profiles, using molecular biology techniques. Differences in bacterial expression were evaluated using the Wilcoxon Rank Sum Test with Benjamini-Hochberg correction. Principal component analysis (PCA) was used to assess bacterial profile similarities and variations. Multi-Step Adaptive Elastic-Net modelling was used to identify a bacterial signature.

#### Results:

Differences in the bacterial profiles were found between the pre-T2D and healthy control groups, and ten bacterial markers were determined to be differentially expressed (Table 1). No significant differences were found when comparing the T2D group and the healthy control group. A bacterial -signature model distinguishing pre-T2D patients from healthy controls, gave an area under the curve (AUC) of 0.88.

#### Conclusion:

This study is promising for the use of gut bacterial signatures in the field of (pre-) Type 2 diabetes. Comparing healthy subjects and pre-T2D patients, differences in bacterial abundance were found, as well as good performance of a bacterial -signature model. However, studies involving larger cohorts are needed. These findings, together with more extensive studies, can serve as a foundation for developing discriminatory bacterial signatures for both pre-T2D and T2D. Such tools may aid in early diagnosis or disease monitoring, preventing risk of progression.

## A-183

### Association Between Circulating Interleukin-22 And Its Receptor Expression In Tuberculosis Patients Of Northwest India

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**Background:** Disease progression of Tuberculosis (TB) is determined mainly by the balance between the microorganism and the host defense systems. T cell-mediated immune response begins after dissemination of *Mycobacterium tuberculosis* in the body. Many distinct types of T helper cells are present at the site of infection. Interleukin-22 (IL-22) helps in cell proliferation, regeneration, and provides protection against microbial diseases. It acts via its receptor IL-22R1 that signals various downstream signaling cascades. IL-22 plays an important role in mycobacterial infection but its role in the progression or control of TB and the underlying mechanism are not fully understood. The aim of the present study was to estimate circulating IL-22 levels and to correlate it with IL-22R1 relative gene expression levels in the peripheral blood of TB patients and healthy controls. **Methods:** 85 sputum positive TB patients and 85 asymptomatic healthy subjects were enrolled in the study taking into account the exclusion and inclusion criteria. After obtaining due informed consent, 5mL venous blood was withdrawn in plain and EDTA vacutainers from all participants enrolled. Serum IL-22 levels were estimated using Human IL-22 ELISA kit (Krishgen BioSystems, India). Total RNA was isolated from whole blood, converted to cDNA and gene expression of IL-22R1 was done using Sybr green real time PCR technology (Thermo Fisher Scientific, USA). Statistical analysis was performed using SPSS. **Results:** The median (IQR) of serum IL-22 was significantly lower in TB patients compared to controls (18.55 (5.08) vs 49.38 (162.88) pg/mL; p<0.0001). IL-22R1 expression was significantly upregulated with a fold change value of 2.04 in TB patients. A strong and significant positive correlation was observed between the protein levels and the expression of its receptor. On ROC analysis, IL-22 discriminated TB patients from healthy controls at 21.67 pg/mL with a sensitivity and specificity of 82% and 82% respectively and an AUC of 0.904. Whereas, relative expression of IL-22R1 discriminated TB patients from healthy controls at 0.18 with sensitivity and specificity of 66%

and 64% respectively. **Conclusion:** IL-22 levels were found to be significantly decreased in patients, with a plausible compensatory increase in its receptor expression. It is crucial for the modulation of tissues in response to TB infection. IL-22 appears to have a good diagnostic efficiency in discriminating TB patients from healthy controls, the exact role of which needs to be further explored.

## A-184

### Genetic diagnosis by clinical exome: Kabuki Syndrome

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**Background:** A 3-year-old patient was referred from Neuropediatrics for genetic counseling due to short stature associated with facial dysmorphism, hypotonia and mild psychomotor retardation. Due to these phenotypic characteristics the patient was clinically diagnosed with Rubinstein Taybi Syndrome. This malformation syndrome of genetic origin is characterized by short stature, mental retardation, microcephaly, facial anomalies, thumbs, thumbs and first toes wide.

#### Methods:

A genetic sequencing study of the CREBB gene related to Rubinstein Taybi syndrome was requested. The result of this study did not detect any variation in the CREBB gene sequence that could be associated with the disease. After several years of follow-up by various specialists, a new referral to genetic counseling was requested to extend the genetic study. The patient currently presents delayed psychomotor, cognitive and language development, joint hyperlaxity with frequent falls and continual spontaneous subluxation of the kneecaps when the knees are extended. He has an aversion to food, still taking most of his food in crushed form. Genetic study of clinical exome directed to phenotype by massive sequencing is requested using the following HPO (Human Phenotype Ontology) terms: Short stature HP:0004322, Abnormal facial shape HP:0001999, Joint hypermobility HP:0001382, Neurodevelopmental delay HP:0012758, Knee dislocation HP:0004976.

#### Results:

The result of this genetic study was the following: The result of this genetic study was the following: The presence in heterozygosis of a nonsense variant classified as pathogenic according to the ACMG (American College of Medical Genetics and Genomics) has been identified. Gene KMT2D: NM\_003482.4:c.11971C>T p.Gln3991\* in heterozygosis with autosomal dominant inheritance.

#### Conclusion:

The variant c.11971C>T p.Gln3991\* detected in the KMT2D gene is a nonsense-type change that predicts the change of the amino acid Glutamine by a premature stop codon at position 3991, resulting in a truncated protein. This variant is described in the databases consulted as pathogenic and related to Kabuki syndrome type 1. Kabuki syndrome type 1 (OMIM: 147920) is a rare neurodevelopmental disorder that presents with multiple congenital anomalies such as intellectual disability, congenital heart defects and renal malformations, persistent fetal padding of the fingers, postnatal short stature, skeletal anomalies and specific facial features (broad and arched eyebrows, elongated palpebral fissures, large prominent and cupped ears, depressed nasal tip...). The syndrome typically manifests with neonatal/infant hypotonia, feeding difficulties, psychomotor retardation and intellectual disability in 90% of patients. The exome is the DNA fraction of the genome that codes for protein production and constitutes approximately 2% of the total human genome. The targeted clinical exome is used for the study of genes that are related to the patient's phenotype. Advances in molecular genetic techniques have allowed the development of new techniques such as massive sequencing, offering the possibility of studying a large number of genes in a single analysis. In our case, the use of the clinical exome has allowed us to establish a new genetic diagnosis of the patient according to his clinical phenotype.

## A-185

### Evaluation of a Novel Buccal Sample collection Device for High Quality Human Genomic DNA

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**Background:** There is a growing trend toward non-invasive, self-administered, remote collection of buccal (cheek) cells, since they are easy to collect, simple to transport at room temperature, and can be a rich source of DNA for genetic testing and forensic applications. This study was designed to investigate the extraction yield and quality of

human genomic DNA (hgDNA) from buccal cells collected with GenSwab™ sample collection devices (Genueri Inc). The extracted DNA was subjected to qPCR analysis to determine the yield of DNA and if any PCR inhibitors are present.

**Methods:** Buccal cells were collected from participants according to the manufacturer's instructions. Samples were preserved by desiccation. Three punches per sample were removed from the dried matrix spots with a disposable 3 mm punch device. Extraction of hgDNA from the discs was performed using QIASymphony SP with the QIASymphony® DNA Investigator® Kit (Qiagen) according to manufacturer's directions. To detect and quantify hgDNA, quantitative real-time PCR was performed using the Investigator® Quantiplex Pro Kit (Qiagen). Standard curves for DNA quantitation were prepared (Applied Biosystems) using control DNA supplied with the kit. The DNA quantitation assay used an internal PCR control (IPC).

**Results:** DNA extraction and quantitation were evaluated using 11 samples of dried buccal cells collected with the GenSwab™ device. The range of Ct data for the samples was 25.18 to 27.03 compared to 27.18 Ct for the positive control. Quantification of extracted DNA ranged from 126.89 to 878.89 pg/μL compared to 106.33 pg/μL for the positive control. DNA degradation index was calculated as the ratio between autosomal short and long PCR fragments. A theoretical value 1 would mean identical amounts of PCR product were detected and no degradation occurred. The indices of samples ranged from 0.75 to 0.90 compared to 0.76 for the positive control, indicating no DNA degradation. Lastly, to check for successful amplification and to identify presence of PCR inhibitors, a 434 bp internal positive control (IPC) was used. Ct value for the IPC was 21.76 compared to range of 21.19 to 21.30 Ct for the samples, indicating there was no PCR inhibition. **Conclusion:** GenSwab™ devices are shown to be a suitable device for collection of buccal cells and should be functionally applicable to downstream methods for hgDNA analysis, including qPCR, Short Tandem Repeats (STR), and Next Generation Sequencing. The results demonstrate substantial extraction yields and high quality hgDNA from desiccated buccal cells collected with the GenSwab™ device. The quality of DNA is highlighted by minimal degradation and the absence of PCR inhibitors.

## A-186

### Early diagnosis of coronary artery disease by micro RNA panel in patients with angina

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#### Background:

Early diagnosis of coronary artery disease (CAD) in patients with angina is a challenge. Currently, diagnosis of CAD in patients with angina is confirmed by angiography, which is invasive and not easily available in developing countries. There is a need to establish a non-invasive biomarker for early diagnosis of CAD. Micro RNA has been found in the blood as stable molecule, which may be used as diagnostic biomarker for angina.

#### Methods:

Diagnostic accuracy study was carried out in tertiary care hospitals, Rawalpindi, Pakistan. A total of 58 patients, aged 25-70 years having chest pain and troponin-I -ve with stenosis  $\geq$  50% were diagnosed by coronary angiography. Fifty-five subjects having coronary artery stenosis less than 50% were included as controls. RNA was extracted by quick RNA whole blood kit and miR-39-3p was supplemented as spike-in control. cDNA was synthesized by applied biological material protocol. Primers of miR-21, miR-33a, miR-133a, miR-145, miR-146a and cel-miR-39-3p were used with master Mix-ms of ABM for analysis of miRNA by real-time polymerase chain reaction on RotorGene.

#### Results:

In patients of CAD aged 55.24±11.61, miRNA analysis on receiver operating characteristic curve revealed more than two-fold, significant up-regulation in miRNA-33a, miRNA-133a, miRNA-146a expression as compared to controls (P<0.05), while no significance was found for miR-21 and miR-145 (Table-1).

miRNA	AUC (95% CI)	Cut off	Sensitivity	Specificity
miRNA-21	0.609(0.488-0.73)	2.05	50%	53%
miRNA-33a	0.884(0.825 -0.943)	3.54	84%	78%
miRNA-133a	0.683(0.584 -0.781)	1.5	60%	53%
miRNA-145	0.417(0.302-0.532)	1.0	50%	52%
miRNA-146a	0.785(0.701 -0.869)	2.24	74%	64%

CombiROC analysis of miR-33a, miR-133a, and miR-146a correctly classified CAD patients with angina as compared to controls (AUC = 0.928, sensitivity 84%, specificity 88%). There was significant ( $p < 0.05$ ) positive correlation between miRNA-33a ( $r = 0.583$ ), miRNA-133a ( $r = 0.361$ ) and miRNA-146a level ( $r = 0.366$ ) with severity of CAD.

#### Conclusion:

The panel of micro-RNA (miRNA-33a, miRNA-133a, and miRNA-146a) has diagnostic role in early detection of CAD in patients with angina. This panel of miRNA may be used as a noninvasive biochemical marker before undergoing angiography for discrimination of healthy individuals from CAD patients.

## A-187

### Performance Evaluation of BioGX Xfree™ COVID-19 Direct RT-PCR Assay

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**Context:** Covid-19 is pandemic infection caused by SARS-CoV-2 virus, as of January 2022 the virus caused more than 5,000,000 deaths worldwide and more than 900,000 in the United State. Identifying infection and preventing spreading of the virus is the key to fight the infection; Real-Time RT-PCR assays are considered the gold standard and several assays have been granted Emergency Use Authorization. We evaluated extraction free assay to help improve turnaround time without sacrificing sensitivity and specificity. **Design:** The BioGX Xfree™ COVID-19 Direct is a real-time RT-PCR test that utilizes primer and probe sequences based on the United States Centers for Disease Control and Prevention (USCDC) assay for detection of SARS-CoV-2 (N1 gene region) and human RNase P. Additionally, a non-naturally occurring single-stranded RNA has been integrated into the lyophilized format to serve as an internal amplification control; The assay time is about 2 hours for 94 samples using direct method with Bio-Rad CFX96 Touch™ platform. The assay can be done either with extraction or direct method; however, the validation was done using direct method. The assay was evaluated for: precision, reproducibility, accuracy, and correlation with existing extraction RT-PCR method using Seegene Allplex assay. The limit of detection was verified by serially diluting quantified inactivity SARS-CoV-2 whole virus. The percentage agreement for the evaluation steps was calculated. Statistical analyses were done using Analyse-it. **Results:** The percentage agreement for precision, reproducibility, and accuracy were 100%. The limit of detection was verified at 1250 copies/mL. The patients' correlation was 100%. **Conclusions:** Laboratories had to overcome many challenges during the COVID19 pandemic including accurate assay, supply shortages, and the demand for faster turnaround. The BioGX Xfree™ COVID-19 Direct offers high precision, accuracy, and sensitivity. The sensitivity of the assay was comparable and even better than other RT-PCR extraction method. The extraction-free method helped alleviating the shortage of consumable to process the extraction and provided significant saving on turnaround time and allowed processing more samples. Although the assay is very easy to perform but like any molecular assay a close attention to avoid contamination should be taken into consideration during training.

## A-188

### Identification of a Novel Splicing Variant and the Other Hidden Variant in *GALNS* by Whole Genome Sequencing and RNA Study in Mucopolysaccharidosis IVA

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**Background** Mucopolysaccharidosis type IVA (MPS IVA; Morquio syndrome type A) is a rare autosomal recessive inherited disorder caused by deficiency in lysosomal hydrolase N-acetylgalactosamine-6-sulfatase (*GALNS*) gene. An early diagnosis followed by early enzyme replacement therapy (ERT) is crucial for better clinical outcome and carrier detection. However, a relatively high number of patients (11.6%) are genetically undiagnosed (Zanetti et al., 2021). Herein, we report a Korean boy with apparent clinical and biochemical features of MPS IVA, whose molecular diagnosis was made clear by WGS and RNA study after an inconclusive result in Sanger sequencing due to allele-drop out. **Case presentation** A 28-month-old boy presented with pectus carinatum, kyphoscoliosis, and joint hypermobility with multiple skeletal dysplasia involving vertebrae and hip joint. Total urinary glycosaminoglycans was elevated with predominant keratan sulfate fraction and leukocyte N-Acetylgalactosamine-6 sulfate sulfatase (GalNac6S) activity was significantly decreased, which were diagnostic for MPS IVA. Sanger sequencing of *GALNS* was performed for molecu-

lar diagnosis, however only one heterozygous novel variant, c.566+3A>T (p.?), with uncertain clinical significance (PM2+PP4 according 2015 ACMG/AMP guideline) was identified. In search of the unidentified allele that is either in the coding region or non-coding region, we conducted whole genome sequencing (WGS) and family study, and found a compound heterozygous mutation of c.1019G>A (p.Gly340Asp) and c.566+3A>T (p.?) in *GALNS*. With subsequent investigation, we discovered that the variant, c.1019G>A, which is a previously reported known pathogenic variant (PM2+PM3\_strong+PP3+PP4), was undetected in the initial sequencing caused by allele-drop-out due to the polymorphism (rs3859024) at the primer annealing location. To confirm the effect of the other variant,

c.566+3A>T, we performed functional analysis by mRNA sequencing. The results showed that this intronic variant causes skipping of exon 5 (r.423\_566del) generating a premature stop codon (p.Trp141\*), and could be finally classified as a likely pathogenic variant (PS3+PM2+PM3+PP4). **Conclusion** We suggest WGS, especially those with probe-based target enrichment platform, as an effective diagnostic tool in patients undiagnosed with conventional sequencing method. And moreover, this case report of a MPS IVA patient with compound heterozygous variants in *GALNS* with a novel splice variant would contribute to the growing body of evidence for the classification of *GALNS* variants.

## A-189

### HAZIS-CirR: a novel enrichment and isolation platform of urinary circulating RNAs

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**Background:** Circulating RNAs, including circulating microRNA (circulating miRNA) and circulating messenger RNA (circulating mRNA), are key regulators of gene expression in physiology and pathology, and have potential utility as diagnostic, prognostic, and predictive non-invasive cancer biomarkers. In prostate cancer (PCa), changes in specific circulating RNA expressions can be used as biomarkers for early cancer detection, but there are unmet needs such as unclear types and roles of PCa-specific circulating RNAs, PCa detection in benign prostatic hyperplasia (BPH) patients, and limitations of conventional methods of sample volume capacity and low circulating RNAs concentration. Here, we report a simple and rapid circulating RNAs isolation technique using homobifunctional hydrazides (HHS) with amine-modified zeolite (AZ), termed as HAZIS-CirR, for analysis of urinary circulating RNAs in clinical specimens. **Methods:** The process of HAZIS-CirR consists of 4 steps: Sample mixing and incubation, Circulating RNAs enrichment, Circulating RNAs washing and Circulating RNAs isolation. Circulating RNA is captured on the AZ surface through covalent and electrostatic coupling between AZ, ADH and circulating RNA during the sample mixing and incubation step. Circulating RNAs captured on the AZ surface are injected into a PVDF syringe filter, debris and unbound molecules smaller than the filter pore size (0.45  $\mu\text{m}$ ) pass through the filter and discarded, and circulating RNAs are concentrated on the filter surface by AZ (about 10  $\mu\text{m}$ ). Residual debris is removed through PBS washing, and circulating RNAs are extracted by breaking the reactive bonds between AZ, ADH, and circulating RNAs using elution buffer (pH 10.6). **Results:** The HAZIS-CirR offers instrument-free enrichment and isolation of circulating RNAs in 20 min without volume limitation, thermo-regulator, and lysis step. The HAZIS-CirR have high RNA capture efficiency (82.03–92.38%) and a detection limit of 20 fM, which is 10 times higher than the conventional method (200 fM), using the *has-mir-21-5p* ss mimics. We confirmed the clinical utility of HAZIS-CirR by analyzing circulating mRNA (PCA3 and TMPRSS2-ERG fusion gene) and circulating miRNA (*miR-21-5p*, *miR-141-3p*, *miR-148a-3p*, *miR-375-3p*, *miR-483-5p*, and *miR-574-3p*) using 89 urine samples for PCa diagnosis. A significant increase was observed in the relative quantification (RQ) of TMPRSS2-ERG gene fusion 3 for mRNA and *miR-141-3p*, *miR-375-3p*, *miR-483-5p*, and *miR-574-3p* for miRNA in the PCa when compared to the BPH patients. Furthermore, we established 3 miRNAs panels, panel 1 (*miR-141-3p*, *miR-375-3p*, *miR-483-5p*, and *miR-574-3p*; PCa versus BPH and normal), panel 2 (*miR-21-5p* and *miR-148a-3p*; PCa versus normal), and panel 3 (*miR-21-5p*, *miR-141-3p*, *miR-148a-3p*, *miR-375-3p*, *miR-483-5p*, and *miR-574-3p*; BPH versus normal), by comparing the levels of miRNAs. **Conclusion:** The clinical utility of HAZIS-CirR is confirmed by analyzing circulating mRNAs and circulating miRNAs in 89 urine samples. Furthermore, three miRNA panels that differentiate PCa from BPH and control, PCa from control, and BPH from control, respectively, are established by comparing miRNA levels. HAZIS-CirR will be used as an optimal and established method for the enrichment and isolation of circulating RNAs as diagnostic, prognostic, and predictive biomarkers in human cancers.

## A-190

## Analysis of HLA A, B, C, DR types associated with CMV-specific cell mediated immunity in seropositive kidney transplant candidates

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**Background:** Cytomegalovirus (CMV)-specific cell-mediated immunity (CMV-CMI) is variable in CMV-seropositive (R+) individuals and has been defined as a predictive biomarker of CMV infection after kidney transplantation (KT). We analyzed whether HLA A, B, C, DR types are associated with CMV-CMI response in R+ KT candidates. **Methods:** A total of 229 CMV transplant candidates were included. The CMV-CMI was measured by enzyme-linked immunospot (ELISPOT) assays against pp65 and IE-1 antigens. **Results:** In KT candidates, pp65-and IE-1-ELISPOT results were [277.5 (233.6-325.8)] and [41.0 (27.2-59.8)] [median (95% CI)] spots per 200,000 lymphocytes. Candidates with HLA-A2 (+) or HLA-A30 (-) had increased CMV-CMI than those with HLA-A2 (-) or HLA-A30 (+), respectively ( $P=0.025$  and  $0.042$ , respectively). HLA-B7 or HLA-B58 was associated with an increase in IE-1 CMV-CMI ( $P=0.027$  and  $0.032$ , respectively). We divided the patients into four groups according to pp65-and IE-1-ELISPOT results (pp65-ELISPOT; <115, 115-276, 277-470,  $\geq 471$ , IE-1-ELISPOT; <9, 9-40, 41-184,  $\geq 185$  spots per 200,000 lymphocytes). Candidates with HLA-A2, -B7, -B54, or -Cw1 tended to be included in the group with higher ELISPOT results. When we analyze the association between Korean HLA haplotypes and CMV-CMI results, candidates with HLA-A2/B27/Cw1/DR1 had the highest pp65-ELISPOT results [456 (244.9-59.3)], and those with HLA-A30/B13/Cw6/DR7 had lowest results [105.5(18.2-264.6)] ( $P=0.017$ ). For IE-1-ELISPOT, A2/B54/Cw1/DR15 haplotype and A30/B13/Cw6/DR7 haplotype had highest and lowest results, respectively [372.3 (56.1-808.1) vs. (10.8 (4.7-94.5),  $P=0.028$ )]. **Conclusions:** We observed the differences in CMV-CMI according to the HLA types and HLA haplotypes. HLA types may help stratify the risk of CMV infection associated with CMV-CMIs in CMV-seropositive KT candidates.

## A-191

## Clinical Performance of the Ultra-High Throughput Amplitude™ platform for Multiplex Respiratory Pathogen detection

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**Background:** Clinical laboratories have been challenged with extremely high testing volumes, especially during COVID-19 surges. The Amplitude™ platform is an ultra-high throughput automated RT-PCR solution that is capable of processing up to 8000 samples in a 24-hour period. We evaluated the clinical performance of the Amplitude™ platform for the simultaneous detection of SARS-CoV-2, Influenza and RSV. **Methods:** A retrospective study was conducted using 321 previously characterized clinical specimens (60 SARS-CoV-2 positive, 50 Influenza A positive, 21 Influenza B positive, 70 RSV positive, 120 triple negative) in a blinded and randomized fashion. Multiplex testing was performed on the Amplitude™ platform using the TaqPath™ COVID-19, Flu A/B, RSV HT Kit. Comparator testing was performed using the Lyra® SARS-CoV-2 Assay (COVID-19), Lyra® Influenza A+B Assay (Influenza A/B) and Lyra® RSV+hMPV Assay (RSV). Performance was assessed by calculating the positive percent agreement (PPA) and negative percent agreement (NPA). **Results:** The multiplex assay on the Amplitude™ platform showed good concordance with the comparator for each of the targets: 95.2% PPA and 100% NPA for COVID-19; 98.5% PPA and 100% NPA for Influenza A/B; and 97.1% PPA and 100% NPA for RSV. A total of 6 discordant samples were tested positive using the comparator method and negative using the TaqPath™ COVID-19, Flu A/B, RSV HT kit. However, four of those six samples had been tested as negative for the specific target when they were first collected. In addition, the Ct values for these samples were close or higher than the average limit of detection. **Conclusion:** The Amplitude™ platform offers an ultra-high throughput processing solution for the accurate detection of respiratory pathogens in a multiplex fashion with added benefits of time and cost. This is applicable for high volume laboratories dealing with increased testing demand such as during COVID-19 surges, especially when associated with the seasonal flu.

Target	Comparator	TaqPath™ COVID-19, Flu A/B, RSV HT Kit			
		PPA	95% CI	NPA	95% CI
COVID-19	Lyra® SARS-CoV-2 Assay	95.2%	86.7%-99.0%	100.0%	96.9%-100.0%
Influenza A/B	Lyra® Influenza A+B Assay	98.5%	92.0%-100.0%	100.0%	98.1%-100.0%
RSV	Lyra® RSV+hMPV	97.1%	89.9%-99.6%	100.0%	98.1%-100.0%

## A-192

## Concurrent Cell Free EGFR Testing and its Influence on Survival Outcome of Non Small Cell Lung Cancer

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**Background:** Mutations in EGFR are the most common mutation type found in non-small cell lung cancer lung carcinoma (NSCLC) and are also most amenable to tyrosine kinase inhibitor (TKI) treatment. Although this mutation may be detected in circulating blood, liquid biopsy is recommended as an alternative to tissue biopsy when the latter is inadequate or unobtainable as per the recent guidelines of 2018 for molecular testing in NSCLC. We evaluated the concurrent testing for these EGFR mutations in tissue and plasma and correlated with the overall survival outcome. **Methods:** Ten ml of blood was collected in EDTA vials within two weeks from histopathological diagnosis of NSCLC (Stage III or IV) from 100 patients prior to the start of any treatment. The treatment was decided based on the mutation status in the tissue. DNA extraction from formalin-fixed paraffin embedded (FFPE) tissue was done using QIAamp DNA FFPE tissue kit. Cell-free total nucleic acid extraction from plasma was done using MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit from Thermo Fisher Scientific. Real-time PCR reaction was performed on Quant Studio™ 6 Flex Real-Time PCR System. EGFR mutation was tested by using TRUPCR® EGFR kit from 3B BlackBio, Biotech India Limited, based on nested ARMS (Amplification Refractory Mutation System) where mutation-specific reactions (exon 18,19,20 and 21) of the EGFR gene and a reference (wild type control in exon 2 without any known polymorphism/mutation) is amplified simultaneously. This kit is validated for formalin-fixed paraffin-embedded (FFPE) and liquid biopsy (cell-free DNA). The study was approved by the institutional ethics committee (2020-1-MD-EXP-14). All statistical analyses were conducted in R version 4.1.1. **Results:** A total of 64 patients showed EGFR mutation irrespective of sample type. 47 patients showed the presence of EGFR mutation in tissue while it was found in 43 patients when tested in plasma with a concordance of 44%. Seventeen cases showed plasma EGFR positivity while the corresponding tissue was wild type. The sensitivity and specificity of cell-free testing for EGFR mutation were found to be 55.3% and 67.9% respectively. 31 of the 47 tissue EGFR mutant cases received TKI based therapy. Univariate Cox regression analysis showed tissue mutation status (OS:HR = 0.4; 95% CI: 0.2-0.7;  $p = 0.003$ ), targeted therapy (OS:HR = 0.29; 95% CI: 0.1-0.8;  $p = 0.02$ ) as the significant factors associated with overall survival (OS). The OS after therapy for Tissue EGFR mutant, EGFR mutant irrespective of sample type, and tissue EGFR wild type was found to be 19 months, 12 months, and 9 months respectively. However, when OS in plasma EGFR positive cases was compared with plasma wild type cases, there was no significant difference ( $p=0.34$ ). **Conclusion:** Several cases of EGFR mutation may be missed if tissue biopsy alone was considered for therapeutic management. These patients would have benefited from targeted therapy if plasma mutation status at baseline had been considered for treatment hence suggesting liquid biopsy testing must be concurrent to tissue biopsy testing and not just an alternative when tissue is not available.

## A-194

## Prevalence of Influenza A and SARS-COV2 coinfection in patients referred to a clinical chemistry laboratory in Brazil

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**Background:** The activity of the Influenza A (H3N2) virus increased in the last weeks of December/2021 worldwide, due to the rise of population mobility and relaxation of protective measures against SARS-CoV2 and other factors. It is reported that 94.2% of COVID-19 patients can be co-infected with one or more pathogens, specially bacteria. The most common reported co-occurrence with other respiratory viruses is with

Influenza virus, which has similar symptoms, transmission mechanisms and seasonal coincidence with SARS-CoV-2. The co-occurrence of COVID-19 with other viral respiratory diseases during a pandemic cannot be ruled out and early suspicion of this co-infection is challenging because of the similarity between both viruses described above. In Brazil, the greatest increasing in trends of H3N2 detections were concomitant with the peak of the SARS-CoV2 omicron variant in southern hemisphere. Thus, the objective of this study was to evaluate the prevalence of Influenza and SARS-CoV-2 coinfection in patients who were referred to our laboratory for a RT-qPCR respiratory virus panel testing. **Methods:** This was an observational study carried out by collecting Data from male and female patients who underwent RT-PCR tests panel for Influenza A, Influenza B, RSV and SARS-CoV-2 in from 01/01/2022 to 01/31/2022 were collected for the laboratory database. The total number of test executed and the positivity for each viruses were calculated and presented as absolute and relative frequency. **Results:** A total of 113,934 RT-PCR respiratory panel were performed during evaluated period, 72,316 (63.47%) returned a positive result for at least one virus. Among the positive tests, SARS-CoV2 was detected in 54,774 (75.74%), Influenza A in 15,124 (20.91%), and the other tested viruses 2,418 (3%). Co-infection of SARS-CoV2 and Influenza A was observed in 1,309 (1.81%) of them, while the co-infections for other viruses can be considered negligible. **Conclusion:** Our findings suggest that, besides the high positivity observed for the test (63.47%) there was no high prevalence of SARS-CoV2 and Influenza A co-infection (1.81%) in patients referred to our clinical chemistry laboratory in Brazil. Additional data on SARS-CoV2 and Influenza A co-infection are necessary and the extension of the evaluation period would increase the robustness to this results and more solid conclusions could be obtained. Thus, this evaluation will be extended for the whole 2022.

## A-196

### Development of Reference Materials for Detection of Hepatitis E Virus (HEV)

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**Background:** Hepatitis E is an inflammatory liver disease caused by the Hepatitis E virus (HEV) and is the common cause of acute hepatitis worldwide. There has been increasing reports of transfusion transmitted HEV in blood donation recipients. There are an estimated 20 million infections per year, 3 million symptomatic cases, and around 70,000 HEV-associated deaths. Although most people infected with HEV recover completely, HEV can cause serious threats to immunocompromised patients - transplant patients at a higher risk for developing chronic infections. Symptoms of HEV infection include fever, jaundice, etc. but these can progress to cirrhosis, liver failure, and overall increased mortality for higher-risk groups. The need for HEV screening in blood banks is gaining importance. Molecular assays have become important tools for the diagnosis and management of donations at blood banks. Blood donations are frequently pooled together to reduce testing costs; however, this results in reduced sensitivity of detection. It is critical for labs to monitor the sensitivity of their testing process for reliable results. LGC Clinical Diagnostics has developed AccuPlex HEV molecular control, which is a non-infectious, stable, and reproducibly manufactured reference material to aid nucleic acid testing across blood banks.

**Methods:** AccuPlex™ Hepatitis E Virus Molecular Control consists of recombinant Sindbis viruses that encompass the entire genome of HEV (7.2 kb). We used Genotype 3a (AB074918 in Genbank) for the product design since it is the prevalent genotype found in infections in Europe and the US. Digital PCR assays for HEV were designed to quantitate viral load. Formulation of the AccuPlex HEV Molecular Control is guided by HEV cp/mL obtained by digital PCR. The final product is targeted as a low positive control very close to the LOD on Roche cobas HEV assay run on the 6800/8800 system and Grifols Procleix HEV Assay run on the Panther testing systems.

**Results:** AccuPlex HEV constructs were formulated in defibrinated human plasma at a concentration of 6.15 E+03 copies/mL (as per testing using QiaAmp viral RNA mini kit and BioRad QX-200 droplet digital PCR system). Serial 2-fold dilutions were made to generate a panel, that was then tested on Grifols Procleix HEV assay in duplicate to verify detection limits. The AccuPlex molecular control material is being developed with a target concentration that is ~3 - 5 times this cut-off.

**Conclusion:** LGC Clinical Diagnostics is developing a stable, replication-deficient molecular control material for use with *in vitro* diagnostic test methods that detect HEV. This study helps ensure that the molecular control will be low positive and useful for monitoring potential changes in sensitivity of the assay. The use of these manufactured quality controls, in contrast to remnant patient samples, ensures not only consistency of supply and performance, but also safe handling. AccuPlex materials can be utilized by diagnostics labs for development, validation, and ongoing QC monitoring of assays for blood screening.

## A-197

### Development of Reference Materials for SARS-CoV-2 Omicron Variant Detection

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**Background:** The Omicron variant is a SARS-CoV-2 variant of concern that was first reported to the WHO in November 2021. According to the CDC, the Omicron variant is more transmissible than COVID-19 and the Delta variant. Newly arising SARS-CoV-2 variant strains are of great concern because it is unclear what effect the mutations have on disease transmission and disease severity. Governments and public health organizations are working to increase surveillance through NGS sequencing and genotyping to understand which variants are circulating and how they are affecting disease outcomes. To ensure the quality of testing, whole process, reproducibly manufactured reference materials are needed. LGC Clinical Diagnostics developed AccuPlex™ SARS-CoV-2 Omicron Variant Reference Material Kit to fulfill this need.

**Methods:** SARS-CoV-2 Omicron Variant Reference Material Kit utilizes proprietary AccuPlex® recombinant virus technology. The reference material contains the entire genomic RNA from SARS-CoV-2 with S and N-gene mutations within the Omicron Variant of concern B.1.1.529. The kit contains a positive vial for the Omicron variant and a SARS-CoV-2 wild type Wuhan sequence (NC\_045512). A negative control vial containing RNase P is also available in the kit. The product contains whole, heat-treated, replication-deficient recombinant viruses in a simulated viral transport media so that the product is a whole process control. The positive vials have a nominal concentration of 1.5E+04 copies/mL and the negative vial is targeted at 5.0E+03 copies/mL. These reference materials were also tested on LGC Biosearch SARS-CoV-2 Variant ValuPanels genotyping assays to confirm the mutations observed in the S-gene region.

**Results:** The nominal concentration of the positive vials was confirmed to be ~1.5E+04 copies/mL (as per testing using QiaAmp viral RNA mini kit and BioRad QX-200 droplet digital PCR system). Using PCR-based SARS-CoV-2 genotyping assay, the mutations in the AccuPlex materials (ΔH69/70V, P681H, H655Y) were detected as expected. The AccuPlex materials behaved similarly to native pathogens on assay lower limits of detection studies, indicating that the reference material is comparable to patient samples.

#### Conclusion:

LGC Clinical Diagnostics has developed a non-infectious, replication-deficient full process reference material for SARS-CoV-2 genotyping by either PCR-based or NGS-based methods. The reference material is targeted to a concentration that is low positive and challenging for variant detection assays. These reference materials can be used for genomic surveillance, development, validation and ongoing QC of variant detection assays aiding labs with their SARS-CoV-2 diagnostic workflows.

## A-198

### Improvement of the quantitiveness of methylation analyses using a methylation-sensitive restriction enzyme

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**Background:** DNA methylation is an epigenetic mechanism for gene expression control that is expected to be a useful biomarker for cancer diagnosis and prognosis. Bisulfite treatment, which is widely used in DNA methylation analyses, has drawbacks, such as DNA fragmentation and loss, and involves complicated processing. Conversely, methylation-sensitive restriction enzyme polymerase chain reaction (MSRE-PCR) is a rapid and simple method without bisulfite treatment that has the advantage of allowing the analysis of small amounts of DNA. We examined this method, but encountered cases of discordance with the gold-standard bisulfite sequencing PCR (BSP); thus, further improvement of this quantitative method is needed. Therefore, we attempted to overcome these problems by correcting the reactivity of the enzyme using a methylation-insensitive restriction enzyme (MIRE) and verified it in a methylation analysis of the *ESM1* gene promoter region. Furthermore, we performed methylation analyses of the *SEPTIN9* and *SFMBT2* gene promoter regions, which may be new methylation markers for bladder cancer, using this method. The results obtained were examined for their relationship with clinicopathological factors.

**Methods:** We researched genomic DNA (gDNA) extracted from T24 cells and EpiScope Methylated HeLa gDNA (Takara Bio), as a control of methylation rate (0% and 100%, respectively), as well as gDNA extracted from 30 fresh-frozen tissue samples

collected during transurethral resection of a bladder tumor (TURBT). We used HapII (Takara Bio) as the MSRE and MspI (Takara Bio) as the MIRE; the latter has the same recognition site as HapII but is not affected by the presence or absence of methylation. After carrying out the enzymatic reaction at 37°C for 60 min, heat treatment was performed at 95°C for 10 min. We performed real-time PCR on the LightCycler 96 (Roche) using primers and a hydrolyzed probe designed for detecting methylation of the target region. The methylation rate was calculated using a conventional formula and a new corrected formula when using MspI, and the results were compared with those of BSP. In addition, 48 TURBT samples were added to the methylation analysis of the *SEPTIN9* and *SFMBT2* promoter regions, which was performed using the conditions used for *ESM1*. We analyzed the methylation rate of *SEPTIN9* and *SFMBT2* using a corrected formula, and researched its association with various clinicopathological factors.

**Results:** Verification of the corrected formula revealed that the concordance rate with BSP was 90.0% (27/30) using the conventional formula and 96.7% (29/30) using the corrected formula when 20% or more of the samples were methylation positive. The investigation of the relationship between the clinicopathological factors of the TURBT samples and the DNA methylation rate revealed that *SEPTIN9* methylation exhibited a significant correlation with recurrence within 1 year, whereas *SFMBT2* methylation showed a significant correlation with tumor grade.

**Conclusion:** Correction using the MIRE improved the concordance rate with BSP and allowed a more accurate quantification of hypomethylated regions. Furthermore, we found an association between the methylation of the *SEPTIN9* and *SFMBT2* promoter regions and various clinicopathological factors of bladder cancer using this method.

## A-199

### Performance Evaluation of TaqMan SARS-CoV-2, Flu A/B, RSV RT-PCR Multiplex Assay for the Detection of Respiratory Viruses

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**Background:** In the midst of this COVID-19 pandemic, co-infection with influenza and respiratory syncytial virus (RSV) can add an additional challenge to detect and differentiate them due to overlapping clinical presentations. A rapid method that can detect these pathogens in a single test can have a significant diagnostic value to improve clinical outcomes. Here we evaluated the performance characteristics of Taqman SARS CoV2, Flu A/B, RSV RT PCR multiplex assay for the detection of SARS CoV2, Flu A/B, and RSV in nasopharyngeal swab and saliva.

**Methods:** A total of four culture fluids of Influenza A virus (H3N2) (A/Wisconsin/67/2005), Influenza B virus (B/Virginia/ATCC4/2009), RSV A2 cpts-248, SARS-CoV-2 (USA-WA1/2020), and quantitative RNA controls of Influenza A virus (H1N1) strain A/PR/8/34 (VR-95DQ), RSV A2 (VR-1540DQ) and SARS-CoV-2 (MN908947.3 Wuhan-Hu-1) from ATCC and Zeptomatrix, NY, USA were used for the validation study. All the validation controls were diluted in ten-fold dilution series from 10<sup>8</sup> to 1 copies/reaction concentrations for LOD determination and generating standard curve to determine efficiency and linear range. Results from dilutions were also used to evaluate the reproducibility. A total of 110 nasopharyngeal and 70 saliva specimens were used for clinical evaluation. All specimens were extracted using automatic Hamilton star and tested using Taqman SARS CoV2, Flu A/B, RSV RT PCR multiplex assay on the Quant Studio 12 Flex. SARS-CoV-2 variant controls (B.1.1.7\_601443, B.1.617.1\_1662307, P.1\_792683, B.1.351\_678597 and B.1.1.529/BA.1) were used to check the accuracy and impact on TaqMan SARS-CoV-2, Flu A/B, RSV RT-PCR multiplex assay. Data were analyzed using QuantStudio™ Design and Analysis Software v2.5 for TaqMan SARS-CoV-2, Flu A/B, and RSV Multiplex Assay method and SDS software v1.4.1 for reference method.

**Results:** All validation controls showed 100% accuracy with CV of precision <5%. In the clinical evaluation, the accuracy of this multiplex assay was 96.38% to 100% (95% CI) in nasopharyngeal and 94.87% to 100% (95% CI) in saliva. Standard curve of TaqMan SARS-CoV-2, Flu A/B, and RSV Multiplex Assay showed linear efficiency between 90-110% and R<sup>2</sup> values is 0.99 for all targets of Influenza A, Influenza B, and RSV. The association of cycle threshold value of SARS-CoV-2 positive samples showed a strong positive correlation between the reference method (Taqpath combo kit assays) and Taqman SARS CoV2, Flu A/B, RSV RT PCR multiplex assay with Pearson correlation (r) of 0.98. TaqMan SARS-CoV-2, Flu A/B, RSV RT-PCR multiplex assay has no impact with SARS-CoV-2 variants including Omicron that has at least 50 mutations for the detection of SARS-CoV-2.

**Conclusion:** The TaqMan SARS-CoV-2, Flu A/B, RSV RT-PCR multiplex assay is an accurate and precise assay, with the high sensitivity and specificity for detection and differentiation of SARS CoV2, RSV and Flu A and B with an intent to aid in diagnosis and management of respiratory infections using nasopharyngeal and saliva samples.

## A-200

### Mechanical loading modulates phosphate related genes in rat bone

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**Background:** Mechanical loading determines bone mass and bone structure, which involves many biochemical signal molecules. Of these molecules, Matrix extracellular phosphoglycoprotein (MEPE) and fibroblast growth factor 23 (FGF23) are involved in bone mineralization and phosphate homeostasis. We aimed to investigate whether mechanical loading of bone affects phosphate homeostasis. We studied the effect of mechanical loading of bone on the gene expression of *Fgf23*, *Mepe*, *Dmp1*, *Phex*, *Cyp27b1*, and *Vdr*.

**Methods:** Sixty 12 week old female Wistar rats were randomly assigned to one of six groups (N=10). The right tibiae of the rats in the five groups (N=10) received a single bout of mechanical loading using a four-point bending load device for 300 loading cycles with a frequency of 2 Hz and a peak load of 50 N and were sacrificed 4, 5, 6, 7 or 8 hours after loading. The left tibiae were kept as contra-lateral controls. The rats in the control group (N=10) were not loaded. From all rats, blood was obtained using cardiac puncture, and serum was separated. Tibiae of the rats were harvested and stored at -80 °C. Proximal and distal ends of the tibiae were removed, and the bone marrow was flushed out using RNase free water. RNA was extracted from the tibia diaphysis using the trizol extraction method. RT-qPCR was performed on tibia mRNA at 4, 5, 6, 7, or 8 hours after mechanical loading for detection of *Mepe*, *Dmp1*, *Fgf23*, *Phex*, *Cyp27b1*, and *Vdr*. Immunohistochemistry was performed to visualize FGF23 protein in tibiae. Serum FGF23 was measured using ELISA and serum calcium and phosphate levels were measured using a Modular P800 clinical chemistry analyzer. **Results:** Four-point bending resulted in a reduction of tibia *Fgf23* gene expression by 64% (p=0.002) and a reduction of serum FGF23 by 30% (p<0.001), six hours after loading. Eight hours after loading, *Dmp1* and *Mepe* gene expression increased by 151% (p=0.007) and 100% (p=0.007). Mechanical loading did not change *Phex*, *Cyp27b1*, and *Vdr* gene expression at any time points. **Conclusion:** We conclude that mechanical loading appears to provoke both a paracrine as well as an endocrine response in bone by modulating factors, which regulate bone mineralization and phosphate homeostasis.

## A-201

### Comparison of Cepheid, Roche, and LDT Platforms for COVID-19 and Influenza Testing

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**Background:** Testing for seasonal respiratory diseases such as influenza has become complicated by the emergence of SARS-CoV-2. Many platforms and assays are available to test for SARS-CoV-2, influenza A, and other respiratory pathogens. Determining which assay is most suitable for patients can be challenging for clinicians. This study compares performance of 3 assays: the Roche Cobas® SARS-CoV-2 & Influenza A/B assay, the Cepheid Xpert®-Xpress-CoV-2-Flu-RSV-Plus assay, and a laboratory-developed test (LDT) for SARS-CoV-2.

**Methods:** 33 Covid positive, 7 influenza positive, and 33 samples negative for SARS-CoV-2 and influenza A were randomly collected from the Cepheid assay and re-run on the Roche and LDT platforms. The Roche Cobas® SARS-CoV-2 & Influenza A/B assay simultaneously targets the E gene and ORF1a/b non-structural region of SARS-CoV-2, the M1/M2 region for influenza A, and the NEP/NS1 genes for influenza B. Similarly, the Cepheid Xpert®-Xpress-CoV-2-Flu-RSV-Plus assay simultaneously targets the N, E, and RdRP genes of SARS-CoV-2; the M, PB2, and PA genes of influenza A; the M, and NS genes of influenza B; and the N gene of RSV A and RSV B. The LDT targets the N1 and N3 regions of the N gene and does not test for influenza. The qualitative results from all testing were analyzed for statistical significance using McNemar's test.

**Results:** Analysis showed no statistically significant difference across the 3 platforms for detecting SARS-CoV-2 and between the Cepheid and Roche platforms for the detection of influenza A. Roche Cobas is positive if ORF1a/b and E-gene are detected and presumed positive if only ORF1a/b is detected. Covid LDT is considered positive if N1 and N3 are detected and inconclusive if only one target is detected.

**Conclusion:** Roche, Cepheid, and LDT SARS-CoV-2 assays were not significantly different in their ability to detect SARS-CoV-2 or influenza A for the Roche and Cepheid platforms.

SARS-CoV-2 or influenza A positive Ct values							
Sample	SARS-CoV-2					Influenza A	
	Cepheid Xpert®	Roche Cobas®		LDT		Cepheid Xpert®	Roche Cobas®
	SARS-CoV-2	SARS-CoV-2 (ORF1a/b)	PanSars (E-gene)	N1	N3	FluA	FluA
1	15.6	15.5	15.4	14.1	12.8	NEG	NEG
2	16.9	17.0	16.7	14.7	14.0	NEG	NEG
3	17.2	18.4	18.0	16.5	13.7	NEG	NEG
4	18.1	17.7	17.9	15.4	14.8	NEG	NEG
5	18.5	18.3	18.2	16.3	15.0	NEG	NEG
6	18.8	19.2	19.4	16.8	16.1	NEG	NEG
7	19.2	21.2	20.5	18.1	17.1	NEG	NEG
8	19.2	21.1	20.9	17.6	16.6	NEG	NEG
9	19.8	19.8	19.8	18.0	16.9	NEG	NEG
10	19.8	20.2	20.1	18.4	17.5	NEG	NEG
11	20.1	20.1	20.0	18.2	17.0	NEG	NEG
12	20.1	19.6	19.7	18.8	17.6	NEG	NEG
13	20.7	20.8	20.8	19.1	18.0	NEG	NEG
14	20.9	21.5	21.2	19.0	18.0	NEG	NEG
15	22.2	23.9	23.8	21.1	20.0	NEG	NEG
16	23.9	24.7	24.8	22.4	21.1	NEG	NEG
17	24.3	24.0	24.0	22.9	21.7	NEG	NEG
18	24.6	23.6	23.4	21.8	21.3	NEG	NEG
19	25.2	24.8	25.0	23.4	22.1	NEG	NEG
20	25.3	26.2	25.9	24.1	23.0	NEG	NEG
21	25.6	25.3	25.5	24.3	23.1	NEG	NEG
22	27.0	27.2	27.3	26.0	25.0	NEG	NEG
23	27.3	27.6	27.5	25.5	24.6	NEG	NEG
24	28.3	27.5	27.8	26.2	24.7	NEG	NEG
25	28.6	29.0	29.1	26.2	25.4	NEG	NEG
26	31.6	31.2	30.5	30.5	29.0	NEG	NEG
27	32.8	31.5	31.4	30.3	28.5	NEG	NEG
28	35.0	31.9	31.7	31.9	31.0	NEG	NEG
29	36.1	34.8	34.0	35.2	35.0	NEG	NEG
30	38.9	36.5	NEG	35.6	35.1	NEG	NEG
31	39.0	36.0	35.1	NEG	NEG	NEG	NEG
32	40.3	38.0	37.1	NEG	36.0	NEG	NEG
33	42.5	36.0	NEG	36.1	35.3	NEG	NEG
34	NEG	NEG	NEG	NEG	NEG	19.4/21.6	28.0
35	NEG	NEG	NEG	NEG	NEG	21.3/23.5	28.7
36	NEG	NEG	NEG	NEG	NEG	22.5/25.1	31.5
37	NEG	NEG	NEG	NEG	NEG	27.9/29.6	32.7
38	NEG	NEG	NEG	NEG	NEG	27.0/29.0	32.6
39	NEG	NEG	NEG	NEG	NEG	35.5/36.6	NEG
40	NEG	NEG	NEG	NEG	NEG	36.3/NEG	NEG

A-202

A 10-year comparative study of karyotypes by G-banding and array-CGH hybridization techniques

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**Background:** Chromosomal abnormalities are associated with a variety of clinical features, such as malformations, intellectual disability, neuropsychiatric changes, and others. Clinical outcome of chromosomal rearrangements generally depends on their location, size, number of genes involved, and function. For patients with suspected chromosomal abnormalities, karyotype analysis using cytogenetic techniques is indicated. Array Comparative Genomic Hybridization (Array-CGH) allows the detection of ultramicroscopic unbalance abnormalities, and its application has grown over the years. In this study, our group aimed to track the use of both methods to assess the concordance of results over 10 years. **Methods:** Results of both methodologies from 2010-2022 from Associação Fundo de Incentivo a Pesquisa - São Paulo (Brazil) were analysed. For G-banding, chromosomes of peripheral blood lymphocytes culture were analyzed microscopically in the metaphase stage. Bands we obtained by Giemsa staining with a resolution of 400 to 550 bands. Array-CGH studies were performed and approximately 750,000 nucleotide probes were dispersed in each genomic sample. A total of 550,000 probes were designed for non-polymorphic regions and 200,436 were dedicated to single nucleotide polymorphism (SNP) detection. The analysis was performed using the Chromosome Analysis Suite v4.1.0.90 (r29400) software. Cohen's-Kappa concordance study was analyzed in EP Evaluator® software (S. Burlington, VT USA). **Results:** In the past 10 years, over 33300 constitutional chromosomal analysis were performed. More than 40% (n=13500) of the patients were below 18 years old (y.o). Out of those, 2520 results showed abnormalities. Almost 300 results had structural abnormalities, such as translocations, insertion, deletion, duplication, isochromosome, ring chromosome, inversion, etc. 128 abnormal results presented balanced abnormalities that could potentially not be detected by array-CGH. Out of 510 array-CGH studies, 49 results were judged pathogenic, 19 were benign, 15 were presumably pathogenic and 88 were considered having a variation of unknown significance (VUS). A total of 62,15% of the findings were normal, and at least 22 of them received more than one classification. We observed that only 67 patients were requested to perform both methodologies. Of those, 51 results were normal and 4 showed abnormalities for both methods. Only 12 cases were reported as normal karyotypes and showed pathogenic or potentially pathogenic abnormalities in the array-CGH. No disagreement was found as abnormal karyotype and normal array-CGH. Cohen-kappa agreement index for this study is 0.34. **Conclusion:** Both methods present important information for diagnostic and genetic counselling and are complimentary. Array-CGH's sensitivity is higher than classical chromosome analysis due to its resolution. However, there are several balanced and/or numeric abnormalities potentially detected only by the karyotype. European Cytogenetics Association and the American College of Medical Genetics recommend the array-CGH as a first-tier test for several anomalies. Our data shows several physicians already request each method properly. For a complete study and evaluation for genetic counselling, karyotype analysis is also important, especially when no abnormality is found in the array-CGH study. Laboratories must report each methodology limitation and suggest complimentary studies when normal results are found.

A-203

Open Format Real-Time PCR Kits Are a Reliable Solution for Testing Needs During COVID-19 Pandemic in Poland

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**Background:**

Since the SARS-CoV-2 Pandemic was declared by the WHO, public centres have been struggling to offer a fast diagnosis for COVID-19. It has been also stated that suspected cases should be screened with nucleic acid amplification tests (NAATs), like real-time PCR. Private laboratories have been seminal to increase the testing capacity. Also, ready-to-use qPCR kits designed for the detection of SARS-CoV-2 genes, like *ORF1ab* and *N* genes, have been key to cover these needs. The aim of this study is to show how Vitassay Healthcare provides a fast and reliable solution for COVID-19 testing with its Vitassay qPCR SARS-CoV-2 kit.

**Methods:**

21,445 nasopharyngeal swabs samples, from both sexes and covering every age range, were collected from May 2020 to August 2021 in different collecting points of Wrocław (Poland). RNA of these samples was extracted either manually or automatically and analysed with two Real-Time PCR CE-IV-certified kits manufactured in Spain: Vitassay qPCR SARS-CoV-2 kit (Vitassay Healthcare SLU) and Viasure SARS-CoV-2 Real-Time PCR Detection Kit (Certest Biotec S.L). During RT-qPCR reaction setup, 5 µl of isolated RNA were loaded on the resuspended ready-to-use master mix and run on the BioRad CFX96.

#### Results:

Data obtained with these two open-format qPCR kits was similar to what has been observed in other populations regarding the age structure ( $\square = 40$  years-old) and sex (16.6% women vs. 14.3% men (Pearson, Chi-square:  $p < 0.01$ ); confirming the reliability of Vitassay. This study also shows that the positivity of tests financed by the National Health Fund (Poland) was higher (25,3%) than the ones paid by a private company or self-paid (10,2%) Interestingly, it was observed a high percentage of infection in social care centres (20,6%), which was lower in a previous study done at the start of the pandemic (13,3%). This demonstrated that institutions are especially sensitive to SARS-CoV-2 infection and extra care is needed.

**Conclusion:** Open format qPCRs for SARS-CoV-2 detection are an accurate and easy method used to cover the need of a broader screening and testing, either if it is for diagnostic reasons, routine screening or travel needs. In addition, this product can also be of further use to study the vaccination effects and virus dynamic.

### A-204

#### Relevance of EQA Programmes in the Assessment of the Performance of qPCR Kits for the Detection of Pathogens Responsible of Tropical Diseases

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#### Background:

Diseases such as Zika, Dengue or Chikungunya are widely spread over tropical and subtropical countries and are a cause of public health concern. The clinical manifestations are usually common, specially during the first stages of the illness, leading to an incorrect diagnosis. Molecular methods based on Real-time Polymerase Chain Reaction are the most accurate diagnostic techniques. Evaluation of the clinical sensitivity and specificity of the diagnostic methods based on PCR technique is required. These evaluations are usually performed with clinical samples, however, availability in other regions than tropical areas, is restricted. To address this, Evaluations of Quality Assessment (EQA) programmes offer a wide range of human-like samples mimicking clinical specimens. Among these, QCMD allows to perform blind comparisons of laboratory testing methods between different facilities from all around the world. Our objective was to assess the performance of Vitassay qPCR Zika + Dengue + Chikungunya, a multiplex for the detection of Zika, Dengue and Chikungunya, and Vitassay qPCR Dengue 1 + 2 + 3 + 4, a test designed for Dengue typing, using reference material from QCMD programmes.

#### Methods:

Samples from QCMD Zika, Dengue or Chikungunya Virus RNA EQA Programmes were purchased from 2017 to 2020. In total, 126 samples were analysed with Vitassay qPCR Zika + Dengue + Chikungunya. These specimens were handled as indicated by the QCMD programme by qualified technicians, being considered as samples from clinical patients. RNA was extracted with QIAamp Viral RNA Mini Kit (Qiagen). Vitassay qPCR lyophilised master mix contains all the reagents needed for a one-step reaction. In brief, master mix was rehydrated with 15 microliters of the resuspension buffer and 5 microliters of RNA eluate were added and samples were run on low-profile Bio-Rad and Agilent thermocyclers. Data was analysed and compared to the results given by the QCMD programmes. In addition, 50 samples were analysed with Vitassay qPCR Dengue 1 + 2 + 3 + 4, following the same protocol. Sensitivity and specificity values were calculated with MetaDisc 4 software.

#### Results:

QCMD reported 33 samples positive for Zika, 48 positives for Dengue and 31 positives for Chikungunya. Results obtained with Vitassay qPCR Zika + Dengue + Chikungunya showed just one false negative for Zika. Sensitivity and specificity for ZIKV are 0.97(0.84-0.99) and 1(0.96-1); for DENV are 1(0.92-1) and 1(0.95-1); for CHKV are 1(0.88-1) and 1(0.96-1), respectively (CI= 95%). With respect to Vitassay qPCR Dengue 1 + 2 + 3 + 4, only one false negative was reported, and showing the following sensitivity and specificity values: DENV-1, 1(0.73-1) and 1(0.90-1); DENV-2, 1(0.66-1) and 1(0.91-1); DENV-3, 0.91 (0.61-0.99) and 1(0.90-1); DENV-4, 1(0.63-1) and 1(0.91-1), respectively.

#### Conclusion:

QCMD programmes offer high-quality reference material to evaluate the accuracy of tests intended for molecular diagnostics, especially when samples availability is restricted.

### A-205

#### Ignoring PCR duplicates does not affect germline variant calling in targeted next-generation sequencing

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**Background:** To prepare DNA for next-generation sequencing (NGS), DNA is fragmented, adapters are ligated to the end of each resulting fragment, which are then PCR amplified, and PCR products are sequenced. Sequence reads that result from two or more copies of the exact same DNA fragment are called PCR duplicates. Many analysis pipelines remove PCR duplicates to mitigate potential biases on variant calling algorithms, because, at worst, PCR duplicates may contain amplification-induced mutations that can be misidentified as a true variant. Thus, we aim to investigate if ignoring the PCR duplicates is associated with a decrease in the variant calling accuracy from germline targeted next-generation sequencing data.

**Methods:** Clinical exome sequencing data used in this evaluation were obtained from our clinical sequencing service database. Only datasets from volunteers who gave informed consent for aggregated data analysis for assay improvement and quality control purposes were used. Twenty datasets with high percentage of PCR duplicates were selected, median (min-max) of 16% (15.3-19.5%). The clinical exome sequencing was executed with lotus DNA library prep, xGEN hybridization capture, xGEN inherited disease panel (all from Integrated DNA technologies) and sequenced using NextSeq-500 (Illumina). Read alignment and variant calling were performed with Dragen Enrichment v.3.9.5 (Illumina) removing and ignoring PCR duplicates. The number of called SNV and INDEL were compared between the above-cited conditions (removed versus ignored PCR duplicates). Positive percent agreement (PPA), analytical positive predictive value (PPV) and the F-measure (harmonic mean of PPA and PPV) for SNVs and INDELS called removing and ignoring PCR duplicates were calculated using VCF-eval (RTG-Tools) and benchmarked considering the data with removed PCR duplicates as reference. To count how many times a duplicated read occurred among the PCR duplicates, frequency distribution of the duplication level of the 20 studied datasets was generated with fastQC (Babraham Bioinformatics).

**Results:** The median (min-max) number of SNV and INDEL called were similar in both tested condition: 8033 (7669-8750) SNV and 406.5 (345-475) INDEL when PCR duplicates were removed versus 8035 (7664-8771) SNV and 411 (339-481) INDEL when PCR duplicates were ignored, respectively. The benchmark between the variants called in each tested condition revealed a median (min-max) for PPA, PPV, and F-measure of 99.65% (99.51-99.83%), 99.69% (99.41-99.85%) and 99.69% (99.49-99.785) for SNV and 94.06% (91.14-96.07%), 93.04% (88.92-97.44%) and 93.46% (90-96.7%) for INDEL, respectively. The frequency distribution of the duplication level revealed a median (min-max) of 11.89% (11-12.15%) for 2 times, 3.7% (3.28-4.14%) for 3 times and 1.38% (1.14-1.87%) for 4 times duplicated, respectively. PCR duplications with counts higher than 4 were negligible.

**Conclusion:** PCR duplicates removing or ignoring did not meaningfully affects the SNV and INDELS calling accuracy. The observed high PPA, PPV and F-measure values supports this conclusion. Lower PPA, PPV and F-measure for INDELS compared to SNV is expected and in agreement with other studies. The most PCR duplicates was duplicated between 2 to 3 times suggesting that this low-level of repetition was not enough to affect the germline variant calling.

### A-206

#### Analysis of Gene Expression in Left Ventricular Dysfunction after Acute Myocardial Infarction

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**Background:** Heart failure (HF) is a progressive syndrome characterized by the inability of the heart to pump enough blood to other tissues, and its main cause is acute myocardial infarction (AMI). Despite advances in the identification of markers associated with left ventricular (LV) dysfunction, the ideal marker for early and accurate

detection of ischemic HF has not yet been found. Therefore, this study aims to evaluate the expression of 13 genes previously associated with AMI in patients with LV dysfunction after AMI compared to patients with normal LV function.

**Methods:** A total of 28 patients who had suffered previous AMI were classified into two groups: LV dysfunction [LV ejection fraction (LVEF)  $\leq$  40%, n = 14] and those with normal LV function (LVEF > 40%, n = 14). *ALOX15*, *AREG*, *BCL2A1*, *BCL2L1*, *CA1*, *COX7B*, *ECHDC3*, *IL18R1*, *IRS2*, *KCNE1*, *MMP9*, *MYL4* and *TREML4* expressions were evaluated by real time reverse transcription-quantitative polymerase chain reaction.

**Results:** Only *KCNE1* expression was decreased in LVEF  $\leq$  40% group ( $p = 0.007$ ). A positive correlation was found between *KCNE1* and LVEF ( $r = 0.557$ ;  $p = 0.001$ ). There was a 12.25-fold risk (95%CI: 1.33 - 113.06;  $p = 0.027$ ) of LV dysfunction for patients with lower *KCNE1* expression. *KCNE1* expression showed high AUROC (AUROC: 0.811, 95%CI: 0.642 - 0.979,  $p = 0.007$ ) in predicting the LV dysfunction.

**Conclusion:** This study suggests that *KCNE1* expression may be associated with LVEF levels and, therefore, with the risk of the LV dysfunction after AMI.

## A-207

### Identification of Candidate Regulatory Variants Within Upstream Regions of Genes Related to Familial Hypercholesterolemia

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**Background:** Familial hypercholesterolemia (FH) is a common inherited metabolic disease with abnormally high low-density lipoprotein cholesterol concentration. Autosomal dominant variants in exonic regions of *LDLR*, *APOB* and *PCSK9* are the main causes of FH, but variant-negative cases indicate that variants located outside of these commonly screened regions might be causing the phenotype. Variants that modify regulatory elements can potentially alter patterns of expression that lead to disease. Thus, the present study aimed to screen for potentially regulatory variants in upstream regions of *LDLR*, *APOB* and *PCSK9* genes in a Brazilian cohort of FH variant-negative individuals.

**Methods:** An Ampliseq for Illumina custom panel was designed to target 3-kb upstream regions (Chr19:11086362-11089361, Chr2:21044074-21047073 and Chr1:55036476-55039475, in hg38) and paired-end sequencing was performed using the Miseq Reagent Nano Kit v2 (n=25). Variant discovery analysis included data pre-processing (FastQC, Cutadapt, SAMtools, BEDtools), short-read mapping (BWA-MEM) and variant calling (GATK-HaplotypeCaller and FreeBayes). Aligned sequences were visually inspected using IGV to confirm quality of variants and a minimum read depth of 20. An integrative approach was applied to prioritize potentially regulatory variants based on regulatory probability prediction score (cepip), functional annotation (rVarBase and VannoPortal), pathogenicity prediction scores, variant related literature, and prediction of altered transcription factor binding.

**Results:** Samples were sequenced to an average coverage of 30x with more than 90% of targets covered. 34 single nucleotide variants (SNVs) were identified (6 in *LDLR*, 15 in *APOB*, and 13 in *PCSK9*). Five SNVs were prioritized as potentially regulatory variants (rs36218923 and rs538300761 in *LDLR*, rs934197 and rs9282606 in *APOB*, and g.55038486A>G in *PCSK9*). All of them were not very common in our sample, presented HepG2-dependent regulatory prediction score above 0.73 and were overlapping active transcription/promoter chromatin states and transcription factor binding sites. However, only *APOB* rs934197 and rs9282606 were predicted to alter transcription factor binding. rs934197 was the only one with previous functional data suggesting that it caused increase in rate of transcription.

**Conclusion:** Our results suggest good candidates for further functional validation assays to confirm their impact in gene regulation. They also highlight the importance of variant screening outside of coding regions not only in *LDLR*, but also in *APOB*.

## A-208

### microRNAs in Left Ventricular Systolic Dysfunction Post-Myocardial Infarction

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**Background:** Acute myocardial infarction (AMI) is a severe cardiovascular disease and leads to progressive cardiac remodeling. The development of left ventricular systolic dysfunction (LVSD) and subsequent heart failure (HF) are a consequence of adverse left ventricular (LV) remodeling. Despite studies advances, there is still a need to understand the molecular mechanisms of LV remodeling. MicroRNAs (miRNAs) are small (~ 22 nucleotides in length) non-coding RNAs that regulate gene expression post-transcriptionally. Evidence indicates that miRNAs from PBMCs are altered in cardiovascular disease, and are implicated in metabolic pathways related to the development of LVSD and post-AMI HF. Thus, our study aimed to investigate PBMC microRNA expression in post-infarction patients and the relation with LVSD. **Methods:** A sample of 25 subjects who had suffered previous ST-segment elevation myocardial infarction (STEMI) at least two months prior to inclusion in the study were classified into two groups: LVSD [Left Ventricular Ejection Fraction (LVEF)  $\leq$  40%, n = ] and those with preserved LV systolic function (pLVSF) (LVEF > 40%, n = ), according to the American Heart Association Guideline for the Management of HF. Blood samples were obtained for clinical laboratory testing and to analyze the expression of 61 miR of PBMC by qPCR. Relative expression was calculated using the 2<sup>- $\Delta\Delta C_t$</sup>  method, normalized by endogenous miR-16-5p. DIANA-TarBase v.7 and DIANA-mirPath v.3 provided the predicted miRNA targets and performed pathway enrichment analysis, respectively. Statistical analysis was performed using SPSS Statistics v.23.0 software (IBM, IL,USA), and p-values < 0.05 were considered statistically significant.

**Results:** We found that miR-7b-5p ( $p = 0.013$ ), miR-326 ( $p = 0.023$ ), and miR-125a-3p ( $p = 0.036$ ), were up-regulated in the AMI patients who developed LVSD (LVEF  $\leq$  40%) versus those who did not (LVEF > 40%). miR-7b-5p ( $r = -0.466$ ;  $p = 0.022$ ) and miR-326 ( $r = -0.415$ ;  $p = 0.049$ ) were negatively correlated with LVEF. miR-7b-5p also correlated with total cholesterol ( $r = 0.443$ ;  $p = 0.044$ ). Predictive bioinformatics analysis demonstrates that some of the targets of miRNAs let-7b-5p, miR-326 and miR-125a-3p are involved in regulatory pathways related to cardiac diseases. Among these target genes, filamin A (FLNA) stands out, involved in proteoglycans pathway (KEGG pathway hsa05205), and which was simultaneously targeted by the three miRNAs.

**Conclusion:** This study suggests that PBMC let-7b-5p, miR-326 and miR-125a-3p are associated with LVSD in AMI patients and possibly plays a potential role in metabolic pathways related to the pathogenesis of ventricular dysfunction observed in these patients.

## A-209

### Panel of SNPs to Assess the Risk and Prognosis of Thyroid Carcinoma in a Mixed Population of Northeastern Brazil

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**Background:** Thyroid cancer (CT) is the most common endocrine tumor and accounts for 1% of all malignant neoplasms. Although the risk factors and the CT screening measures are known, there is still no defined genetic profile that associates to the development of this disease, as well as to prognostic in the mixed-race population of Rio Grande do Norte (RN)/Brazil. In this sense, high-throughput genotyping studies look for single nucleotide polymorphisms (SNPs) that may be related to increased risk of CT and worst prognosis. Therefore, this study aimed to evaluate a panel of selected SNPs would be associated with CT predisposition and prognosis in a mixed population of northeastern Brazil.

**Methods:** Thyroid biopsies were selected from 279 CT patients from the bank of the Pathology laboratory of the Liga Norte Riograndense Contra o Câncer-RN/Brazil (Case group) and peripheral blood samples were collected from 301 healthy donor volunteers from the blood Hemovida/RN-Brazil (Control group) for DNA extraction. Genotyping of a panel of 84 SNPs was performed in both groups using the MassARRAY SNP system (Agena Bioscience San Diego, USA).

**Results:** The SNPs rs10759944, rs7850258, rs116909374 and rs34566348 were associated with the risk of developing CT. Regarding the factors associated with CT prognosis, initially the association of the SNPs panel with the tumor cell type was performed. It was observed that the dominant (CT+CC, OR = 6.05;  $p = 0.002$ ) and recessive models (CT, OR=7.83;  $p < 0.001$ ) of rs2236487 were associated with risk of papillary carcinoma. In addition, it was found that the dominant (AG+AA, OR=11.36;  $p = 0.001$ ) and overdominant models (AG, OR=12.7;  $p < 0.001$ ) of rs1571443 were associated with risk of follicular carcinoma. Subsequently, when evaluating the association of SNPs with tumor size, it was found that the codominant (AG, OR=0.46; and GG, OR=0.2;  $p = 0.002$ ), dominant (AG+GG, OR=0.41;  $p = 0.001$ ), recessive (GG, OR=0.34;  $p = 0.032$ ) and overdominant models (AG, OR=0.54;  $p = 0.028$ ) of rs31872 were associated with protection from the development of tumors above 2 cm. Then, when evaluating the association of SNPs with tumor staging, it was found that the codominant (CT, OR=2.92; and TT, OR=3.31;  $p < 0.001$ ), dominant (CT+TT, OR=2.98;  $p < 0.001$ ) and overdominant models (CT, OR=2.51;  $p = 0.001$ ) of rs10238549 were associated with the risk of developing stages I and II. On the other hand, the codominant (CT, OR=0.34; and TT, OR=0.24;  $p < 0.001$ ), dominant (CT+TT, OR=0.31;  $p < 0.001$ ), recessive (TT, OR=0.44;  $p = 0.041$ ) and overdominant models (CT, OR=0.49;  $p = 0.010$ ) of rs7800391 were associated with protection from stage I and II development. Furthermore, it was evaluated to association of SNPs with recurrence and the presence of metastasis. In this evaluation was observed that the overdominant model (AT, OR=8.68;  $p < 0.001$ ) of rs949908 was associated with the protection of persistent CT, and the codominant (GT, OR=1.33 and GG, OR=15.73;  $p = 0.005$ ) and recessive (GG, OR=13.8;  $p = 0.001$ ) models of rs487880 were associated with a risk of developing metastasis.

**Conclusion:** The present study, a pioneer in a mixed population of northeastern Brazil, suggests the association of SNPs as markers of predisposition and prognosis of CT in this population.

## A-210

### Clinical Evaluation of Thioredoxin 1 in the Blood as a Novel Biomarker to Detect Breast Cancer

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**Background:** For a long time, there has been an unmet need to find a means to detect breast cancer (BC) with blood for the mutual benefit of women and clinicians. We have reported that the serum level of thioredoxin 1 (Trx1) could be a biomarker to assess the risk of BC. This novel BC detection tool was thought to be an alternative way to improve the diagnosis accuracy of BC. A pivotal clinical trial has been performed to validate its clinical utility with support from the Promotion of Innovative Businesses for Regulation-Free Special Zones, Ministry of SMEs and Startups (MSS, Korea).

**Methods:** An ELISA kit, DxMe BC, to estimate the level of serum Trx1 has been developed. The performance of DxMe BC kit to assess the risk of BC was evaluated. A total of 1,032 sera have been analyzed in this pivotal clinical trial including biopsy confirmed BC patients (n=308), women without cancer or other breast related symptoms (n=336), and other malignancies (n=388) such as stomach, lung, colorectal, cervical, and ovarian cancers. The blood levels of Trx1 from various pathological and physiological status of BC patients compared to control groups were analyzed. The combined analysis of Trx1 level with mammography was performed to see how well Trx1 level could correct previously mis- or incompletely judged mammograms. All measurements were duplicated and statistically analyzed by ROC analysis, one-way ANOVA, and unpaired t-test.

**Results:** The mean levels of Trx1 from blood from women without breast-related disease and benign breast tumor were 6.48±2.83 and 7.06±3.00 ng/ml, respectively. However, that of biopsy confirmed BC patients was 29.90±10.04 ng/ml, which was a difference big enough to discern BC with the cut-off value set at 11.4 ng/ml. The clinical sensitivity and specificity of this trial were 96.43% and 97.32% (AUC 0.985±0.005,  $P < 0.001$ ), respectively. The level of Trx1 could detect BC at stage 0

and I as well as more advanced stages. It could differentiate BC regardless of age, luminal types, pathological types, Ki67 activity, p53 expression, and TNM stage of BC. The blood level of Trx1 in various malignancies was also measured to verify the specificity. It showed that Trx1 levels were lower than the cut-off value in cervical, lung, and ovarian cancer patients but higher in colorectal and stomach cancer patients. The recovery rate of misjudged mammography was 93% when Trx 1 level and mammography were analyzed together, with 99.03% sensitivity and 100% specificity.

**Conclusion:** Analyzing 1,032 blood samples, the level of Trx1 could discern BC regardless of the characteristics of BC. It also recovered 93% of mis- or incompletely judged mammography. It also has been validated to be an effective detection tool to assist with BC diagnosis in every stage. Thus, the blood level of Trx1 can be a novel aid to detect BC from blood.

## A-211

### Analytical Performance of SARS-CoV-2 RT-qPCR Assay on Advanced Mobile Real Time PCR Device

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**Background** Liberty16 has been clinically validated as a flexible and accessible device system for running the affordable SalivaDirect™ real time PCR detection assay for SARS-CoV-2, especially in low resource settings (USFDA, 2020; Vogels et al., 2021; Yolda-Carr et al., 2022). The need for diagnostics that advance clinical care and public health has never been greater, and therefore we developed an advanced version of mobile real time PCR device, to expand testing capacity and enable pathogen detection with multiplex assay and new speed whilst retaining the existing simplicity of use. **Methods** In a preliminary study we treated ZeptoMetrix spike-in saliva samples, containing inactivated SARS-CoV-2 virus from approximately 22 to 0.6 copies/μL, according to SalivaDirect™ protocol with heat-inactivation (without proteinase K) and tested on Liberty16 and our newly developed PCR device. Subsequently, the pre-determined lowest detectable concentration of inactivated virus was spiked into negative saliva samples for a robustness study (n = 24). The data was statistically analysed with a statistics computer software, GraphPad Prism 9.3.1. **Result** No significant difference was found in the results applying Liberty16 SalivaDirect™ protocol versus our in-house developed assay running on advanced version of mobile real time PCR device. Furthermore, a positive relationship was found between these two methods ( $R^2 = 0.9336$ ;  $P < 0.0001$ ), suggesting that their analytical performances are basically similar in detecting SARS-CoV-2 viruses. Additionally, the LOD of our in-house developed assay has been further confirmed as 0.6 copies/μL using ZeptoMetrix control material containing inactivated SARS-CoV-2 viruses. **Conclusions** Our new PCR machine development potentially expands testing capacity without sacrificing the assay's detection sensitivity and retains the unique features which are best fit to a low-resource setting. Taking SalivaDirect™ assay for an example, the throughput may increase from 6 to 14 samples/run by running N1 and RP as a duplex test. And theoretically, with the application of our newly developed PCR machine, there is an opportunity to increase sample daily throughput and turn-around-time compared to the Liberty16 single-channel with standard cycling conditions, ranging from 230% to 700% (depending on the selected thermal cycling protocol).

## A-212

### Analytical Specificity of the SalivaDirect™ Assay on the Liberty16 for Detecting SARS-CoV-2 B.1.1.529 (“Omicron”)

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#### Background:

The newly emerged Omicron variant of SARS-CoV-2 has numerous mutations that are not found in other variants of concern (VOCs). Despite acquiring extended functions in adapting to the host-cell environment, the viral genetic variation exerts a potential negative impact on the molecular test, which in turn, compromises public health and safety. On the other hand, the Liberty16 has been clinically validated as a flexible and accessible device system for running the affordable SalivaDirect™ real time PCR detection assay for SARS-CoV-2 especially in low resource settings (USFDA, 2020; Vogels et al., 2021; Yolda-Carr et al., 2022). As Liberty16's manufacturer, we continuously monitor and verify SalivaDirect™ assay performance, ensuring that the test remains a reliable tool for COVID-19 screening.

#### Methods:

Based on in-silico sequence analysis, we found that Omicron's mutation at position 28,311 overlaps with the CDC 2019-nCoV\_N1 probe binding region. In order to ver-

ify the performance of CDC 2019-nCoV-N1 primers-probe set in detecting the Omicron variant of SARS-CoV-2, plasmids containing Wuhan/WH01/2019 (wild-type) and B.1.1.529 (Omicron) sequences were serially diluted and subsequently detected for SalivaDirect™ RT-qPCR detection on Liberty16 using commercially procured reagents. Both the standard (Vogels et al., 2021) (95°C, 10s; 55°C, 30s) and fast cycling protocols (Yolda-Carr et al., 2022) (95°C, 2s; 55°C, 5s) were tested. The data were further statistically analyzed using a statistics computer software, GraphPad Prism 9.3.1.

#### Results:

Linearity analysis of a 6-log dilution series revealed no statistical difference between the two amplification plots detecting wild-type and Omicron sequences ( $P = 0.7963$ ). The sensitivity of Omicron sequences detection was 100% ( $n = 20$ ) when using  $2 \times \text{LoD}$  (24 copies/ $\mu\text{L}$ ). Apart from that, our data demonstrate that the N1 targets could be effectively detected across 5-log dilution series (from approximately  $3 \times 10^3$  to 30 copies/ $\mu\text{L}$ ) of the mutant and wild-type plasmid DNA using either standard or fast thermal cycling protocols.

#### Conclusion:

Our findings provide analytical support for reports that the mutations in the Omicron variant have little or no impact on SalivaDirect™ assay in terms of amplification efficiency and detection sensitivity using either standard or the recently reported fast Liberty16 SalivaDirect™ thermal cycling protocols.

## A-213

### Multivariate Analysis of the Effects of Specimen Tube Type and Relative Percent Tube Fill Volume on Cerebrospinal Fluid A $\beta$ 42, pTau181, and pTau181/A $\beta$ 42 ratio determinations.

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**Background:** In cerebrospinal fluid (CSF) decreased amyloid  $\beta$ (1-42) (A $\beta$ 42) and increased phosphorylated Tau (pTau181) concentrations are associated with AD pathological changes and may be used as an alternative to imaging studies to assess amyloid deposition in the brain. Clinical use of these markers, however, has been impeded by potential variation associated with pre-analytical processing, particularly for amyloid  $\beta$  proteins. Here, we investigate the effect of several pre-analytical variables on the recovery of A $\beta$ 42, pTau181, and the pTau181/A $\beta$ 42 ratio.

**Methods:** Three CSF pools with varying concentrations of A $\beta$ 42 (pg/mL; high = 1514, mid = 1051, low = 874) were prepared and then pipette aliquoted into specimen containers. A $\beta$ 42 and pTau181 were measured using Roche Elecsys assays on a Cobas® 6000. Baseline measurements of each pool, and subsequent determinations were obtained in triplicate after vortexing. Tube types evaluated included two low-bind polypropylene (PP) tubes (2.5 mL Sarstedt 63.614.699, 1.5 mL Sarstedt 72.703.600) and one PP tube (5 mL Sarstedt 62.504.037). Fill volumes of 100%, 80%, 50%, 40%, and 30% of the manufacturer's recommended tube capacity were evaluated. Three different storage conditions were evaluated (1hr ambient, 24hrs refrigerated upright, and 24hrs refrigerated sideways to simulate sample shipment). All data were modeled using linear regression analysis to assess the effect of each pre-analytical variable on A $\beta$ 42 recovery relative to baseline. The significance of each independent variable was tested in a multivariate model.

**Results:** There was little effect on A $\beta$ 42 (1.4+/-0.7%), pTau181 (0.96+/-0.43%), and pTau181/A $\beta$ 42 ratio (0.23+/- 1.3%), following aliquoting into 100% full tubes relative to baseline patient pools regardless of tube type or storage condition. The multivariate model which included the factors tube fill percentage, storage condition, and pool concentration, revealed that A $\beta$ 42 recovery was most strongly affected by tube fill percentage ( $p < 2e^{-16}$ ). A comparison of A $\beta$ 42, pTau181 and pTau181/A $\beta$ 42 ratio levels between 100% full and 40% full tubes identified a significant loss of A $\beta$ 42 ( $p = 5.133e^{-14}$ , mean difference = 56.4 pg/mL 95% CI = 44.2-68.7) and a change in pTau181/A $\beta$  ratio ( $p = 5.156e^{-12}$ , mean difference = -0.00092, 95% CI = -0.0012-0.0007) but not pTau181 ( $p = 0.9187$ , mean difference = 0.009 pg/mL, 95% CI = -0.164-0.181). Multivariate analysis revealed that each percent reduction in tube fill percentage resulted in a 0.12% increase in the pTau181/A $\beta$ 42 ratio. The observed intra-assay precision %CV of the pTau181/A $\beta$ 42 ratio was 4.67% at a pTau181/A $\beta$ 42 ratio value of 0.035. Taking this into consideration, an increase in the pTau181/A $\beta$ 42 ratio of +1SD and +2 SD of the assay precision would be expected at 61% and 22% fill volumes, respectively.

**Conclusions:** In the tubes and conditions evaluated in this study, the pTau181/A $\beta$ 42 ratio is significantly increased by reducing tube fill volume. However, in these specimen pools the mean bias was  $\leq 1$  SD of assay precision when tube relative fill volume was greater than 61%. The observed increase in pTau181/A $\beta$ 42 ratio should be accounted for in specimen tubes with reduced fill volume to avoid potential misinterpretation of the results.

## A-214

### TaqMan™ SARS-CoV-2 Genotyping Assays for Quick Identification of Delta, Omicron BA.1 and BA.2 SARS-CoV-2 Variants

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**Background** Over the course of the COVID-19 pandemic, several SARS-CoV-2 variants, including Alpha, Delta and most recently Omicron, emerged and quickly spread worldwide becoming the dominant strains by replacing the previously circulating strains. Based on the risk posed to public health, these variants have been designated as variants of concern (VOC) and their surveillance plays a crucial role for successfully implementing measures to prevent the spread of such variants. Whole genome sequencing (WGS) is considered as the gold standard for variant identification; however, it is not easily scalable to include high proportion of positive samples (<5%), has a turnaround time of several days and is expensive. In this study, we evaluated the performance of a panel of 8 TaqMan™ SARS-CoV-2 genotyping assays in combination with the S-gene Target Failure (SGTF) that can be used for detection and differentiation of SARS-CoV-2 VOC including Omicron and Delta, the results of which can be made available in <24h, significantly faster than WGS. **Methods** 102 SARS-CoV-2 positive samples collected in Austria in the period of September 2021-January 2022 were included in this study. All samples underwent genotyping with 8 TaqMan assays: L452R, P681R, N501Y, K417N, P681H, G339D, Q493R and Q954H and lineages were assigned based on the mutation profile also including SGTF from TaqPath™ COVID-19 CE-IVD RT-PCR kit in a blinded fashion. SARS-CoV-2 lineage assignment was compared against WGS generated using the Ion Torrent S5 Next Generation Sequencing method. The Omicron BA.1, BA.1.1 sub-lineages detected by WGS were grouped as BA.1-like based on results from the genotyping panel. **Results** In the samples tested, the genotyping approach correctly detected Delta and Omicron variants in 100% of the cases, when compared to WGS. Of 102 samples, 56.8% were identified as Delta (N=58), 41.2% as Omicron BA.1-like (N=42) and 2% as Omicron BA.2 (N=2). The SGTF of the TaqPath™ COVID-19 CE-IVD RT-PCR kit was present in all samples with the Omicron BA.1-like VOC, while it was absent in samples with the Delta or BA.2 variants, indicating that it can be used as a proxy to provide an initial indication of the VOC present in the sample. WGS data showed that within the 58 samples with Delta VOC, 15 different Pango sub-lineages could be detected, including, among others, the AY.4.9, AY.43 and AY.46, indicating that the genotyping approach can correctly assign the Delta VOC on a wide range of sub-lineages. **Conclusion** Using the bipartite workflow of (i) SGTF from TaqPath COVID-19 RT-PCR kit as a proxy for H69\_V70Del, followed by (ii) analyzing positive samples with the mutation panel, we were able to assign Omicron and Delta VOC lineages to positive SARS-CoV-2 samples within 24h which can enable swift reaction by the health care system to implement public health measures. This genotyping approach is highly precise and easily implementable, for detection of known VOCs and can complement WGS testing which can be reserved for identification of those positive cases undetermined using the genotyping approach such novel emerging variants.

## A-215

### Effect of Upper Respiratory Tract Site of Collection for the Molecular Detection of SARS-CoV-2: Anterior Nasal versus Nasopharyngeal Swabs

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**Background** Nasopharyngeal swab (NPS) is considered the reference sample for detection of respiratory viruses, including SARS-CoV-2. NPS is collected from the nasopharynx which requires a trained healthcare professional and can cause patient discomfort. Anterior nasal swab (ANS) offers an alternative that is more comfortable to the patient and also allows for self-testing. In this study, we evaluated the performance of ANS compared to NPS for the detection of SARS-CoV-2 infection. **Methods** The prospective study included 381 samples, collected from symptomatic individuals experiencing COVID-19 and Flu-like symptoms from February-May 2021. Paired samples were analyzed using two RT-PCR methods, TaqPath™ COVID-19, FluA, FluB Combo Kit and Lyra® SARS-CoV-2 Assays. Positive percent agreement (PPA) and negative percent agreement (NPA) was calculated between the two modes of collection for each of the two PCR methods. **Results** PPA between the two sampling modes was 77.4% using the TaqPath assay and 83.5% using the Lyra assay. In ANS that failed to detect SARS-CoV-2, average Ct value for these samples was 30.99 for TaqPath assay (reported LOD Ct 32.76) and 22.84 for Lyra assay (reported LOD Ct

25.18). Most ANS unable to detect SARS-CoV-2 had Ct values close to the reported LOD, suggestive of low viral titers in these specimens. The NPA between the two sampling modes was 99.3% using either PCR method. **Conclusion** While both collection methods are suitable for detection of SARS-CoV-2, ANS tends to be slightly less sensitive than NPS especially in patients with low viral titers. Such patients may represent probably at early or late stage of viral infection. Preference for the mode of collection depends on a number of factors including patient population, testing frequency, and access to trained healthcare professionals.

		Nasopharyngeal swab			
		Positive	Negative	Total	
Anterior Nasal swab	TaqPath™ COVID-19, FluA, FluB Combo Kit	Positive	65	2	67
		Negative	19	295	314
		Total	84	297	381
	Positive Percent Agreement, PPA (95% CI)		77.4% [66.9%-87.9%]		
	Negative Percent Agreement, NPA (95% CI)		99.3% [97.6%-99.9%]		
	Lyra SARS-CoV-2 Assay	Positive	66	2	68
		Negative	13	299	312
		Total	79	301	380
	Positive Percent Agreement, PPA (95% CI)		83.5% [73.5%-90.9%]		
	Negative Percent Agreement, NPA (95% CI)		99.3% [97.6%-99.9%]		

A-217

Evaluation of the analytical performance of four SARS-CoV-2 rapid antigen detection tests for laboratory diagnosis of COVID-19 in Brazil

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**Background:** The emergence of the SARS-CoV-2 omicron variant provided a rapid increase in the number of infected individuals, and consequently a huge growth in the demand for diagnostic tests. Therefore, due to this crescent demand, is increasingly necessary to expand the offer of faster, simpler, and more accurate tests for SARS-CoV-2 detection. Rapid tests (RTs) for the qualitative detection of SARS-CoV-2 (Ag) antigen can play an important role in expanding the supply of tests, helping in disease management. Nevertheless, this work aimed to evaluate the analytical performance of four RTs for the qualitative detection of SARS-CoV-2 Ag available in the Brazilian market. **Methods:** Samples were previously defined as positive (SARS-CoV-2 detected; Cq (cycle quantification) up to 30) and negative (SARS-CoV-2 non-detected), based on RT-PCR (gold standard) confirmation. The kits SARS-CoV-2 Ag Rapid Test (VivaDiag, China), COVID-19 Ag SE Rápido (Vida Biotecnologia, Brasil), Novel Coronavirus Antigen Rapid Test (Bioscience, Brasil), and COVID Ag (Gold Analisa, Brasil) were assessed for their analytical performance and the results compared to RT-qPCR (golden standard) technique. **Results:** The results can be observed in Table 1. **Conclusion:** All RTs evaluated showed specificity greater than 96%. GOLD Analyze (77%) showed the best sensitivity between RTs evaluated when considering all samples (Cq up to 30). All tests obtained sensitivity above 90% considering samples with Cq lower than 20. Thus, the rapid tests evaluated can be important tools to support the expansion of testing and diagnosis, especially for individuals with high viral load.

RTs evaluated	Samples	Negative (RT-qPCR)	Negative (RTs)	Specificity	Positive (RT-qPCR)	Positive (RTs)	Sensitivity
VivaDiag	60	25	24	96%	35	22	63%
Bioscience	51	25	24	96%	26	18	69%
VIDA biotecnologia	60	30	30	100%	30	22	73%
GOLD Analisa	60	30	27	90%	30	23	77%

A-218

Lactose Intolerance by Oral Swab Genotyping: validation of a direct-to-consumer test

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**Background:** Lactose is a disaccharide composed of glucose and galactose. Its digestion and absorption are mediated mainly by the lactase enzyme whose activity is genetically controlled and largely determined by the LCT gene. The persistence of the lactase enzyme in adulthood is associated with many polymorphisms in a region of DNA located about 14Kb above the lactase gene (LCT). The 13910C>T polymorphism (rs4988235) has been associated with lactase activity in adulthood, exhibiting an autosomal dominant inheritance pattern. Possible combinations for this polymorphism are CC homozygote (lactase non-persistence), CT heterozygote (lactase persistence), and TT homozygote (lactase persistence). The frequency of these genotypes varies according to the population studied, being around 35.8% for the CC genotype, 48.1% for the CT genotype, and 16.1% for the TT genotype. Non-persistence of lactase can cause nonspecific signs and symptoms such as abdominal pain, nausea, flatulence, and diarrhea after milk consumption. Some studies show an association of CT and TT genotypes with obesity. The evaluation of these polymorphisms can be performed both in blood samples and in oral swab samples. Interest in genetic testing is increasing as more people become curious about their ancestry and health-related genetic predispositions. Rapid technological advances in recent decades have made it possible for genetic tests to be performed cheaply, quickly, and directly by consumers. The direct-to-consumer (DTC) genetic testing market has increased significantly in recent years. Information on ancestry, health, pharmacogenomics, fitness and nutrition can be easily acquired, increasingly empowering the end consumer with their data. **Objective:** To validate a direct-to-consumer Lactose Intolerance test by real-time PCR genotyping methodology using TaqMan probes for allelic discrimination of these polymorphisms through oral cell analysis using oral swab samples. **Methods:** Blood and oral swab samples were collected from 46 volunteers who agreed to participate in the validation by signing the Informed Consent Term - TCLE. Two patients already had the diagnosis of lactose intolerance and the others were collected "blindly". DNA isolation was performed using the MagMax™ DNA Multi-Sample Ultra 2.0 Kit on the KingFisher Flex (ThermoFisher Scientific, EUA) automated platform according to the manufacturer's instructions. Genotyping for rs4988235 was performed using TaqMan SNP Genotyping Assays (Life Technologies, Foster City, CA) at 7500 Fast Real-Time PCR System (Life Technologies). The results were analyzed using TaqMan Genotyper Software version 1.0.1. **Results:** After carrying out the tests, the results were compared between the 46 blood samples (methodology already validated and offered by the Pardini Group) and oral swab. Carriers of the rs4988235 CC genotype predominated (31/46, 67.4%), the heterozygotes were 14/46 (30.4%) and the remaining homozygotes for the T allele were 1/46 (2.2%). The results showed 100% agreement and reproducibility compared to the routine performed for whole blood samples. **Conclusion:** The study demonstrated that the qPCR assay is a sensitive and reliable LCT genotyping assay on oral swab samples, and can be a safe direct-to-consumer test option.

A-219

Molecular Panel to detect and differentiate SARS-CoV-2 variants by RT-qPCR

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**Background:** Since late 2019, the COVID-19 pandemic has been the center of attention worldwide, accumulating nowadays more than 400 million cases and almost 6 million deaths. Several public and private research centers have been directing great efforts to allow the monitoring of SARS-CoV-2 variants in real-time. Among all characterized variants, the identification of Variants of Concern (VOCs) is of special interest to health authorities. The attributes of a VOC include the increasing transmissibility and/or disease severity, significant reduction in vaccine efficacy, or failures in diagnostic detection. The gold standard for the identification of VOCs and other new variants remains the massive viral genomic sequencing. However, this technique requires highly specialized equipment and trained technicians in analysis, in addition to the relatively high cost, which restricts its availability in several regions. In this sense, variant genotyping using quantitative real-time PCR (qPCR) has become an important alternative to support the monitoring of variants in a fast, sensitive, and specific way. Thus, this work aims to describe the validation of a molecular panel for genotyping SARS-CoV-2 variants. **Methods:** The RNA isolation was performed using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher, Mas-

sachusetts, USA), according to the manufacturer's instructions. Through the TaqMan SARS-CoV-2 Mutation Panel, we selected a set of eight non-synonymous Spike protein mutations (G339D, K417T, K417N, L452R, E484K, E484Q, N501Y, and P681R) intending to identification the main circulating variants so far described. Genotyping was conducted by quantitative real-time PCR (RT-qPCR) with the iTaq Universal Probes One-Step kit (Bio-Rad, California, USA) according to the manufacturer's instructions. Each reaction was performed using 4.5 µL of RNA (with a CT value of less or equal to 30), 5 µL of iTaq universal probes reaction mix (2X), 0.25 µL of iScript advanced reverse transcriptase, and 0.25 µL of specific SNP probe (40X; TaqMan SARS-CoV-2 Mutation Panel) in a final volume of 10 µL. Amplification was performed under the following cycling conditions: 50°C for 10 minutes; 95°C for 3 minutes; 45 cycles of 95°C for 15 seconds and 60°C for 1 minute; 60°C for 1 minute. All samples used in the validation were previously submitted to whole-genome sequencing for comparability of results. **Results:** The set of mutations used in our panel was able to identify and differentiate the Gamma, Zeta, Beta, Alpha, Kappa, Delta, and Omicron variants. There was no discordant result comparing the classification obtained by whole-genome sequencing. Samples that presented Cq (Cycle Quantification) values greater than 30, showed inconclusive results in both the RT-qPCR and sequencing methodologies. **Conclusion:** Genotyping of SARS-CoV-2 variants by RT-qPCR demonstrated to be a fast, efficient, and lower-cost alternative (compared to the gold standard). The assay showed better performance in samples with high viral load (Cq < 30), correlated with the period of greatest transmissibility potential. Thus, the methodology in question allows the identification of the main circulating variants in real-time, proving to be an important support for the genomic surveillance of COVID-19 in the world.

## A-220

### New concern in COVID-19 pandemic: Co-infection of SARS-CoV-2 with other respiratory pathogens.

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**Background:** The pandemic of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has impacted the health public worldwide, requiring major adjustments to healthcare systems. While the concern remains focused on SARS-CoV-2, other respiratory pathogens also continue to circulate. An example was the Flu outbreak that affected several countries such as the USA and Brazil in early 2022, which led to the creation of the term "Flurona" to designate the co-infection caused by influenza and SARS-CoV-2. Due to the similar transmission characteristics and common clinical manifestations, it is difficult to differentiate and identify the co-infection with these respiratory pathogens without specific diagnostic tests. Thus, the information about COVID-19 and influenza coinfection, as well as other respiratory pathogens, is limited. Screening studies report more cases, suggesting that unless screening patients with COVID-19, the coinfection remains undiagnosed and underestimated. **Methods:** The study was conducted from December 1, 2021, to January 11, 2022, using 2,218 nasal swab samples from different regions of Brazil. Samples were subjected to the Alinity m system with the Alinity m Resp-4-Plex Assay (Abbott) according to the manufacturer's instructions. This system consists of an integrated flow of nucleic acid extraction and polymerase chain reaction preceded by reverse transcription (RT-qPCR), capable of detecting and differentiating RNA from Influenza Virus A, Influenza Virus B, Respiratory Syncytial Virus (RSV), and SARS-CoV-2. **Results:** In none of the samples, the influenza B virus was detected. The analysis showed that the number of co-infection cases increased from December 2021 (38/803; 4.73%) to the first half of January 2022 (95/1,415; 6.71%), in all combinations of the three main respiratory syndromes viruses in the Brazilian population: Influenza A, SARS-CoV-2, and RSV. Flurona cases (SARS-CoV-2 and Influenza A co-infection) were the most represented in our sample (78/2,218; 3.52%) followed by Influenza A and RSV coinfection (38/2,218; 1.71%). RSV and SARS-CoV-2 co-infection was observed in 0.45% of cases (10/2,218) and the triple co-infection of SARS-CoV-2, RSV, and influenza A were presented in 0.32% of samples. **Conclusion:** Although a low proportion of COVID-19 patients coinfecting with other respiratory pathogens has been observed, this finding is of great epidemiological relevance once overlapping diseases can complicate diagnosis, treatment, and prognosis. Besides that, as the number of diagnostic tests that can detect several respiratory viruses simultaneously is restricted, the rate of co-infection in COVID-19 patients may be sub-estimated.

## A-221

### One-step in-house RT-qPCR method standardization for detecting PML-RARa fusions

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**Background:** The translocation that characterizes acute promyelocytic leukemia (APL) involves the fusion of the promyelocytic leukemia (PML) gene at 15q22 with the alpha retinoic acid receptor gene (RARa) in 17q21. While RARa gene presents one break site in intron 2, the PML Gene has three distinct breakpoint cluster regions (bcr): bcr1 (long isoform) in intron 6 (between exons 6 and 7), bcr2 (variable isoform) in exon 6, and bcr3 (short) in intron 3 (between exons 3 and 4). Given the advantages associated with quantitative real-time PCR (qPCR), such as fast and reliable diagnostic tests, this technique represents an important tool in clinical laboratories to diagnose and monitor patients with APL history. In this context, this work aimed to validate an "in-house" qualitative method to detect PML-RARa fusions, using one-step RT (reverse transcriptase) qPCR. **Methods:** Total mRNA from 27 samples with known diagnostic was extracted by the Trizol (Thermo Fisher) method according to the manufacturer's instructions. Primers for detecting each isoform, as well as the probe, were designed using the PrimerBlast tool. All samples were quantified by NanoDrop 2000 spectrophotometer (Thermo Fisher). All assays were performed using TaqMan RNA-to-Ct 1-Step kit (Thermo Fisher). The inventoried ABL1 assay (Thermo Fisher) was used as endogenous control. Using a pool of samples of each isoform and a pool of healthy volunteers samples (negative control) were performed: sequence tests, standardization of RNA input, primer, and probe concentration, evaluation of reaction efficiency. After tests standardizing, the samples were individually evaluated to assess whether the result observed by the in-house method was in agreement with the expected result. **Results:** All designed sequences worked well by each specific isoform detection. The best RNA Input for the reaction was 200ng. For the three isoforms, the final standardized primer concentration was 300nM (Forward)/300mM (Reverse) and 150nM for probe concentration. The efficiencies reactions were 92.6% and 90.6% for bcr1 and bcr3 isoforms respectively, within the limit recommended by the literature (between 90 and 110%). However, the bcr2 efficiency reaction was 117.2%, above the recommended. For all samples, there was ABL1 amplification (endogenous control). There was no unspecific amplification in samples from healthy volunteers. In samples previously classified as bcr1, besides the curve given by bcr1 primers set, were observed two later curves, which originated from bcr2 and bcr3 primers set. Samples classified as bcr2 showed two distinct profiles: (i) curve amplification of bcr2 isoform (60% of samples); (ii) presence of an additional later curve, given by bcr3 primers set for (40% of samples). As for the samples classified as bcr3, there was amplification only when using the bcr3 primer set. **Conclusion:** All of the known positive samples showed amplification of one or more isoforms and there were no cases of unspecific amplification in negative controls. Thus, the test described here was able to identify the investigated PML-RARa fusions qualitatively, however, unable to differentiate the isoforms.

## A-222

### One-step RT-qPCR method standardizing for detecting the RUNX1-RUNX1T1 fusion in the acute myeloid leukemia

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**Background:** Acute myeloid leukemia (AML) with translocation t(8;21)(q22;q22.1) and *RUNX1-RUNX1T1* gene fusion is one of the most common subtypes of AML with recurrent genetic abnormalities and is associated with a favorable outcome. In this context, a fast and reliable diagnostic test represents an important tool in clinical laboratories to diagnose these AML. This work aimed to validate a qualitative method to detect the *RUNX1-RUNX1T1* gene fusion, using a one-step RT (reverse transcriptase) qPCR. **Methods:** We evaluated the performance of the *RUNX1-RUNX1T1* (Hs03024752\_ft) inventoried TaqMan assay (Thermo Fisher Scientific). Total mRNA from ten negative and five positive samples for fusion was extracted by the TRIZOL reagent according to the manufacturer's instructions. The inventoried *ABL1* assay (Thermo Fisher Scientific) was used as endogenous control. Both assays were performed using TaqMan RNA-to-Ct 1-Step kit (Thermo Fisher Scientific). Using a pool of positive samples of fusion and a pool of negative samples were performed on: sequence verification tests, standardization of RNA input, evaluation of reaction efficiency and determination of the detection limit (LOD). All analyzes took place first with the *ABL1* control in a different reaction well from the fusion reaction and then in the same well. **Results:** The qPCR *RUNX1-RUNX1T1* assay demonstrated good performance for the detection of this specific fusion. The best RNA input for the reaction was 20 ng. The efficiency reaction with *ABL1* in separate wells and in the

same reaction was 95.5% and 100.2%, respectively, it was within the limit recommended by the literature (between 90 and 110). The LOD found in both cases (with *ABL1* in separate reactions and in the same) was  $10^3$ . There was unspecific amplification in negative samples with  $Cq > 35$ . **Conclusion:** The TaqMan assays with RT one-step protocol evaluated were sensitive, fast, and applicable as a tool for detection of *RUNX1-RUNX1T1* gene fusion. The  $Cq > 35$  was defined as a cut-off to consider a sample as positive.

### A-223

#### Performance Evaluation of a Molecular Assay for Detection CBFB-MYH11 Fusion Transcripts

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**Background:** Inversion on chromosome 16, also known as *inv(16)(p13.1q22)* or *t(16;16)(p13.1;q22)* CBFB-MYH11, is found in approximately 5% of Acute Myeloid Leukemia (AML) cases. These cases are considered a clinical pathological genetic entity and are included in the category “AML with recurrent genetic abnormalities” in the World Health Organization classification. At the molecular level, *inv(16)t(16;16)* results in the fusion protein CBFB -MYH11, which interferes with the differentiation process of hematopoietic cells. Because of the variability of genomic breakpoints within CBFB and MYH11, more than 10 CBFB-MYH11 fusion transcriptional variants of different sizes have been reported. More than 85% of fusions are type A and 5%-10% are type D and type E fusions. Clinically, patients with *inv(16)* show a relatively favorable prognosis, although almost half of the adult patients relapse, and around 50% have reduced 5-year survival. The fast and useful molecular test such as real-time PCR is important to identify the main CBFB-MYH11 fusion transcripts, as it helps in the diagnosis and risk stratification in patients with AML. **Objective:** To describe the analytical and clinical validation of real-time PCR with reverse transcription assay (RT-qPCR) for detection of isoforms A, D, and E CBFB-MYH11 fusion. **Methods:** The primer-probe sets were designed using the Primer-Blast tool for the main fusion isoforms, A, D, and E. RNA isolation was performed with the Trizol method (Thermo Fisher, Massachusetts, EUA) according to manufacturer instructions. Assay performance was evaluated using commercial quantified positive control and clinical samples. The analysis parameters included: (i) Standardization of the primer-probe set; (ii) Determination of amount RNA concentration; (iii) Analytical sensitivity (Limit of detection); (iv) Intrassay and interassay precision. All reactions included the endogenous control *ABL1*. **Results:** The assay demonstrated different performances for each isoform. A primer-probe concentration matrix was performed aiming to achieve the lowest Cycle Quantification ( $Cq$ ) and highest  $\Delta Rn$ . Defined concentrations ranged from 100 to 600 nM for probes and 150 to 250 nM for probe. The total sample input per reaction was set at 10 ng. The limits of detection of the test were 100 copies/reaction for isoform A, 1,000 copies/reaction for isoform D, and 10,000 copies/reaction for isoform E with a 95% confidence interval. The regression equations obtained show good amplification conditions with a positive correlation between the variables, with a coefficient of determination ( $r^2$ ) of 0.99. The experiments performed to evaluate the precision demonstrated optimal repeatability and reproducibility. **Conclusions:** An accurate diagnosis is essential to clinical management and prognosis classification of AML patients. Here, we describe a rapid and sensitive method for detecting isoforms A, D and E of the CBFB-MYH11 fusion. This validation provided data indicating a high reaction performance for isoforms detection. However, the assay needs to be evaluated in a clinical context.

### A-224

#### Validation of one-step in-house RT-qPCR method for detecting two relevant gene fusions for the acute lymphoblastic leucemias

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**Background:** Acute leukemias are genetic diseases caused by translocations or mutations, which dysregulate hematopoiesis towards malignant transformation. Gene fusions are frequently seen in leukemia and several of the recurrent gene fusions are required for subgrouping of leukemia and prognostication, according to the WHO classification. Acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) are the most common and aggressive malignancies of the hematopoietic system. The gene fusions *TCF3-PBX1* and *ETV6-RUNX1* are expressed, respectively, as a result of the *t(1;19)* and *t(12;21)*, chromosomal translocations important for diagnostic and prognostic in childhood acute lymphoblastic leukemia. Therefore, fast and reliable diagnostic tests represent an important tool in clinical laboratories to diagnose these leukemias. In this context, this work aimed to validate a qualitative method to detect the *E2A-PBX1* and *ETV6-RUNX1* gene fusions, using one-step RT (reverse

transcriptase) qPCR. **Methods:** We evaluated the performance of the *TCF3-PBX1* (Hs03024664\_f1) and *ETV6-RUNX1* (Hs03043640\_f1) inventoried TaqMan assays (Thermo Fisher Scientific). Total mRNA from five negative and five 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 a pool of positive samples of each fusion and a pool of negative samples (healthy volunteers) 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 and later in the same reaction. **Results:** Our results showed that both assays 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: 94.8% and 92.3% for *TCF3-PBX1* and *ETV6-RUNX1* assays, respectively; b) with *ABL1* in the same reactions: 95.3% and 101.5% for *TCF3-PBX1* and *ETV6-RUNX1* assays, respectively. The literature recommends the limit of efficiency was between 90% and 110%. The LODs with *ABL1* in separate reactions were  $10^4$  and  $10^5$  for *TCF3-PBX1* and *ETV6-RUNX1* assays, respectively. However, the LODs with *ABL1* in the same reactions were  $10^3$  and  $10^4$  for *TCF3-PBX1* and *ETV6-RUNX1* assays, respectively. There was no unspecific amplification in negative samples. **Conclusion:** The TaqMan assays with RT one-step protocol evaluated are highly sensitive, fast, financially acceptable, and applicable as a tool for the rapid detection of *TCF3-PBX1* and *ETV6-RUNX1* gene fusions.

### A-226

#### Performance of PCR-based Genotyping Panel and Whole Genome Sequencing for SARS-CoV-2 Variant Identification in Low Viral Load Samples

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**Background:** Whole genome sequencing (WGS) has been the conventional method for SARS-CoV-2 genomic surveillance. Historically, low viral loads/high cycle threshold ( $Ct$ ) values have been a technical limiting factor for WGS. In addition, due to vaccination measures and natural immunity from infection, newer SARS-CoV-2 variants are associated with lower viral burden which can negatively impact the performance of WGS in such samples. PCR-based genotyping panel is an alternative approach for identification of known SARS-CoV-2 variants with a theoretically better analytical limit of detection. The aim of this study was to compare the performance of the two above-mentioned variant identifications approaches, specifically in SARS-CoV-2 samples with low viral loads ( $Ct > 25$ ). **Methods:** Genotyping analysis was performed on 60 SARS-CoV-2 positive samples collected at the Life Science Testing Center at Northeastern University from December 2021 to February 2022. RNA was extracted using the MagMax™ Viral/Pathogen II Nucleic Acid Isolation Kit. SARS-CoV-2 detection was confirmed using the TaqPath™ COVID-19 Combo Kit and samples were segregated using  $Ct$  values ( $Ct < 25$ ,  $Ct > 25$ ). Variant determination was conducted using two methods: 1) PCR-based genotyping using a panel of 8 TaqMan SARS-CoV-2 single nucleotide polymorphism (SNP) assays (Q493R, K417T, d.H69V70, T95I, P681R, L452R, G339D, Q27x) were used for the discrimination of Delta and Omicron Variants of Concern (VOC) processed on the QuantStudio6 Pro RT-PCR instrument; 2) WGS using the Ion Torrent GeneStudio-S5 Plus system. **Results:** The PCR-based genotyping panel analysis was able to accurately assign lineages in 98.33% (59/60) of all samples while WGS was able to assign lineages in 68.33% (41/60) of all samples. In samples with high viral loads ( $Ct < 25$ ), the performance of PCR-based genotyping and WGS was comparable with lineage assignment in all cases. In low viral load cases ( $Ct > 25$ ), PCR-based genotyping was able to assign SARS-CoV-2 lineages in 94.73% (18/19) whereas WGS was unable to determine SARS-CoV-2 VOC status in samples with  $Ct$  values  $> 25$ . Of the 60 samples, the PCR-based genotyping panel identified the VOC as follows: (delta N=4), (omicron BA.1 N=39), (omicron BA.2 N=16). **Conclusion:** PCR-based genotyping is the preferred method for SARS-CoV-2 VOC identification especially in samples with low viral loads. While WGS perform well in samples with high viral burden ( $Ct < 25$ ), there are analytical limitations for its applicability in low viral load ( $Ct > 25$ ) samples. Since different SARS-CoV-2 strains can be associated with different viral loads in the background of the host-mediated immune response, lineage determination across the entire range of viral loads is important to accurately conduct SARS-CoV-2 strain surveillance.

**A-231**

**The real situation of the Covid-19 pandemic in a laboratory in northern Brazil during 2021**

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**Background:** COVID-19 is an infectious disease that started in China and spread worldwide, a global pandemic, caused by the Virus SARS-CoV-2, is the cause of severe acute respiratory syndrome (SRAG). Rt-PCR (Reverse Transcription - Polymerase Chain Reaction) is a real-time Reverse Transcription Chain Reaction test that verifies the presence of genetic material from the virus, confirming that the person meets COVID-19. Rt-PCR (gold standard) tests have the diagnostic function, being the definitive test according to the World Health Organization (WHO)1. It is based on the detection of viral RNA in swab samples of the nasopharynx and oropharynx and should be done in the first days of symptoms (3rd - 7th day). The RT-PCR test has some limitations, due to several factors, including pre-analytical ones, which include inadequate collection, sample quality, in addition to conservation and inadequate transport. Analytical factors involve possible viral mutation, presence of PCR inhibitors in the sample, or viral load below the detection limit of the test. Biological factors depend on the type of material used and positivity is higher in low respiratory tract samples; the period in which the samples were collected and the fluctuation of the viral load.

**Methods:** Results were collected in the laboratory database by the LabSystem-SHIFT system, in analyses already processed, for a retrospective research, and the confidentiality of patient identification forms was protected. The period January 1, 2021 to February 22, 2022

**Results:**

	jan/21	fev/21	mar/21	abr/21	mai/21	jun/21	jul/21	ago/21	set/21	out/21	nov/21	dez/21	jan/22	fev/22
DETECT-ADO	980	966	2122	410	238	74	63	22	17	25	33	44	2701	345
NÃO DETECT-ADO	3091	2235	4212	1987	1651	1197	931	1162	929	819	777	1239	2449	889
INDETERMIN-ADO	3	1	3	1	6	9	2	3					2	
TOTAL	4074	3202	6337	2398	1895	1280	996	1187	946	844	810	1283	5152	1234

**Conclusion:**It was possible to observe that there was a significant decrease in the number of positive cases during the year 2021, a high upward curve in the month of Jan 2022, led many people to despair in search of tests, because when many people would have already relaxed in care and social distancing, it was a false feeling that there was no longer danger of infection.

**A-232**

**Validation of Influenza A, Influenza B and SARS-CoV-2 kit: positive results parameters observation in clinical samples of Rio de Janeiro, Brazil.**

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**Background:**Since the ending months of 2019, the COVID-19 pandemic has brought various levels of impact for different parts of the world and different cultures. Two years later, the first days of december 2021 brought a new wave of COVID-19 cases, as a result of the emergence of the omicron variant in Brazil, followed by several concomitant cases of Influenza A (H3N2) - especially in Rio de Janeiro. To analyze the mix of cases in Rio de Janeiro and identify the virus type, a respiratory viral panel test has been developed to objectively categorize cases for Influenza A (susceptible to the H3N2 strain), Influenza B and SARS-CoV-2. This work presents the process and validation of the the analysis for Influenza A, Influenza B and SARS-CoV-2 kit, as well as, observe the positive results for these parameters in a cohort of individuals from Rio de Janeiro-Brazil.

**Methods:**These results were obtained from RNA, isolated from samples collected by nasopharyngeal swab, and analyzed with the RNeasy kit SARS-CoV-2 (MGI Tech Co., China), strictly following the manufacturer's instructions. The isolated mRNA was transcribed into cDNA using MGIEasy Nucleic Acid Extraction Kit (MGI Tech Co., China) - following the manufacturer's protocol. MGIEasy Nucleic Acid Extraction Kit is designed for isolating viral RNA from various sample types using super-paramagnetic bead technology, and compatible with the multiplex automated extraction platform, MGISP-960. RT-qPCR was performed using Molecular

kit SARS-CoV-2 (E/RP) (BioManguinhos, Brazil) on the 7500 real-time PCR system thermo-cycler (LineGene 9600 Plus Fluorescent Quantitative Detection System - Bioer, China).

**Results:**Within the patients with Influenza, all samples were positive only for Influenza A. These samples were analyzed by the Respiratory Viral Panel amplification kit, where 2160 known analyzes come from patients of Rio de Janeiro, a city that experienced an outbreak of Influenza A (H3N2 variant). From the validated results, Influenza A infection was present in 224 patients, co-infection with SARS-CoV-2 was observed in 75 analysis and no presence of Influenza B. In addition to this comparative results, the analyzes were performed concomitantly with the use of the EDx amplification kit for the detection of the SARS-CoV-2 virus, standard use at UNADIG-RJ, and showed more than 99% accuracy.

**Conclusion:** Our results showed high accuracy by the respiratory viral panel amplification kit for the detection of Influenza A, Influenza B and SARS-CoV-2 viruses as well as high specificity index for the detection of Influenza A virus in Rio de Janeiro.

**A-233**

**Congenital Neutropenia With Deficiency of SRP54**

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**Background:** Congenital neutropenia are rare genetic disorders that can be caused by mutations in multiple genes. Mutations in the gene SRP54 (cromosome 14q13) which are inherited in a autosomal dominant pattern can caused a severe congenital neutropenia type 8 with similar characteristics to Shwachman-Bodian-Diamond syndrome, first described in 2017. The SRP54 protein encodes a GTPase from the cytoplasmic ribonucleoprotein complex which is involved in targeting new proteins to the ER membrane. It means having low levels of neutrophils accompanied by a higher risk for infections early in life. It also encompasses blood disorders, hipocellularity in bone marrow, impaired granulocytes maturation and neurological deficit.

**Methods:** Patient presentation: A 2 year old girl with recurring skin and respiratory infections, gluteal abscesses, arterial aneurysm of left elbow, acute otitis media, microcytic hypochromic anemia, preseptal cellulitis in right eye and neutropenic condition of 400 cells/μL en 2015 with no evidence of anti-granulocytes antibodies. No family history of an immunodeficiency disorder.

The patient was treated with granulocyte-colony stimulating factor (G-CSF) (Filgrastim) 10 mcg/kg every other day, increasing the dose in october 2016 due to the low levels of neutrophils. After new recurrent infecctions, the abscess evolved to cellulitis and miositis. A genetic study of immunodeficiency was requested in 2017. It was performed congenital neutropenia and chronic granulomatous disease gene sequencing panel. The following genes were analysed: ELANE (severe congenital neutropenia 1), GFI1 (severe congenital neutropenia 2), HAX1 (severe congenital neutropenia 3), G6PC3 (severe congenital neutropenia 4), VPS45 (severe congenital neutropenia severa 5), G6PT1 (glycogenosis Ib), TAZ (Barth syndrome), C16ORF57 (poikiloderma with neutropenia), WAS (Wiskott Aldrich syndrome), JAGN1 (JAGN1 deficiency), ROBLD3 (P14/LAMTOR2 deficiency) y GATA2 (MonoMac syndrome).

**Results:** In this study, it was detected the GFI1 mutation (GFI1 deficiency- SCN2) c.472G>A in heterozygosis. The fact that this mutation is considered neutral and is not located at the end of the gene (where all the associated deficiency GFI1 mutations appear) discards this mutation as the cause of clinical symptoms.

In 2020, neutrophil levels were 90 cells/ μL, requiring an elective appendectomy or suspected appendiceal mucocele. In 2021, it was performed an exome sequencing genetic testing by NGS with platform NexSeq 500 sequencing system (Illumina) with a targeted analysis of 444 genes associated to primary immune deficiency diseases. It was detected a pathogenic variant c.337G>C (p.G113R) of SRP54 gene in heterozygosis.

**Conclusion:** The updating and inclusion of new genes and mutations in genetic studies has made possible the detection of the pathogenic mutation in SRP54 gene, providing a final diagnosis in diseases in which these mutations had not previously been detected.

## A-234

**Importance of the multidisciplinary approach in the diagnosis and follow-up of minority diseases**

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**Introduction:** A 13-month-old male under study due to failure to thrive. No family history of interest, only child of healthy non-consanguineous parents. Pregnancy with retarded intrauterine growth (RIG), delivery at 40 weeks, low birth weight (2,380 grams). Hospitalized in the neonatal period for poor appetite and poor intake without vomiting with a diagnosis of hyponatremic dehydration and malnutrition at discharge. Negative heel prick, correct vaccination. On several occasions he was admitted to the emergency department for fever of several days of evolution and after one month of life he was admitted due to enterovirus meningitis. During admission, cardiac murmur and hiperttransaminasemia were detected in complementary tests, and he was referred to cardiology and gastroenterology services.

**Development:** Male admitted to complete metabolic study due to suspected liver disease. On examination: weight 7,890 Kg(P1) and length 73cm(<P1). After performing different complementary tests the following results were obtained:•Abdominal ultrasound with signs of possible liver disease.•Hypertrophic cardiomyopathy.•Karyotype 46XY.•Neutropenia (Neutrophils 2,2%;RI 25-65%)(0,30x10e9/L; RI 1,5-8).•Hypertransaminasemia(AST 84 UI/L;RI<40UI/L)(ALT 62 UI/L;RI<30UI/L).•Increased lactic acid (8,28mmol/L;RI 0,40-1,80), pyruvic acid (88,20µmol/L;RI30-70µmol/L), beta-hydroxybutyrate (1484,50µmol/L;RI 15-700µmol/L), normal acetoacetate, free fatty acids (1700µmol/L;RI 200-600µmol/L) and normal acetoacetate.•Increased urinary 3-methylglutaric acid and 3-methylglutaconic acid.

Neutropenia was confirmed in later studies, becoming absolute, so a bone marrow puncture was performed: a complete blockage of neutrophilic granulopoiesis was observed, which was present but not mature. Treatment was started(Filgrastim and prophylactic azithromycin).In order to know the primary cause of 3-methylglutaconic aciduria a genetic study was performed. After performing next generation sequencing (NGS) a deletion (c.302\_311delACATCTGCTT)(p.D101Afs\*35) was detected in hemizygos in the *TAZ* gene, not previously described in the literature but considered potentially pathogenic because it gives rise to a premature stop codon that interrupts the synthesis of the protein. Maternal genetic study was extended and the same mutation was detected.The *TAZ* gene is associated with Barth syndrome (BTHS) with X-linked recessive inheritance. BTHS or 3-methylglutaconic aciduria type 2 is an inborn error of phospholipid metabolism characterized by dilated cardiomyopathy, skeletal myopathy, neutropenia, growth retardation and organic aciduria. It usually appears in infancy and is caused by mutations in the *TAZ* gene encoding the tafazzin protein, a mitochondrial acyltransferase involved in cardiolipin metabolism.Conclusions: Phenotypic variability presents a major challenge to the diagnosis of BTHS: some patients never develop neutropenia, while others lack increased 3-MGCA and a minority have no cardiomyopathy. It is a multisystem disease in which heart disease and infections are life-threatening complications. For this reason, a multidisciplinary approach that allows early diagnosis, avoids future complications and provides adequate follow-up is essential.

The laboratory is fundamental for diagnosis and follow-up of the disease. In addition, it is essential for genetic counseling in future pregnancies of male children. In this case, the parents had a second son whose genetic diagnosis was made at birth and the disease was detected in the first days of life. Thanks to early treatment, no severe symptoms developed. In addition, a new potentially pathogenic mutation not previously described in the literature has been found.

## A-235

**Primary ciliary dyskinesia. Genetic diagnosis in a patient with suspected primary immunodeficiency**

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**Background:** Primary ciliary dyskinesia (PCD) is a rare disease (incidence of 1/15,000-1/30,000 live births) caused by ciliary structural defect that leads to respiratory disorders (bronchiectasis, respiratory infections, etc.) and, in some cases, reduced fertility, hearing loss and/or chronic sinusitis. It is genetically heterogeneous but the development of molecular biology has contributed to the identification of more than 20 genes related to structural components of the cilia or their regulatory proteins whose pathogenic mutations are associated with DCP, including: DNAH5, DNAI1, DNAI2, DNAL1, NME8/TXNDC3, CCDC114, ARMC4C, CDC39, CCDC40, CCDC164,

CCDC65, DNAH11, DNAAF1/LRRC50, DNAAF2/KTU, DNAAF3, CCDC103, HEATR2, LRRC6, ZMYND10, DYX1C1, SPAG1, C21ORF59, RSPH4A, RSPH9, RSPH1.However, up to 33% of diagnosed patients do not have mutations in any of the genes mentioned, and currently the genetic study of this type of patient is not performed routinely, which makes diagnosis difficult and delayed.

**Methods:** we analyze genes related to primary immunodeficiencies and the whole exome by using next-generation sequencing (NGS) with the NexSeq 500 sequencing system platform (Illumina). Results were confirmed by SANGER sequencing.

**Case:** a 33-year-old man studied for bronchiectasis and mixed ventilatory failure secondary to recurrent pulmonary infections since neonatal age. Family history of consanguineous parents and a brother with respiratory disease, sperm immobility and diagnostic suspicion of LRBA Deficiency stand out. In 2019, he was referred to the Transplant Unit for evaluation, being ruled out as a candidate for lung transplant due to the patient's underlying disease (possible unknown immunodeficiency).In 2020, a molecular study was carried out by NGS of the patient and his brother, detecting the mutation c.7912T>C (p.Trp2638Arg) in the LRBA gene in heterozygosis in the case of the patient and in homozygosis in that of his brother. This genetic variant is defined as Uncertain Significance and was not described as associated with LRBA deficiency, so functional studies would be required to confirm functional alterations of the protein. In addition, it is an autosomal recessive inheritance pathology, so LRBA deficiency was ruled out in the patient.In 2021, a new molecular study was carried out by NGS of the whole exome, finding two genetic variants in the RSPH1 gene, both described as pathogenic and associated with DCP. SANGER sequencing confirmed the presence of both mutations in different alleles (compound heterozygosis).The molecular confirmation of the diagnosis of PCD justifies patient's respiratory pathology, which rules out the presence of a PID that contraindicates lung transplantation, so the patient has been proposed again as a candidate.

**Conclusion:** PCD is a rare disease that mainly causes lung disease (in some severe cases, requiring lung transplantation), infertility and/or hearing loss. Its genetic association is very heterogeneous and genes whose pathogenic mutations are associated with this disease continue to be found. The genetic study, together with clinical suspicion, is essential to reach an accurate diagnosis of these patients, rule out other pathologies and direct treatment.

## A-237

**Inconsistent Variant Interpretation Between Original Results and Prenatal Molecular Diagnostic Center.**

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**Background:**The advent of genome era poses challenges to the traditional medical system. Variations in patients with rare single-gene neurogenetic diseases are mainly detected by NGS. The bottleneck and difficulty of genetic disease diagnosis have switched from the inaccessibility of detection technology to the interpretation of sequencing results. Multiple studies suggest the inconsistency rate of interlaboratory variant interpretation reached about 10~30%, while few studies performed on prenatal diagnostic prospect. In this study, we aimed to summarize the reasons underline the inconsistency of variant retest and reevaluation results between prenatal diagnostic laboratory and original results. It would be a benefit to optimize the health care services in the field of prenatal genetic counseling and prenatal diagnosis. **Methods:**Pathogenicity of variants was interpreted by molecular geneticists and clinicians according to the guidelines of the American College of Medical Genetics and Genomics (ACMG). The definition of the inconsistency between the verification result and the original report refers to any kind of inconsistency in variant detection, pathogenicity interpretation and inheritance pattern analysis. The method of variant detection depends on the type of DNA changes. PCR-Sanger sequencing was used for SNVs and small fragment indels within 20bp, while qRCR and MLPA for large fragment insertion/deletion. The consistency of pathogenicity classification was compared with the three-tier differences, which means different among pathogenicity (P/LP), unclear significance (VUS) and benign (B/LB). Besides probands' samples, those from parents (all families), siblings and other relatives (when necessary and available) were collected for genetic variant verification and co-segregation analysis. **Results:**Totally 17(6%) cases of 286 families affected with rare monogenic diseases were found to have different results during variant verification procedure. These cases were composed of 4(23.5%) with variant detection errors, 13(76.5%) inconsistent in interpretation and 1(6%) with non-Mendelian inheritance pattern. Detection errors were related with pseudogene, GC-rich sequence, and false positive results of NGS proved by Sanger sequencing. Overall, classifications of 18 variants were changed among the 13 cases with different interpretation. The transformed classification results were 14 cases of LP→VUS/LB/B, 3 VUS→P/LP, 1 VUS→VUS/LP, plus 1

VUS→LB/B. The reasons underlying the discrepancy were difference in classification system(n=3), using phenotype evaluation(n=4), AF criteria (n=6), co-segregation evaluation(n=8), computational prediction criteria(n=5), and literature review(n=6). One case could be caused by more than one different reason mentioned above. The last case with atypical inheritance pattern was originally considered as AR inheritance, but maternal uniparental disomy after additional genetic analysis. **Conclusion:** Due to the potential inconsistencies, we recommend that families affected by monogenic diseases with reproductive plans should be referred to prenatal genetic counseling as early as possible to avoid the different results postponed the subsequent prenatal diagnosis.

### A-238

#### Diagnosis of primary hereditary dyslipidemias through the laboratory

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**Background:** Familial hypercholesterolemia (FH) is caused by genetic defects involving the LDLR, APOB, and PCSK9 genes. They are transmitted as AD with complete penetrance and can present: 1. In a homozygous form, where affected individuals lack receptors for LDL cholesterol or do not function properly, causing very high concentrations of LDL cholesterol (500-1000mg/dL) from birth. The estimated prevalence is 1:1,000,000. 2. In heterozygous form, it is the most frequent and affected individuals have mutated one allele of the gene and LDL cholesterol between 190-500mg/dL. A prevalence between 1/200 to 1/500 is estimated. It is the most frequent cause of premature cardiovascular disease: the risk of suffering from this type of disease is between 3 and 13 times higher than that of the general population. It is considered an underdiagnosed and undertreated disease

**Methods:** In our laboratory, we have a tool to screen patients at high cardiovascular risk: in those with LDL cholesterol > 250 mg/dl, a comment is activated in the LIS (Laboratory Information System), so that we can diagnose the highest % of cases of FH. At present, the genetic study is carried out by massive sequencing of a panel of 7 genes, including the main ones responsible for HF, as well as other less frequent dyslipidemias. Regarding the genetic diagnosis of FH, promoter and coding DNA sequences and exon-intron junction regions of the LDLR, APOB, PCSK9, APOE and STAP1 genes are analyzed

**Results:** During the years 2018-2021, the genetic study of 30 patients with high suspicion of hereditary dyslipidemia (high values of LDL cholesterol) was carried out. Secondary causes had previously been ruled out. In 12 of the patients, the genetic result was negative, that is, no pathogenic and/or probably pathogenic variants were identified to confirm the clinical suspicion. In 14 patients, the result of the genetic analysis was positive. The most frequently found variants were NM\_000527.5 (LDLR):c.1027G>A (p.Gly343Ser) and NM\_000527.4(LDLR): c.1845+1g>c(p.Glu615fs\*25), in heterozygosis. In the remaining 4 cases, the result of the genetic study was inconclusive, that is, variants classified as Significance Uncertain (VOUS) were identified in the different databases consulted. Finally, it was possible to carry out an allelic segregation study in the relatives of 5 of the cases reported as positive.

**Conclusion:** Almost half of the patients were diagnosed early with HF, the most frequent according to the bibliography. In cases of hypercholesterolemia where the genetic study was negative, the possibility of polygenic primary hypercholesterolemia should be assessed. On the other hand, those patients with inconclusive genetic results should be evaluated with an allelic segregation study in order to try, as far as possible, to interpret the variants identified as VOUS in their family context. Highlight the great role of the clinical laboratory in the early diagnosis of hereditary dyslipidemias, which will allow us to treat these patients early, avoiding the appearance of cardiovascular events throughout their lives; as well as the identification of those direct relatives with genetic predisposition.

### A-240

#### A novel variant of RAG2 Gene in a parent, potential risk factor for neonates with Severe Combined Immunodeficiency

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**Background:** Severe Combined Immunodeficiency (SCID) is a congenital disorder and the most severe form of potentially fatal primary immunodeficiency. Several mutations in genes such as IL2RG, JAK3, IL7RA, RAG 1 RAG2, and ADA and their pathogenic mechanisms associated with SCID have been identified. Inherited hypo-

morphic mutations of Recombination-activating gene (RAG) 1 and 2 seem to be the most common cause of different forms of SCID especially leaky SCID and Omenn syndrome (OS).

**Methods:** A whole exome sequencing (NGS) of a couple was done to identify the cause of SCID. Nine in silico SNP prediction tools were used to identify the damaging effects of novel variant found in the couple

**Results:** In this case study, we report a couple with normal history and non-consanguineous marriage presented with 3 consecutive neonatal death to due combined immunodeficiency along with exfoliative erythroderma, respiratory infection, lymphadenopathy, and hepatosplenomegaly. Whole exome sequencing of the parents revealed 2 different variants RAG 2 gene; a novel variant (Exon 3, c.137T>C, p. Phe-46Ser) in father and previously known rare variant (Exon 3, c.218G>A, p.Arg73His) in mother which was reported to cause OS. Functional differences through SNPs can lead to deleterious effects; hence this novel variant was analyzed for its pathogenicity using both sequence and structure based approaches. Nine in silico SNP prediction tools identified this variant as damaging/deleterious with the high confidence score. A significant deviation in the energy minimization score was also observed for this variant upon analyzing its effect on the protein stability. These results were found to be consistent when compared with the known variants for the respective congenital disorder. Protein-protein interaction analysis revealed that the protein associated with this novel SNP is a part of core complex and acts as hub connecting 9 proteins and alteration in the respective protein may lead to dissociation of the several signal cascades.

**Conclusion:** In conclusion these analyses suggest that this novel variant may serve as potential target for different proteomics studies and diagnostic interventions.

### A-304

#### Analysis of membrane transporter genes as prognostic tools and predictors of response to chemoradiation therapy in patients with cervical cancer

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**Background:** Cervical cancer (CC) is the fourth most common type of cancer in women worldwide. Statistics show that in 2020, there were estimated 604,127 new cases, of which 14,480 would be of invasive cervical cancer type. Improvement in survival remains the goal of the treatment in the clinical setting, and treatment varies by stage, metastasis, or recurrence. Although some advances have been made in cervical cancer screening technology, the survival rate and response to treatment remain unfavorable. Membrane transporters are a large group of proteins responsible for the maintenance of proper amounts of molecules inside and outside the cells. Among them, we can highlight the ATPase transporter family, involved in stimulating chemotherapy resistance and platinum accumulation in whole cells and vesicular compartments of cisplatin, carboplatin and oxaliplatin in neoplastic cells. The solute carrier protein (SLC) groups also stand out among those transporters playing critical roles in the movement of cytotoxic agents across the neoplastic cell membrane. The potential to determine efficacy, toxicity and possible resistance to chemotherapy gives these transporters a promising role as predictive biomarkers for cervical cancer. **Objective:** The present study aims to identify possible ATPase transporter and SLC genes that present a correlation between their differential expression pattern and the manifestation of resistance to chemoradiotherapy treatment in patients with cervical cancer, leading to more accurate decision-making and more effective therapy. **Methods:** Fluorescence-activated cell screening (FACS) was used to isolate non-stem cells (NCCSCs) from cervical cancer biopsies. The genomic library of samples from 21 responders (R) and 10 non-responders (NR) patients was constructed and sequenced using the Illumina platform. Expression analysis was performed with DESeq2, considering as differentially expressed genes (DEGs) those with  $\log_2(\text{foldchange}) > 1$  or  $< -1$  and  $\text{padj} < 0.05$ . Wald's statistical test and p-value adjustment with the Benjamini Hochberg method were also performed with DESeq2. **Results:** Preliminary results demonstrate two large groups of genes with opposite differential expression profiles. The first group, composed mainly of the SLC35 and ATP13 families, had overexpressed genes in NR compared to R. The second group, mostly represented by the SLC25 and ATP6 family, was overexpressed in R compared to NR. The results achieved suggest that both groups are promising predictors of response to chemoradiotherapy. The diversity of biochemical functions of the SLC and ATPase transporter families, and their implications for chemoresistance and chemosensitivity of various classes of chemotherapeutic agents such as those based on platinum, make them a potential prognostic tool for cervical cancer.

## Special Patient Populations

### A-241

#### Novel Neuroimmune MicroRNA Panel as a Potential Early Diagnostic Marker of Autism Spectrum Disorders in Egyptian Children With Autism

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**Background:** Autism spectrum disorders (ASD) are complex neurodevelopmental disorders with contributing genetics and epigenetics factors. It involves moderately to severely disrupted functioning in social skills, expressive and receptive communication, and repetitive or stereotyped behaviours and interests. Studies on ASD pathogenesis have shown that neuroimmune microRNAs share a direct role in the ASD onset. MiRNAs play important roles in neurogenesis, synaptogenesis, and neuronal migration. There is unmet need to carryout studies to identify novel diagnostic markers that would transfer the early detection of ASD from clinical to molecular level. This study aimed at assessing the diagnostic accuracy of circulating miR-146a-5p, miR-106b-5p and miR-148a-5p as potential diagnostic markers of ASD.

#### Methods:

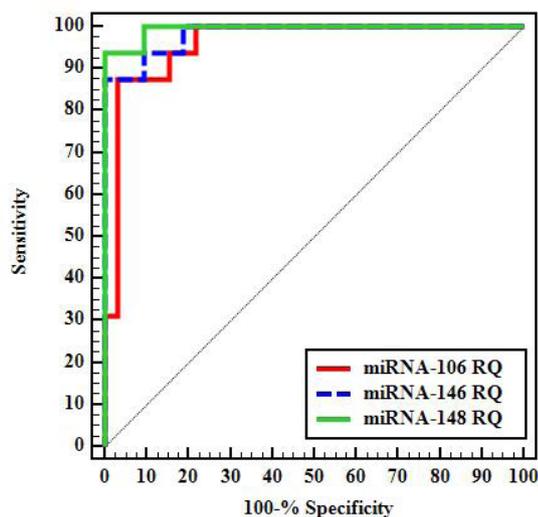
Forty-eight children were included in the study. They were divided into thirty-two healthy and sixteen with ASD based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). The severity of the core symptoms of ASD was assessed using the Childhood Autism Rating Scale (CARS). Circulating miR-146a-5p, miR-106b-5p and miR-148a-5p expressions level were determined using real-time quantitative polymerase chain reaction. The diagnostic performance of the studied miRNAs was evaluated by the Receiver operating characteristics (ROC) curve analysis.

#### Results:

Children with ASD had a median age of diagnosis of 2.5 years with a median CARS of 30. Circulating miR-146a-5p, miR-106b-5p and miR-148a-5p were significantly upregulated in children with autism (median = 5, 3.9, 10) compared to the healthy children (median = 1.2, 1.3, 1.1) respectively,  $p < 0.001$  respectively. All the studied miRNAs were found as significant discriminators of ASD by the ROC curve with an area under the curve (AUC) of (0.982, 0.959, 0.994) respectively and p-value of  $< 0.001$  respectively. None of the studied miRNAs showed any statistically significant correlation with CARS.

#### Conclusion:

Circulating neuroimmune miR-146a-5p, miR-106b-5p and miR-148a-5p could be used as a potential diagnostic miRNA panel for early detection of children with ASD.



### A-242

#### Pediatric reference interval verification for special chemistry, immunoassay, and cancer markers on the Abbott Alinity ci system

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**Background:** The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has developed an extensive database of reference intervals (RIs) for several biomarkers on various analytical systems, including special chemistry, immunoassay and cancer markers used to inform pediatric clinical decisions. In the current study, pediatric RIs were verified for 13 assays on the Abbott Alinity system based on the analysis of samples collected from healthy children and adolescents (birth-18 years) and comparison to comprehensive RIs previously established for Abbott ARCHITECT assays.

**Methods:** Analytical performance of Alinity chemistry and immunoassays was first assessed through precision, linearity, and method comparison. Subsequent, 100 serum samples from healthy children recruited with informed consent were analyzed for 13 Alinity assays (i.e. cancer antigen 19-9, cancer embryonic antigen, C3, C4, cortisol, C-peptide, DHEA-S, glucose, immunoglobulin A, G, M, lactic acid, total prostate specific antigen). The percentage of test results falling within published CALIPER ARCHITECT reference and confidence limits was determined. Reference intervals were considered verified if  $\geq 90\%$  of laboratory test results fell within previously established confidence limits.

**Results:** All assays demonstrated acceptable performance on the Alinity ci system. Of 13 assays assessed, 12 met the criteria for verification with  $\geq 95\%$  of laboratory test results falling within previously established ARCHITECT limits for most assays. Several pediatric reference values were below the limit of detection for cancer markers (i.e. cancer embryonic antigen, cancer antigen 19-9, total prostate specific antigen). Only 54% of pediatric samples fell within the recommended Abbott ARCHITECT for lactic acid, and thus a new Alinity-specific reference interval is needed.

**Conclusion:** These data demonstrate marked concordance between ARCHITECT and Alinity systems for 12 assays, as well as the robustness of previously established CALIPER RIs in healthy children and adolescents. Expanding the utility of the CALIPER database ([www.caliperdatabase.org](http://www.caliperdatabase.org)) to include Alinity assays for special chemistry and cancer markers will assist clinical laboratories using this new platform and contribute to sustaining improved clinical decision-making in pediatric populations.

### A-243

#### Pediatric reference intervals for serum calprotectin in the CALIPER cohort of healthy children and adolescents: A potential biomarker for neonatal and pediatric bacterial infection

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**Background:** Calprotectin is an antimicrobial peptide that is released from granulocytes and mononuclear phagocytes immediately after host-pathogen interaction. Measurement of serum calprotectin in neonates and children suspected with bacterial infection has been suggested to improve outcomes due to higher diagnostic sensitivity relative to other markers of infection (e.g. C-reactive protein, procalcitonin). The objective of the current study was to analytically evaluate an automated serum calprotectin assay (GCAL®, Gentian AS) and establish accurate pediatric reference intervals in the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) cohort of healthy neonates, children, and adolescents.

**Methods:** A three part analytical validation was completed in accordance with CLSI EP05-A3, EP06-A, EP09-A3 guidelines, including: 1) precision (two controls, 5 runs per day for 5 days); 2) linearity (10 levels run in triplicate and assessed via linear regression), 3) method comparison (n=40, GCAL® on Abbott Architect and Roche cobas). 300 healthy children and adolescents (0 to <19 years) were recruited from the community by CALIPER. Health information, anthropometric measurements, and blood samples were collected. Sera were analyzed using an automated serum calprotectin assay (GCAL®, Gentian AS) on an Abbott Architect ci8000 system. Data were analyzed in accordance with CLSI EP28-A3c guidelines. Health-associated data were also compared to sera collected from neonates presenting to the emergency department with suspected bacterial infection (n=70).

**Results:** The calprotectin immunoassay demonstrated acceptable precision (control 1 mean (CV): 1.04 mg/L (7.5%), control 2 mean (CV): 10.1 mg/L (1.5%)) and was determined to be linear across the analytical reporting range (slope: 0.97, intercept: 0.12, R<sup>2</sup>: 0.999, range: 0-19 mg/L). Method comparison evaluation demonstrated reproducible performance on two separate systems with a small negative bias of -0.18 mg/L

(slope: 0.95, intercept: 0.08, R<sup>2</sup>: 0.97, range: 0-19 mg/L). No statistically significant age and/or sex-specific differences were observed across the pediatric age range and one reference interval was derived (<0.15-2.97 mg/L). Significantly higher concentrations were observed in children with obesity (p<0.05) and neonates with suspected bacterial infection relative to healthy subjects (p<0.05).

**Conclusion:** In the current study, an analytical validation of an automated serum calprotectin immunoassay (GCAL®, Gentian AS) was completed and pediatric reference intervals were established for the first time in the CALIPER cohort. Presented data contribute to our knowledge of serum calprotectin levels from birth to adolescence in healthy individuals relative to disease states (e.g. obesity, bacterial infection). Future work will focus on delineating the kinetics of calprotectin response including diagnostic and prognostic value relative to other biomarkers of infection (e.g. procalcitonin, and C-reactive protein) in neonatal bacterial infection of potential clinical implementation.

**A-244**

**Serological Antibody Response to SARS-CoV-2 Vaccination in a Large Cohort of Canadian Children, Adolescents, and Adults**

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**Background:** Monitoring immune protection post-administration of an mRNA SARS-CoV-2 vaccine is essential to inform public health initiatives globally. Quantitative anti-SARS-CoV-2 antibody immunoassays present potential utility as surrogates of breakthrough infection risk. Significant evidence gaps exist in our knowledge of humoral immune response to mRNA SARS-CoV-2 vaccination in children and adolescents. Children have been differentially impacted by the SARS-CoV-2 pandemic, demonstrating discrepant symptoms and immune response to naïve infection relative to adults. It is thus essential to investigate age-specific differences in antibody response to SARS-CoV-2 vaccination.

**Methods:** 644 participants (312 adults, 332 children) were enrolled in this cross-sectional study with informed consent (age range: 6-79 years, male: 34%, female: 66%). Participation required completion of a health questionnaire and blood donation. A subset of participants were longitudinally monitored over a five month period (Aug-Dec 2021). A control cohort of 168 individuals with no history of SARS-CoV-2 infection or vaccination were evaluated to assess specificity. Sera were assayed by two immunoassays DiaSorin LIAISON SARS-CoV-2 TrimericS IgG and Abbott AdviseDx SARS-CoV-2 IgG II assays.

**Results:** Serokinetic antibody response of participants who received two doses of an mRNA vaccine at the time of collection demonstrated a strong negative correlation with time post-dose, ranging from 0 to 308 days (n=394, percent positive agreement: 96%). Anti-SARS-CoV-2 IgG titres in participants who received a booster dose increased ten-fold in longitudinally monitored participants (n=60, percent positive agreement, 100%). A statistically significant difference was observed between pediatric (mean±SD= 2037±1515 BAU/mL) and adult (1444±1277 BAU/mL) antibody titres, adjusting for time post-dose. A specificity of 98% was observed for participants with no history of SARS-CoV-2 infection or vaccination. A strong correlation between titres on the AdviseDx and TrimericS assays was determined, with high proportional bias (slope: 3.53, intercept: -350 BAU/mL, Pearson R: 0.92).

**Conclusion:** This is the largest evaluation of commercially available quantitative SARS-CoV-2 antibody assays in a cohort of Canadian children, adolescents, and adults. Findings suggest children have higher antibody titres as compared to adults post-administration of an mRNA vaccine. However, significant variation in antibody titres was observed. Future work is needed to relate antibody presence to functional immune response as well as risk of breakthrough infections.

**A-245**

**Analysis of 5 years of Ottawa outpatient lipid data reveals realistic, fit for use, between-week intra-patient biologic variations twice that of normal subjects.**

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**Background:** Discovery of elevated cholesterol with accompanying risk factors is followed by lipid monitoring with or without therapy. As out-of-range limits do not accompany cholesterol monitoring, it may be advantageous to use the patient's mean cholesterol bounded by statistically derived biological variation limits. We apply a unique approach (PMID:35137000) to determine longer term (weeks to years) biological variation from serial intra-patient measurements.

**Methods:** We obtained 5 years (2014-2019) of Ottawa Hospital outpatient Siemens Vista paired cholesterol, with fasting:random samples =22,296:23,804; HDL, 21,558:22,460; LDL, 20,659:21,402; nonHDL, 17,211:18,676; and cholesterol/HDL ratio, 14,769:13,283. We determined the weekly inpatient variation of each test, fasting and random, separately. For each test, if either drawn a week later and within 120 (or more than 120 minutes) of its previous sampling time, we tabulated consecutive pairs of inpatient results by intervals of separation: 0-1 weeks, 1-2 weeks, 2-3 weeks . . . , up to 95-96w. We also determined the average patient variation over weeks 50 to 54 which seem to represent a recurring stable nadir.

**Results:** The inpatient variations are summarized in the Table. While the 52 week patient variation is lowest, even this variation is almost twice that of the IFCC normal subject-derived variation. There is not much difference between the fasting and random sampling variations. Almost uniformly if the consecutive inpatient samplings occur in the same 120 minutes interval, the variation is lower.

**Conclusion:** The IFCC's short, same time sampling studies of normal subjects ignore the frequent occurrences of nonidentical sampling times and subsequent diurnal variation, lipid-lowering therapy and the seasonal variation of lipids. Our larger long term variation estimates represent reality and can be used for reference change calculations in patients on statin therapy. While the lower 52 week variation might represent stable patients measured yearly, this nadir in variation represents minimal seasonal variation.

Test	Between-sampling	Between-sampling	52 w average	IFCC AM Fasting
	times>120 min	times<120 min		
Cholesterol, F	11.9%	11.2%	10.0%	5.3%
Cholesterol, R	11.2%	10.7%	10.3%	5.3%
HDL, F	13.0%	10.4%	10.1%	5.7%
HDL, R	11.6%	11.0%	11.0%	5.7%
LDL Cholesterol, F	19.0%	18.1%	15.6%	8.6%
LDL Cholesterol, R	18.8%	18.3%	16.9%	8.6%
NonHDL, F	17.5%	15.5%	14.3%	5.7%
NonHDL, R	15.9%	14.5%	14.3%	5.7%
Cholesterol/HDL, F	15.7%	12.7%	12.6%	??%
Cholesterol/HDL, R	14.9%	13.5%	13.4%	??%
<b>Average between week variation (up to 96 weeks)</b>			F=Fasting, R=Random	

**A-246**

**Comparison of clinical characteristics between two pediatric groups: confirmed Anti-NMDA receptor mediated autoimmune encephalitis patients versus clinically suspected but Anti-NMDAR negative**

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**Introduction:** The N-methyl-D-aspartate receptor (NMDAR), is a receptor of glutamate manifested in neurons directly. The typical clinical phenotype of anti-N-methyl-D-aspartate receptor (NMDAR) antibody mediated encephalitis has typically been described in adults. The hallmark are neuropsychiatric presentations. Patients also experience seizures, status epilepticus and autonomic instabilities which could even be life threatening. The paediatric clinical phenotype is evolving. It has many similarities with adult population but also some crucial differences.

**Objective:** Since NMDAR mediated antibodies testing is available in CSF and serum by Immunofluorescence microscopy, a good characterization is essential to aid clinicians to request the test. The objective of the study is to come up with a clinical phenotype to aid clinicians suspect anti-NMDAR antibody mediated encephalitis especially for paediatric patients.

**Methods:** In this study, we selected patients <18 years of age for whom anti-NMDAR antibody testing was requested in serum or CSF. We categorized the cohort in 2 groups: Group I (positive patients) and Group II (negative patients) and compared their clinical characteristics.

**Results:** Group I included 07 patients (03 males and 04 females), with mean age of 9.6 years. Group II patients included 43 patients. The typical clinical characteristics of group I included 02 patients with focal neurological deficits, 04 patients with seizures. All of them gradually developed neuropsychiatric symptoms. 06 of 07 patients showed dyskinesia. No patients displayed any autonomic instabilities during the hospital stay. None of the positive patient uncovered any underlying tumor pathology. PET-CT of almost all the patients depicted hypometabolism or hypermetabolism in brain consistent with encephalitis. MRI interpretation revealed no intracranial abnormality except for 02 patients who showed small focal cortical signal and some neuronal loss. Only 01 patient depicted corticogyril swelling in right basifrontal region. EEG reports of 02 patients showed mild diffuse encephalopathy. No evidence of subclinical seizures was found in rest 05 patients. Other clinical points noted were vitamin D deficiency, non-specific thyroid abnormality, etc. but were not clinically related to NMDA. Group II patients eventually were diagnosed with various epilepsy

syndromes or unclassified, possibly autoimmune encephalitis. Group II patients displayed little or null neuropsychiatric symptoms but had cognitive problems. Group II did not display dyskinesias.

**Conclusion:** In conclusion, the clinical features associated with anti-NMDAR antibody mediated encephalitis in pediatric population are seizures, neuropsychiatric symptoms, focal neurological deficits and dyskinesias in our cohort. None of our patients had any underlying neoplastic etiologies. Paediatric clinical phenotype shows some overlap with the adult phenotype. Unique feature in our small cohort were focal neurological deficits in 2 patients. Our cohort also reiterates that anti NMDAR antibody mediated encephalitis in paediatrics is much less likely to be paraneoplastic.

## A-247

### Evaluation of serum S100 protein as biomarker for evaluating progression and prognosis of Intra Cerebral Hematoma

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**Objective:** S100 is a calcium binding protein most abundant in neuronal tissue. This protein is expressed in glial and Schwann cells and has both intracellular and extracellular effects. Several studies have evaluated the use of S100 protein as a reliable biomarker for different types of strokes. In this study we have evaluated the serum S100 protein levels and GFAP levels to determine its role in assessment of hematoma progression and clinical outcomes in adult patients in western India.

**Methods:** In our study we determined serial serum S100 level in patient with intracerebral hematomas at time of admission and at the time of consecutive scans and correlated the trends with over all radiological and clinical outcomes. In addition to CT imaging, serum S100 level has been validated as alternate modality to modify treatment plan or for better prognosis. 40 patients with age more than 18 years with Intra cerebral haemorrhage (cases) along with 40 healthy individuals (controls) were studied.

**Results:** In our study, there was gender preponderance with male's developing ICH in a ratio of 3:1. In this study, we found a significant increase in serum levels of S100 protein in patients (cases) than in healthy populations (controls). Paired t-test was used to calculate the p value. The p value was found to be 0.0035 ( $p < 0.05$ ). The duration of symptoms was <6 hours in highest percentage of participants (81.6%), followed by 12.2% participants having duration of 6-12 hours and 6.1% having duration of >12 hours. There was a significant association of CT volume with location and S100 levels ( $p < 0.05$ ). Highest percentage of participants with cerebral cortex had volume >20 cc<sup>3</sup> followed by mid-brain and then thalamus and basal ganglia. None of the patients who had cerebellum affected had volume >20 cc<sup>3</sup>. CT scans were done at the time of admission (CT1), and consecutive next 2 days (CT2 and CT3) to assess the changes in the size of hematoma over the time. There was

no significant difference in CT 1 and CT 2 volume ( $p > 0.05$ ). However, CT volume decreased significantly from CT 2 to CT 3 ( $p < 0.05$ ). Trend analysis was performed for the levels of S100 protein in patients with ICH. A significant raise was observed in the S100 values with correspondence to CT scan findings. Highest value of S 100 was seen at the time of processing of 2<sup>nd</sup> sample.

**Conclusions:** S-100 protein appears to be a relevant biomarker for the assessment of the patients with the Intra Cerebral Hematoma. Hence, considering the cost and ease of performing the biochemical test, S100 can be used as a relevant test in assessing the progression of the ICH. S100 may be used in a pre-hospital setting to determine the presence of ICH. Increased levels of S100 will prompt for early treatment and intervention. Current study illustrates and defines the potential of S100 protein to be established as a serum biomarker for haemorrhagic stroke.

## A-248

### Does fatty acid content influence plasmalogen levels? Implications for the laboratory evaluation of peroxisomal disorders

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**Background:** Plasmalogens are glycerophospholipids characterized by a vinyl-ether bond with a fatty alcohol at the sn-1 position, typically C16:0 (palmityl), C18:0 (stearoyl) or C18:1 (oleoyl), a polyunsaturated fatty acid at the sn-2 position, most commonly docosahexaenoic acid (DHA) or arachidonic acid, and a polar head at the sn-3 position, ethanolamine in most tissues. Since the first two steps of plasmalogen

synthesis occur in peroxisomes, patients with defects in peroxisome biogenesis (PBD) exhibit markedly reduced plasmalogens. We developed a liquid-chromatography/tandem mass spectrometry (LC-MS/MS) method to quantify 18 intact ethanolamine plasmalogens (pl-PEs) in packed red blood cells (RBCs) to aid the diagnosis of PBDs. Unlike traditional methods that assess total C16:0 and C18:0 plasmalogens as ratios to the corresponding fatty acid, our method measured individual pl-PEs. Since pl-PEs are composed of fatty acids, fatty acid deficiency or supplementation could potentially alter plasmalogen levels and affect the results of clinical pl-PEs testing in patients with PBDs, who present with profoundly reduced DHA, or in infants receiving special formula. To address this concern, we correlated the levels of pl-PEs with the abundance of fatty acid in RBCs, which represents long-term nutritional intake.

**Methods:** RBCs were washed with normal saline and lysed by freezing. Plasmalogens were extracted in methanol containing internal standards. Eighteen species were quantified using a XEVO TQ-XS Mass Spectrometer in conjunction with Ultra-High Performance Liquid Chromatography (Waters). Fatty acids (FAs) were quantified by gas-chromatography/mass spectrometry as previously described. Plasmalogens and FAs were measured in RBCs from 155 normal controls and 94 patients referred for clinical FA testing to assess for fatty acid deficiency or monitor for nutritional therapies. Data was analyzed using Prism v.8.3.0 software (La Jolla, CA). The study was approved by the Institutional Review Board of the University of Utah.

**Results:** pl-PE species containing arachidonic acid, DHA and docosatetraenoic acid (DTA) were the most abundant, representing on average 48%, 15% and 23% of total plasmalogens, respectively. Species containing eicosapentaenoic acid (EPA) or linoleic acid were present only in small amounts. Species containing arachidonic acid displayed the lowest inter-individual variability (<20%) and no correlation to this fatty acid. In contrast, other pl-PE species correlate with the corresponding RBC fatty acid. Particularly, EPA pl-PE strongly correlated with RBC EPA levels ( $r_s = 0.761$ ,  $p < 0.0001$ ) and increased with supplementation to represent up to 23% of total plasmalogen (range in controls: 0.2 to 6.5%). However, total amount of plasmalogens did not correlate to the total amount of fatty acids or with any individual fatty acid, both in control and clinical samples, including those with essential fatty acid deficiency or receiving supplementation.

**Conclusion:** Although fatty acid levels may impact the abundance of some individual plasmalogen species, total plasmalogen levels are not significantly affected by fatty acid deficiency or supplementation. Therefore, the quantitation of plasmalogens in RBCs by LC-MS/MS would aid in diagnosis of PBDs.

## A-249

### Preliminary Investigation into the Prevalence of G6PD Deficiency in a Pediatric African American Population using a Near-Patient Diagnostic Platform

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**Background:** Glucose-6-phosphate dehydrogenase deficiency (G6PDD) is an X-linked disorder and the most common enzyme deficiency in humans, with a high prevalence in persons of African, Asian, and Mediterranean descent. Neonates with undetected G6PDD are at risk for extreme hyperbilirubinemia. We previously developed a low blood volume, quantitative test for G6PD on a digital microfluidic (DMF) platform to rapidly analyze whole blood samples near the patient in a clinical setting.

**Methods:** We performed a retrospective study of residual, de-identified whole blood specimens collected at the Children's Healthcare of Atlanta-Egleston Campus to study the prevalence of G6PD deficiency in the African-American population. Residual and de-identified whole blood specimens (n=89) were obtained from distinct pediatric African American patients under an IRB approved protocol. Samples were stripped of patient identifiers prior to testing on the DMF platform. Only race, ethnicity, liver enzyme test results when available (obtained by standard laboratory methods), date of collection, age, and gender information was collected specific to this study. Specimens were selected randomly from leftover samples. Samples were analyzed on the DMF G6PD platform within 48 hours of sample collection at CHO to obtain G6PD values (U/gHb). **Results:** A total of 8 G6PD deficient samples (5 male and 3 female) were identified from 89 patient samples (Table). The age range of all subjects was 17 days old to 20 years of age, and the G6PD values ranged from 0.9 to 19.5 U/gHb. The incidence of G6PDD in this pediatric African American cohort was 8.9% (11.9% male and 6.3% female). **Conclusions:** The near-patient DMF G6PD assay was used to estimate the incidence of G6PDD deficiency in a pediatric African American cohort. A future study is planned to correlate whether G6PD deficiency in this population is associated with abnormal liver function tests.

**Table.** Mean G6PD values and frequency of deficient samples in the overall sample population and differentiated between male and female subjects

	Overall	Male	Female
Number of Samples	89	42	47
Age Range	17 days old to 20 years of age	4 months old to 17 years of age	17 days old to 20 years of age
Mean G6PD Value (U/gHb)	10.05	9.70	10.38
Range of G6PD Values (U/gHb)	0.9 to 19.5	0.9 to 18.6	1.2 to 19.5
Deficient Samples (n)	8	5	3
Deficiency Frequency	8.9%	11.9%	6.3%

**A-250**

**Comparison of an Automated Multi-Analyte System with the Manual ELISA Method for Celiac Disease Antibody Detection and Evaluation of the Updated ESPGHAN Guidelines**

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**Background:** Celiac disease (CD) screening is primarily based on the detection of anti-tissue transglutaminase (tTg) IgA. Since IgA deficiency is common in CD patients, anti-tTg IgG testing should be considered in IgA-deficient individuals. The latest guidelines from the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) advocate for biopsy-free CD diagnoses in patients with greater than or equal to 10 times the upper limit of normal ( $\geq 10 \times$  ULN) of anti-tTg IgA levels. Assays to detect anti-tTg antibodies are commonly available as single tests in clinical laboratories and are offered in a reflex format. Aptiva®, an automated system that uses the particle-based multi-analyte technology, received FDA clearance recently for CD testing. It enables a one-step detection of CD antibodies and exhibits high throughput capabilities. The aims of this study are 1) to evaluate the diagnostic characteristics of anti-tTg IgA and IgG assays and 2) to assess the clinical performance of anti-tTg IgA assay at  $\geq 10 \times$  ULN in the diagnosis of CD using the novel multi-analyte system as compared to the manual enzyme linked immunoassay (ELISA) method. **Methods:** Residual sera from 322 and 242 subjects with tTg IgA and tTg IgG tests, respectively, obtained using the QUANTA Lite® R h-tTg ELISA (Inova Diagnostics, San Diego, US) method were available and tested on the Aptiva® Celiac Disease IgA and IgG reagents (Inova Diagnostics, San Diego, US). Positive percent agreement (PPA) and negative percent agreement (NPA) were determined between the two methods. Of the total, 66 subjects were identified with clinically-defined CD through biopsy and 70 of them were classified as ‘negatives’ by chart-review. At the manufacturer-established cut-points and at anti-tTg IgA levels  $\geq 10 \times$  ULN, the following clinical performance characteristics were calculated: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). **Results:** Anti-tTg IgA showed an overall qualitative agreement of 93% (PPA:92% NPA:97%) and anti-tTg IgG displayed an agreement of 64% (PPA:43%, NPA:98%) between the two methods. Anti-tTg IgA showed high clinical sensitivity (95%, 91%) and specificity (93%, 95%) on Aptiva® (PPV: 92% NPV: 96%) and QUANTA Lite® (PPV: 94% NPV: 92%), respectively. For anti-tTg IgG sensitivities and specificities were 64%/71% and 97%/88% for Aptiva® (PPV:95% NPV:76%) and for QUANTA Lite® (PPV:84% NPV:78%), respectively. At the anti-tTg IgA levels  $\geq 10 \times$  ULN, clinical sensitivity and specificity was 71%/100% on Aptiva® (PPV:100% NPV:56%) and 58%/100% on QUANTA Lite® (PPV:100% NPV:48%), respectively. **Conclusion:** Anti-tTg IgG displayed an overall lower agreement between the two methods with a high specificity on Aptiva® but a slightly better sensitivity on QUANTA Lite®. Anti-tTg IgA performed comparably and exhibited an excellent accuracy for CD diagnosis using both methods. At the anti-tTg IgA levels  $\geq 10 \times$  ULN, Aptiva® displayed superior performance compared to QUANTA Lite®. These data support the use of the multi-analyte automated assay as a viable alternative for anti-tTg detection. Awareness to method specific cut-off is recommended while considering a biopsy-free approach for CD diagnosis.

**A-251**

**Comparison of Random Urine Protein/Creatinine Ratio with 24-hr Urine Protein in Suspected Pre-eclampsia**

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**Introduction:** Preeclampsia is characterized by new-onset hypertension after 20 weeks gestation with one or more features including proteinuria, maternal organ dysfunction, or fetal growth restriction. The gold standard for the assessment of proteinuria is 24-hour urine collection, however, this method is time-consuming and sometimes inaccurate due to incomplete collection. The American College of Obstetricians defines proteinuria as the excretion of  $\geq 300$  mg of protein per day or alternatively, a urine protein/creatinine ratio (PCR) of  $\geq 0.3$  mg/mg. There are mixed conclusions on the clinical utility of the protein/creatinine ratio in pre-eclamptic patients. Therefore, the goal of our study was to evaluate the correlation between urine PCR and proteinuria in a 24-h urine collection in our patient population. **Methods:** A total of 51 women with suspected pre-eclampsia from the maternal-fetal clinic of our institution were retrospectively studied, to compare the concordance of random urine PCR with 24-h urine protein. Results of 24-h urine protein and random urine PCR were obtained from patients’ medical records. The correlation between the urine PCR in random urine samples and protein excretion in the 24-h urine collection was determined by Deming Regression analysis and Pearson correlation on EP evaluator and SPSS respectively. **Results:** The median maternal age of the studied cohort was 30.8 (IQR, 26 -36) years, and the median body mass was 35.1 (IQR, 31-39.7) Kg/m<sup>2</sup>. Median gestational age at evaluation 31.8 (IQR, 29-34) weeks. Thirty-six patients (70.6%) had proteinuria by 24-h urine protein  $\geq 300$ mg/24hr. There was a significant positive correlation between the numerical values obtained by 24-h urine protein and the protein/creatinine ratio (R=0.88, P<0.001). Concordance analysis showed 81.1% positive agreement for proteinuria between methods ( $> 300$  mg/24hr and  $> 0.3$  mg/mg) and 71.4% negative agreement. Results were not concordant for 11 (21%) of patients, with 7 (13.7%) being positive for proteinuria by 24-hour urine but not PCR, and 4 (7.8%) being positive by PCR but not 24-hour urine. **Conclusion:** Overall, urine protein/creatinine ratio was well correlated with 24-hr urine protein and could be an effective and complaint screening tool to indicate proteinuria in pre-eclamptic patients.

**A-252**

**Investigation of thyroid testing in pregnant women at a large safety net hospital**

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**Background:** The choosing wisely campaign recommended ordering only thyroid stimulating hormone (TSH) for initial screening of thyroid function in the general population based on the perceived inverse log-linear relation between TSH and free thyroxine (fT4). It was recently reported that TSH and fT4 follow a negative sigmoidal relationship depending on age and gender. In pregnancy the presence of thyroid peroxidase antibodies (TPOAb) may further complicate this relationship. Therefore, in clinical practice, more thyroid tests than a single TSH are ordered for screening in pregnancy. Here, we sought to investigate if TSH alone is sufficient to identify thyroid dysfunction as the first line of screening test in pregnancy in a large safety-net academic medical center.

**Methods:** First thyroid test results, including fT4, fT3, TSH, and TPOAb of pregnant women in their first trimester without a prior diagnosis of thyroid abnormality in a year’s duration were retrieved. TSH was measured using electrochemiluminescence immunoassay (Roche COBAS 6000). Competitive chemiluminescence immunoassay was employed to analyze fT4, fT3 and TPOAb on the COBAS 8000 (Roche Diagnostics, IN, USA). Statistical analyses were performed using Excel and Graph Pad prism.

**Results and Discussion:** In all, 174 thyroid tests were co-ordered as initial tests, comprising 75-fT3, 153-TSH, 8-TPOAb with fT4 always on the order (174). Thirty-four percent had normal TSH and at normal fT4, fT3 and TPOAb if ordered, suggestive of normal thyroid function. None of the patients had normal TSH but abnormal fT4 and fT3, strongly buttressing the independent utility of TSH as a single initial screening test for thyroid dysfunction. Seven percent of patients had low TSH and high fT4 while 0.5% had high TSH and high fT4 indicating primary and secondary hyperthyroidism respectively. Nearly 3% of the pregnant women had high TSH and low fT4 whereas 0.51% recorded low TSH and low fT4, an indication of primary and secondary hypothyroidism correspondingly. Results showing normal fT4 and fT3 in the background of low TSH, as well as those showing normal T4 and T3 in the setting of high TSH made up 8.7% and 15% of all abnormal thyroid test results accordingly,

expressive of subclinical hyperthyroidism and subclinical hypothyroidism. Six percent of the initial thyroid tests ordered was for only fT4 without either TSH or fT3, and 12% excluded TSH.

**Conclusion:** Our data underpins the significance of TSH as the first test for thyroid investigation not only in the general population, but also in pregnancy. Ordering multiple tests in addition to TSH is redundant for the initial assessment of thyroid disorders. The future direction of this study is to analyze the pattern of relationship between TSH and fT4 in pregnancy to support the usage of TSH only for thyroid dysfunction screening in pregnancy.

## A-253

### Glycated Albumin During Pregnancy: Preliminary Reference Intervals for a Midwestern U.S. Population and Usefulness as a Predictor of Adverse Neonatal Events

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**Background:** Glycemic control is important for pregnant women with diabetes mellitus to decrease risk of an adverse neonatal event. However, for many subjects in our clinics, compliance with multiple, daily fingersticks is low. Glycated albumin (%GA) has become more widely available in the U.S. and is a useful marker of intermediate-term glucose control; thus, it may be useful in these subjects. A few reference interval studies in pregnant women have been reported, but these are outside the U.S. and have not included African-Americans or a high percentage of overweight or obese women. We attempted to establish a preliminary reference interval for our population and examine the ability of %GA to predict adverse neonatal events (ANE) in a prospectively recruited cohort of women with gestational diabetes (GDM) or type 2 diabetes (T2D).

**Methods:** Subjects were recruited from March 2019 to November 2020 under IRB approval at Washington University in St. Louis, MO. Those completing the study with a live birth and a serum sample included 15 with T2D and 21 with GDM. For those with GDM, the median BMI was 30 kg/m<sup>2</sup> and for those with T2D, 37.4 kg/m<sup>2</sup>. We obtained 28 healthy controls from our university's biobank, the Women and Infants Health Specimen Consortium, matched to our prospectively collected cohort for age, ethnicity, BMI, and gestational age. These were used to establish a preliminary reference interval using a percentile bootstrap interval with  $\alpha = 0.05$ . Percent glycated albumin was calculated from measurements of glycated serum proteins (GSP) and albumin using the GlycoGap assay (Diazyme) with the Roche albumin assay (bromocresol green) according to Diazyme's published equation. Nonparametric statistics were used for data analysis. Receiver operator curves (ROC) were constructed to assess the usefulness of %GA to predict an ANE in the prospective cohort.

**Results:** Reference intervals obtained in this manner were much narrower than those previously published. The median %GA and 95% confidence interval were 10.85 (10.5, 11.7) for trimester 2 and 10.10 (9.4, 10.7) for trimester 3. Of the 64 live births, 13 (31%) experienced an adverse neonatal event (ANE), with the most common event being macrosomia (9), followed by hypoglycemia (5), respiratory distress (5), and hyperbilirubinemia (1). A few births exhibited multiple ANE. Measurements of %GA during trimester 2 were excellent at predicting the occurrence of an ANE (ROC AUC = 0.948). However, trimester 3 measurements were not as useful (AUC = 0.762). There was a correlation between %GA and birthweight in both trimester 2 ( $\rho=0.346$ ,  $p=.014$ ) and trimester 3 ( $\rho=0.403$ ,  $p=.003$ ).

**Conclusion:** To our knowledge, this is the first study reporting preliminary %GA reference intervals in a population of U.S. pregnant women with elevated BMI. %GA in both trimesters 2 and 3 shows a weak to moderate correlation with birthweight. However, %GA measured during trimester 2 is much better at predicting risk of an ANE. We wish to acknowledge Jyoti Arora, Washington Univ., for statistical analysis and funding from Washington University Diabetes Research Center's Pilot & Feasibility Grant Program (NIH P30 DK020579).

## A-254

### Ursodeoxycholic acid treatment in intrahepatic cholestasis of pregnancy: a case for monitoring

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**Background:** Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific hepatic disease with an incidence of 0.32-5.6% in the US. ICP is characterized by pruritus and elevated levels of serum bile acids (BAs). High maternal serum BAs

are associated with adverse perinatal outcomes, including preterm delivery and still-birth. Ursodeoxycholic acid (UDCA), a secondary bile acid normally present only in trace amounts, has been shown to have an anticholestatic effect by stimulating primary bile acids excretion. It is widely used in ICP and considered safe. The goal of this study was to evaluate changes in bile acid concentrations after UDCA treatment in women with ICP. **Methods:** Retrospective data analysis was performed on de-identified patient results from bile acid testing, which reports total (conjugated/unconjugated) concentrations of cholic (CA), chenodeoxycholic (CDC), deoxycholic (DCA), ursodeoxycholic (UDCA) acids, and total BAs. A total of 119 patients (age 31±6 yo) with high levels of total BAs (>7.0 μmol/L) and diagnostic codes indicating liver/biliary tract disorders in pregnancy were included in the study. All patients had at least one bile acid measurement before and after UDCA treatment. **Results:** The mean pre-treatment serum concentrations of total BAs, CA, CDC and DCA were 27.7 μmol/L (normal ≤7.0 μmol/L), 15.4 μmol/L (normal ≤1.9 μmol/L), 8.2 μmol/L (normal ≤3.4 μmol/L) and 3.8 μmol/L (normal ≤2.5 μmol/L), respectively, and only traces of UDCA were detected (mean 0.3 μmol/L; normal ≤1.0 μmol/L). Samples collected after treatment had on average 28-fold higher UDCA (mean 8.9 μmol/L). In 71% patients, UDCA treatment reduced CA, CDC and DCA levels, and the effect was consistent among multiple measurements. Unexpectedly, 17% of all patients had one or more measurements where CA, CDC and/or DCA values on treatment were >10% higher than their pre-treatment levels. The severity of cholestasis or the timing of follow-up measurements did not explain the differences in responses to UDCA. However, changes in BAs correlated with serum UDCA concentrations ( $p<0.0001$  for CA and CDC;  $p=0.014$  for DCA). To illustrate, samples with UDCA <5 μmol/L showed -70%, -63% and -35% decrease in CA, CDC and DCA on average compared to the pre-treatment levels, respectively. In samples with 5-10 μmol/L and 10-20 μmol/L UDCA the changes were less pronounced: -57%, -43% and -8% and -37%, -18% and +5% on average, respectively. The differences in BA concentrations between these UDCA concentration groups were also statistically significant. Finally, in samples with UDCA >20 μmol/L CA, CDC and DCA concentrations were 179%, 150% and 28% higher on average than pre-treatment and 692%, 1394% and 289% higher on average than in post-treatment samples with UDCA <5 μmol/L suggesting a potential toxicity of high UDCA on biliary function. **Conclusions:** UDCA treatment was associated with reduced CA, CDC and DCA levels in most patients with ICP. However, the beneficial effect was less pronounced or even reversed with higher serum concentrations of UDCA. Primary and secondary bile acids should be closely monitored during the treatment. Clinical studies are necessary to define optimal serum levels of UDCA to alleviate clinical symptoms and improve biochemical parameters.

## A-255

### Pediatric Reference Limits for 10 Commonly Measured Autoimmune Disease Markers

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**Background:** The objective of this study was to establish pediatric reference limits for autoimmune disease markers in the CALIPER cohort of healthy children and adolescents to support their interpretation. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) is a national study of healthy children aiming to close gaps in pediatric laboratory medicine by establishing a robust database of pediatric reference intervals for pediatric disease biomarkers (caliperdatabase.org). **Methods:** Healthy children and adolescents (n=123), aged 1-19 were recruited as part of the CALIPER study with informed consent. Serum autoantibody testing was conducted using chemiluminescent immunoassays on the BIO-FLASH automated analyzer (Biokit, Barcelona, Spain) including anti-dsDNA IgG, anti-Sm IgG, anti-RNP IgG, anti-SSB/La IgG, anti-Ro60 IgG, anti-Ro52 IgG, anti-cardiolipin IgG, anti-MPO IgG, anti-PR3 IgG, and anti-tTG IgA. Pediatric reference limits representing the 95<sup>th</sup>, 97.5<sup>th</sup>, and 99<sup>th</sup> percentiles were calculated using the non-parametric rank method according to Clinical Laboratory Standards Institute C28-A3 guidelines. **Results:** The proportion of samples with results above the assay reportable range were: anti-dsDNA (26/119; 22%), anti-Sm (16/121; 13%), anti-RNP (1/120; 0.8%), anti-SSB/La (0/120; 0%), anti-Ro60 (0/122, 0%), anti-Ro52 (0/121, 0%), anti-cardiolipin IgG (105/117, 90%), anti-MPO (29/118, 25%), anti-PR3 (11/119, 9%), and anti-tTG IgA (34/120, 28%). Pediatric reference limits and associated 90% confidence intervals were established for all 10 markers and found to be below the manufacturer's assay cut-offs. **Conclusions:** Robust pediatric reference limits for 10 commonly clinically utilized autoimmune markers established herein will allow for improved laboratory assessment of pediatric patients using this assay platform worldwide.

**Table 1: Pediatric Reference Limits for 10 Commonly Measured Autoimmune Disease Markers**

Analyte	95th percentile (90% CI)	95th percentile (90% CI)	95th percentile (90% CI)
Anti-Cardiolipin IgG	12.2 (10.2, 14.6)	14.3 (12.6, 16.9)	15.9 (15.8, 18.0)
Anti-dsDNA IgG	15.5 (14.5, 17.7)	16.5 (14.1, 18.9)	18.6 (18.4, 21.2)
Anti-SSB IgG	3.3 (3.3, 4.1)	3.3 (3.3, 4.1)	3.9 (3.3, 4.1)
Anti-RNP IgG	3.5 (3.5, 4.2)	3.5 (3.5, 4.2)	4.1 (3.5, 4.2)
Anti-Ro52 IgG	2.3 (N/A)	2.3 (N/A)	2.3 (N/A)
Anti-Ro60 IgG	4.9 (N/A)	4.9 (N/A)	4.9 (N/A)
Anti-Sm IgG	7.6 (7.2, 17.3)	14.3 (7.2, 17.3)	16.9 (7.2, 17.3)
Anti-tTG IgA	5.2 (4.8, 10.4)	7.5 (4.8, 10.4)	9.9 (4.8, 10.4)
Anti-MPO IgG	7.8 (6.3, 10.2)	9.2 (5.9, 11.2)	12.3 (12.0, 15.5)
Anti-PR3 IgG	3.1 (0, 3.8)	8.8 (5.7, 14.8)	11.8 (11.7, 15.8)

N/A: unable to estimate, majority of results equal to the lower detection limit of the assay

**A-256**

**The presence of anosmia and dysgeusia was associated with the perception of weight loss in patients with COVID-19, but not the worst prognosis**

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Background: COVID-19 is an infectious disease, which ranges from asymptomatic to critical cases, and can lead to death. Among the symptoms reported, the loss of smell (anosmia) and taste disorder (dysgeusia) have attracted attention for its relevance for screening the disease. Since these symptoms affect appetite and the desire to eat, they may pose a risk to nutritional status, contributing to weight loss, losses in the supply of nutrients and even the fragility of their immunity. Therefore, the study aimed to evaluate the association between the presence of anosmia and dysgeusia and the perception of weight loss of individuals diagnosed with COVID-19. Methods: A cross-sectional study was conducted between May and October 2020. The study included 770 individuals (>18 years) diagnosed with COVID-19. Clinical data were collected by in person interviews or by telephone contact, and by access to the medical records of hospitalized patients. Participants were allocated into two groups according to the presence (n= 393) or absence (n= 377) of dysgeusia and anosmia symptoms. Data were transferred to an electronic entry tool, Research Electronic Data Capture (REDCap, Vanderbilt University), and analyzed by Statistical Package for the Social Sciences (IBM SPSS Statistics 26). The Kolmogorov-Smirnov test was used to determine the normality of the variables. For descriptive analysis, data were represented by median (interquartile range - IQR; 25th-75th percentile) or percentage (number of patients). The Chi-square and Mann-Whitney tests were applied, followed by the univariate logistic regression test. P values <0.05 were considered statistically significant. This research was approved by the Ethics and Research Committee of the Federal University of Rio Grande do Norte (UFRN) - Brazil, and a consent form was obtained from each volunteer. Results: Among the participants who presented symptoms, there was a higher frequency of women and a younger age than those without symptoms. The presence of anosmia and dysgeusia was also associated with lower disease severity and lower incidence of hospitalization, as 71.7% developed mild disease and only 21.6% of participants with symptoms were hospitalized. Individuals who had the symptoms of dysgeusia and anosmia reported greater perceived weight loss compared to those who did not have the symptoms. Univariate logistic regression analysis showed a 5.4 greater odds of experiencing perceived weight loss among subjects with dysgeusia and anosmia compared with those without symptoms (OR= 5.4; 95% CI= 3.9 - 7.60; p-value: 0.00). Conclusions: The survey showed that the presence of anosmia and dysgeusia were more prevalent in younger, female, and individuals with mild symptoms, and appear to be associated with higher odds for perceived weight loss in COVID-19 patients. Otherwise, these symptoms were not associated with worse prognoses.

**A-257**

**Performance of the InteliSwab® COVID-19 Rapid Test in Pediatric Population 2-14 Years of Age**

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**Background:** Rapid COVID-19 antigen tests are a valuable tool for diagnosis of COVID-19 both in healthcare and non-healthcare settings, particularly in children where the rate of vaccination is still low.

**Methods:** The usability and performance of InteliSwab® in children aged 2-14 years was established in a cohort of 19 eligible symptomatic children paired with parents who signed the informed consent/assent forms and were concurrently enrolled across four testing sites. The parent (operator) collected the nasal sample from their child subject, performed the test, and read the test results unassisted as though in a home-setting using only the information available within the test kit Instructions for Use (IFU). An additional nasal sample for PCR reference method testing was collected by study staff. Performance of each step/task described in the IFU was observed and all use task failures were documented. Study participants provided feedback regarding ease of use and satisfaction with the test via a written user questionnaire. To demonstrate safety and ease of use of InteliSwab® in the targeted pediatric population, a separate human factor study (HFS) was performed with an additional thirty (30) eligible healthy children/parent pairs. Study moderators made note of the execution of each study task by the test operators. A validated scale [Face, Legs, Activity, Cry, Consolability (FLACC) scale] was used to assess the children’s tolerability of the swabbing procedure.

**Results:** When compared with results of FDA Emergency Use Authorized PCR tests, InteliSwab® had a positive percent agreement (PPA) of 90% (95% CI: 60%-98%), a negative percent agreement (NPA) of 100% (95% CI: 70%-100%) and accuracy of 95% (18/19) (95% CI: 75%-99%). There was only one false negative result in a specimen with a low viral load (Ct results >29). The overall success rate for all test procedure tasks (N=304) was 97% (295/304). The majority of respondents rated the overall impression with InteliSwab® as satisfactory/favorable (100%) and agreed/strongly agreed that it was easy to use (>97%).

During the HSF study, observers noted that 98% (176/180) of the 6 critical sub-tasks were performed correctly. Observations using the FLACC scale identified high level of tolerability during the swabbing. Over 80% of child subjects were observed to have had a relaxed and comfortable experience or only mild discomfort. Three child subjects demonstrated moderate pain; 1 child subject demonstrated severe discomfort. All discomfort/pain resolved within 1-2 minutes.

**Conclusion:** The data presented represent one of the largest cohorts of pediatric population tested with a rapid COVID-19 antigen test both for performance and ease of use. The OraSure InteliSwab® COVID-19 Rapid Test is an accurate, safe, and easy to use test for COVID-19 diagnosis by untrained and unproctored lay users in children aged 2-14 years in non-healthcare settings, such as home, school or daycare.

**A-261**

**Very early dyslipidemia in congenital generalized lipodystrophy**

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**Background:** Congenital generalized lipodystrophy (CGL) is a rare disease that affects 1: 10,000,000 live births. This condition is inherited in an autosomal recessive pattern. These individuals have total or near total absence of subcutaneous tissue since birth. This condition is associated to severe insulin resistance and, in general, these patients develop early metabolic disorders such as diabetes and hypertriglyceridemia, at risk for episodes of pancreatitis. Others clinical manifestations are hepatic steatosis and hepatic cirrhosis. The aim of this study was to describe when the first lipid abnormalities appear in CGL patients. **Methods:** We studied 16 CGL patients in follow-up at a referral center for lipodystrophy in the state of Ceará-Brazil. A medical record review was carried out to collect information related to the clinical history of patients and the survey of lipid profile dosages. Data were analyzed in STATA 11.2 program. Results were expressed as median (min and max).

**Results:** 56% male patients; CGL family history was present in 21.43% and inbreeding in the family 64.29%. Regarding comorbidities, all individuals had hypertriglyceridemia and low HDL-cholesterol; 7 (46.7%) had DM and 7 (46.7%) had hypercholesterolemia. Hypertriglyceridemia and low HDL-cholesterol appeared early in the course of the disease, with the median age at diagnosis of hypertriglyceridemia at 11.5

months of age (1-54) and those with low HDL-c at 8 months of age (1-24), with the following laboratory findings values: HDL-c levels of 23 mg / dL (16 - 31), TG levels of 456,5 mg / dL (141 - 1914). Among those with hypercholesterolemia, the median age at diagnosis was 18 months of age (12-28), with levels of 188 mg / dL (175 - 253). **Conclusion:** The patients with CGL have important metabolic disturbances since childhood, with multiple cardiovascular risk factors. Thus, it is necessary an early therapeutic intervention in this population, in particular dietary interventions in early childhood, to minimize the complications inherent to these condition.

## A-262

### Point of care testing with a viscoelastic assay at different timepoints in perioperative haemostasis management of orthotopic liver transplant from donors after cardiac death

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**BACKGROUND:** Viscoelastic testing has been suggested to help manage coagulopathy in orthotopic liver transplant (OLT). The liver transplantation surgery with a thromboelastometry algorithm could reduce blood loss and transfusion volume, improve patient outcomes and reduce cost. However, results from different viscoelastic devices (ROTEM, ClotPro) are not readily comparable and more studies are needed to establish cut-off values in special patient populations. Moreover, there has been a significant effort to increase the existing deceased donor pool. This effort has resulted in a greater use of liver allografts following donation after cardiac death that are still challenging. The aim of the present retrospective analysis was to compare test results of a POCT assay (ClotPro) with a tailored transfusion algorithm in the perioperative period at three different timepoints between a group of OLT recipients with donation after brain death (DBD) and another group of OLT recipients with donation after cardiac death (DCD). **METHODS:** Analysis was conducted for one year (2021). Patient characteristics (age, sex, MELD score), retransplant rate and death causes were extracted into a spreadsheet. Informed consent was obtained for all patients. We conducted a comparative analysis on two separate patient groups: DBD and DCD in ClotPro® (Enicor GmbH, Munich, Germany), a thromboelastometry analyzer. We measured several parameters used in clinical practice: EX test (CT, A5, A10, MCF) and FIB test (A5, A10, MCF). Blood samples were tested immediately after collection in the operation room and results placed into it at three different timepoints (dissection/pre-anhepatic phase, anhepatic phase and reperfusion). The transfusion management was decided according to preestablished guidelines and cut-off values. Transfusion events were considered in terms of transfusion rates: red blood cells (RBCs) fresh frozen plasma (FFP), platelets (PLTs) and fibrinogen concentrates. A descriptive analysis was carried out using qualitative variables, represented in the tables as absolute frequencies and percentages. Quantitative variables were expressed using mean and standard deviation (SD). Differences between groups were tested using the Mann-Whitney U-test, the Pearson's  $\chi^2$  -test or Fisher's exact test, as appropriate. All statistical analyses were conducted using software with the Statistical Packaged for the Social Sciences (SPSS version 19.0, Chicago, IL, USA). **RESULTS:** 66 patients undergoing OLT was included. Patient baseline characteristics and transfusion events in terms of transfusion rates (RBC, FFP and platelet count transfused) and fibrinogen concentrates used (grams) in perioperative coagulation management showed no significant differences. Viscoelastic parameters indicated the presence of a normal hemostasia in most patients. However, we found high median results of coagulation time (CT) and low median clot strength parameters (A5, A10 and MCF) of EX test in DBD group at reperfusion phase. **CONCLUSION:** We found no significant differences in the two groups. The small differences found at reperfusion phase are not important, we cannot conclude differences in the management of haemostasis between the two groups of liver

transplant recipients. We hypothesize that other variables like extraction technique, methods used to preserve the allograft and later complications have a significantly greater impact than perioperative hemostasis management in retransplant rates and death.

## A-263

### Should we stop performing OGTT in patients with a history of bariatric surgery? - An analysis of side effects and usefulness of the test

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**Background:** Bariatric surgery is considered a successful way to achieve and maintain weight loss. The screening for diabetes mellitus (DM) with an oral glucose tolerance test (OGTT) is challenging in patients post bariatric surgery, because it is usually not well tolerated with many side effects. Furthermore, the diagnosis of DM is difficult in this population due to the few studies showing which results would be the best indicators. The authors aimed to evaluate the percentage of hypoglycemia and other side effects (as early dumping) in patients with a history of bariatric surgery who were submitted to OGTT, and the relationship of these side effects with the type of bariatric surgery or if the patient was pregnant; as well as to assess the usefulness of this test for the diagnosis of DM in this population. **Methods** Observational study with analysis of Laboratory information system (LIS) database and Functional Test Monitoring Forms. We included adult patients with a history of bariatric surgery who were submitted to the OGTT with medical monitoring in one of the laboratories of the DASA in Rio de Janeiro between the years 2018 and 2021. Side effects evaluated: hypoglycemia (blood glucose <70mg/dl), early dumping (gastrointestinal symptoms in the first 30 minutes) and late dumping (hypoglycemia symptoms at 120 minutes). Diagnosis of DM by OGTT in the pregnant cohort: blood glucose at 60 minutes  $\geq$ 180 mg/dl or  $\geq$ 153 mg/dl at 120 minutes and in the non-pregnant cohort: blood glucose  $\geq$ 200 at 120 minutes. **Results** We evaluated 55 patients, 50 (91%) women, of whom 35 were pregnant (64%), aged 24 to 70 years. Overall, 30/55 (54.5%) had some side effect during the OGTT, of these 24/30 (80%) had hypoglycemia and of these 10/24 (42%) were symptomatic hypoglycemia also classified as late dumping. Early dumping was present in 13/55 (24%). Diagnosis of DM established by OGTT in only 7/55 (13%). There were no differences in the pregnant cohort. The comparative analysis of side effects depending on the type of bariatric surgery could only be performed on 22 patients that informed the type of surgery. Of these, 18/22 (82%) had undergone Y-Roux Gastric Bypass, 4/22 (18%) had Gastroplasty in "Sleeve. Early dumping occurred in 4/4 (100%) of Sleeve-patients, while only 6/18 (34%) of Y Roux-patients. On the other hand, hypoglycemia occurred in 6/18 (34%) of Y-Roux patients but only in 1/4 (25%) of Sleeve patients. **Conclusion** More than half of the patients with a history of bariatric surgery undergoing OGTT had side effects in the analyzed cohort, and the most frequent was hypoglycemia. The biggest concern in the Sleeve group of patients is the early dumping. Hypoglycemia is more frequent in the Y Roux group. On the other hand, only 13% had a diagnosis of diabetes established by the OGTT. We suggest that OGTT should not be performed in patients after bariatric surgery, since it induces many side effects and has a questionable usefulness for the diagnosis of diabetes in this population.

## A-264

### Assessment of Non-Invasive Prenatal Test Implementation in the Prenatal Screening Program

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**Background:** The appearance of Non-Invasive Prenatal Diagnosis (NIPD) is leading a change in prenatal aneuploidy screening programs strategy. It is currently included in the public health system population screening as a contingent model to the First Trimester Combined Screening (FTCS). If the FTCS is positive but the early morphological evaluation is normal, performing a NIPD and reversal of the invasive technique is proposed. Pregnancy is considered low risk if NIPD result is negative, while a positive result requires confirmation by invasive conventional karyotype. This strategy allows a significant reduction in the number of invasive techniques without reducing the final results quality. The aim of this study was to assess the NIPD implementation on a pregnant women population with high-risk screening in the Regional Screening Program for Congenital Anomalies.

**Methods:** Observational retrospective study, carried out from pregnant women included in the Prenatal Screening Program for Congenital Anomalies between January - December 2021. FTCS is performed in two steps: determination of free  $\beta$ -HCG and PAPP-A between 8 and 13+6 weeks (dated by date of last period) and ultrasound between 10+4 and 13+6 weeks to determine gestational age by measuring the crown-rump length (CRL) and nuchal translucency (NT). PAPP-A and free  $\beta$ -HCG levels were determined by chemiluminescence in a Cobas 6000-E 601 analyzer (Roche Di-

agnostics Spain). Measurement of CRL and NT was performed by sonographers certified according to the Fetal Medicine Foundation standards. Both the genetic diagnosis (invasive conventional karyotype) and DPNI were performed in external laboratories. The parameters analyzed were: Detection Rate (DR), False Positive Rate (FPR), number and results of invasive techniques and number of voluntary reversals. Second trimester biochemical screening was excluded. For the risk estimation, the siPACAC software was used. The cut-off point for invasive tests was 1/280.

**Results:** A total of 5,914 FTCS will be implemented in single pregnancies. 366 (6.1%) had a high risk estimate (>1/280). The overall DR for FTCS with NIPD was 86% and FPR for FTCS was 4.1%. A total of 289 true negatives NIPDs and 72 invasive tests (57 negative and 15 positive) were performed. Five pregnant women did not continue with the established protocol for a positive FTCS.

**Conclusion:** This new strategy, with high sensitivity and specificity, has managed to significantly reduce the number of invasive techniques performed and problems derived from them. The NIPD is a screening test with a high demand by pregnant women, which is demonstrated in the reduction of reversals by more than 70%. Due to this, the number of pregnant women who leave the public health system after a positive FTCS, to undergo a NIPD as an alternative to an invasive technique, decreases.

**A-265**

**Prenatal disorder- Does race matter?**

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**Background:** Prenatal screening plays a pivotal role in identifying pregnancies at risk for serious disorders. This provides an opportunity to identify existing health risks in the maternal population that may include diabetes, abnormal weight, effects of smoking and inadequate nutrition. Herein, our primary goal is to identify the key determinants that contribute to persistent disparities in maternal health further affecting the pregnancy outcome. We will also discuss the need to eliminate racial and ethnic factors that still exist in the screening of the maternal population. **Methods:** At our institution, we performed a 5-year statistical analysis of second-trimester prenatal screens. Firstly, we identified several unique disorders in down syndrome, trisomy 18, and open neural tube defect. Furthermore, we performed a breakdown analysis of the variables that might have affected the fetus. **Results:** A total of 8,849 women were screened during a 5-year period. The race demographic in the population were African Americans (41%) and non-African Americans (59%). The below table breaks down the data with respect to disorders and variables: diabetes, abnormal weight, smoking status. **Conclusion:** The variance of positivity rate between African American and Non-African American populations is less than 1%. Therefore, it is important to eliminate racial and ethnic factors in prenatal screening. Our data shows that there is a significant percentage of patients who tested positive for Down Syndrome that have a previous history of diabetes. It is imperative to ensure that extensive research is performed on this anomaly.

Variables	Neural Tube Defect Disorder (%)	Trisomy 18 Disorder (%)	Down Syndrome Disorder (%)
African American Population (Positivity Rate)	4.78%	0.58%	1.11%
Positivity Rate - Non-African American Population	3.85%	0.29%	2.18%
Diabetic Status	4.01%	2.78%	17.24%
Smoking Status	2.14%	5.56%	3.45%
Overweight (>280 lbs) Status	5.08%	5.56%	9.20%

**A-272**

**Optimizing Cortisol After Glucagon Stimulus in Children: When and How Many Times Should We Collect Blood Samples to Accurately Exclude Hypocortisolism**

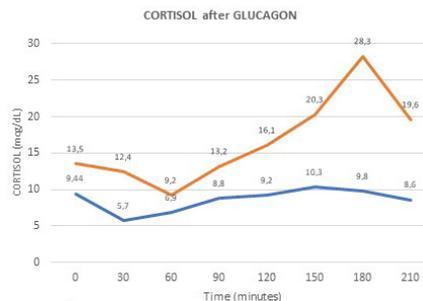
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**Background:**

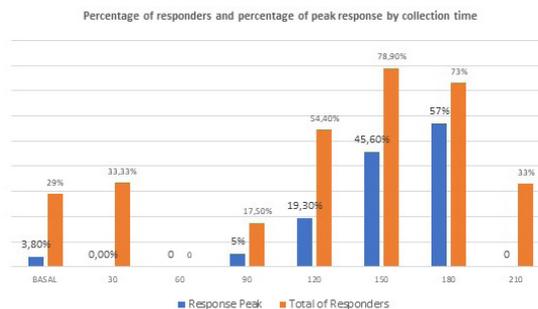
The insulin tolerance test is still considered the gold standard test in the investigation of short stature, allowing simultaneous access to the somatotrophic and corticotrophic axis. The administration of insulin represents, however, not an entirely risk-free procedure. In this context, the Glucagon stimulation test (GST) has been increasingly indicated. The greatest limitation of this test is its long duration and multiple specimen sampling. The aim of our study was to examine whether the GST could be performed with fewer samples without compromising its diagnostic value.

**Materials and Methods:** We retrospectively reviewed 107 children submitted to GST in the context of the simultaneous investigation of growth hormone deficiency and hypocortisolism. Glucagon was administered subcutaneously at a dose of 0.03 mg/kg. Blood samples for Cortisol levels were taken at 0, 30, 60, 90, 120, 150, 180, and 210 minutes after the stimulus. A test was considered responsive when Cortisol ≥ 16.3 mcg/dL.

**Results:** Fifty-seven (53%) patients showed a normal response to the stimulus. The mean age of our patients was 11 years (range 9 months to 16 years) and 56% were male. Median Cortisol values before and 30, 60, 90, 120, 150, 180, and 210 minutes after GST in both groups [responders (blue line)] and non-responders (red line) are shown in graphic 1. In graphic 2, we studied only patients who showed a positive response to the stimuli. In this graphic, the peak response to the stimulus and the sampling times patients presented a response above 16.4 are analyzed. **Conclusions:** Cortisol stimulation test with Glucagon can be optimized, without scarring sensitivity, by collecting only four specimens, at times 90, 120, 150, and, 180 minutes. Adopting this procedure, the punctured time is reduced to just one and a half hours instead of 3 hours, resulting in a much more comfortable test.



Graphic 1



Graphic 2

## Toxicology and Therapeutic Drug Monitoring

### A-273

#### Prevalence of concurrent detection of novel psychoactive substances and antipsychotics treatment.

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**Background:** Novel psychoactive substances (NPS) are synthetically manufactured designer drugs, often known as internet drugs or a more popular but misleading common term as legal highs. These are complex and diverse analogues of either the existing controlled substances and pharmaceutical products or newly synthesized chemicals, created to mimic the psychoactive effects of licensed medicines and other controlled substances. By their number, nature and composition, NPS pose significant challenges for clinicians in drug services and, more broadly for forensic toxicologists, healthcare systems and drug control policy globally. Hence these have been described as a 'growing worldwide epidemic'. The public health threat from their use stems from poor knowledge of their effects and to a larger extent their inadequate testing in the human population.

**Methods:** With this concern, we are providing LC-MS/MS testing of more than 160 Novel psychoactive substances in urine and oral fluid samples. Our aim is to alert the clinicians for the use of these compounds in order to assist them in accurate assessment of an individual receiving treatment that is exhibiting the effects of these substances. In this context, we have collected data from NPS LC-MS/MS testing and Antipsychotic drugs LC-MS/MS testing in the patient samples. Individuals receiving treatment for conditions requiring antipsychotics are often impacted by mental illness, and use of non-prescribed substances can have significant impacts on medication non-adherence or risk for adverse effects, such as overdose in this patient population. For this study 5168 patient samples positive for at least one of the NPS substance were evaluated for co-positivity with antipsychotic drugs.

**Results:** Our findings have indicated the prevalence of NPS use along with presence of antipsychotic drugs in patient samples. It has been found that out of 5168 NPS positive samples, 335 patients (6.5%) were found to also have at least one of the antipsychotic medication present.

**Conclusion:** Although use of NPS compounds is often thought of in relation to treatment of individuals with opioid or substance use disorder, however, the co-positivity for NPS with antipsychotics seen in this data indicates that individuals being treated for mental illness may also have a propensity for misuse of these illegal substances as well. Due to concerns for potential overdose as well as for the negative impact on medication adherence that may occur secondary to the use of NPS compounds, clinicians need to be aware of the opportunity for concurrent use of NPS and antipsychotics. Further, the use of NPS may aggravate symptoms of mental illnesses, such as schizophrenia or bipolar disorder, and create additional obstacles for patients and providers seeking to meet treatment goals. Detection of NPS use in behavioral health populations provides valuable information for clinicians evaluating patients and assists in determining treatment decisions and clinical outcomes.

### A-274

#### Does Labetalol Trigger False Positive Drug Testing Results?

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**Background:** Hypertension is a common health concerns, affecting at least 10% of pregnant women in the United States. Several medications are used for management of hypertension in pregnancy. Labetalol, an alpha- and beta-adrenergic antagonist, has been blamed for causing false positive results in urine for amphetamine (AMP) and methamphetamine (MAMP), likely through cross-reactivity with immunoassays, due to structural similarity. Pregnant women and infants born to women prescribed labetalol during pregnancy may be tested for drugs including AMP and MAMP. In this study we tested labetalol in four specimen types, with several immunoassays and laboratory developed tests performed with liquid chromatography tandem mass spectrometry (LC-MS/MS), with a focus on assays designed to detect AMP and MAMP.

**Methods:** Urine, plasma, umbilical cord tissue and meconium aliquots were obtained from residual clinical specimens (de-identified and drug-free). Labetalol hydrochloride (Millipore Sigma), dissolved in methanol was used to prepare spikes in triplicate per specimen type: 1 µg/mL (2.7407 µM), 18.25 µg/mL (50 µM), and 36.5 µg/mL (100 µM). Triplicate aliquots containing corresponding concentrations of methanol

alone, served as matrix controls. These 18 samples were tested by previously validated AMP and MAMP immunoassays: urine (Emit II Plus, cutoff 300 ng/mL), plasma (Immunalysis, cutoff 20 ng/mL), and meconium (Immunalysis, cutoff 30 ng/g). Other drug classes including opioids, cocaine, barbiturates, benzodiazepines, and cannabinoids were also tested by immunoassay. Established LC-MS/MS analyses designed to target AMP and MAMP, among other drugs, were also performed with plasma (cutoff 20 mg/L), urine (cutoff 200 ng/mL for MAMP, 50 ng/mL for AMP), umbilical cord tissue homogenate (cutoff 5 ng/g) and meconium (cutoff 20 ng/g).

**Results:** For meconium, concentration-related apparent positive results were obtained by immunoassay for both AMP and MAMP. No apparent positive results were obtained with the other immunoassays tested or with any of the LC-MS/MS tests. That said, some evidence of ion suppression, retention time shifts and ion mass ratio errors that could affect detection of other drug analytes was observed.

**Conclusion:** Our results demonstrate that high concentrations of labetalol may trigger apparent positive AMP and MAMP results by immunoassay but do not trigger positive results in several LC-MS/MS assays performed with urine, plasma, meconium, and umbilical cord tissue. As such, AMP and MAMP are not expected in infants born to mothers that are treated with labetalol during pregnancy.

### A-275

#### Internal Versus External Calibration for Determination of Plasma Methotrexate by LC-MS/MS

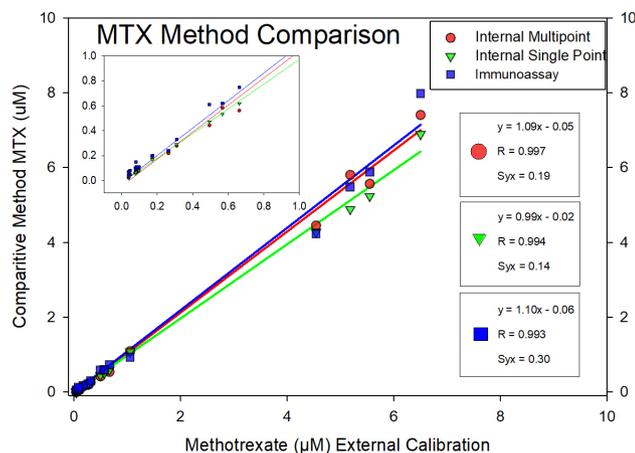
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**Background:** Methotrexate (MTX) is employed to treat a variety of cancers. MTX concentration is typically assessed via immunoassay because this process can be automated for rapid turnaround. Hardware, personnel, and analytic constraints limit the use of LC-MS/MS in time-sensitive clinical settings. We compared the precision and accuracy of MTX measurement using multi-point external calibration to internal calibration (single and multi-point) in an effort to simplify and expedite measurement of MTX by LC-MS/MS.

**Methods:** MTX was determined by ARK immunoassay on a Roche Cobas c502 or using a Waters Xevo TqS tandem MS system. Methanolic plasma extracts were analyzed (ESI+) using a BEH C18 column with an elution gradient of 5% to 90% methanol (2 mM ammonium acetate/0.1% formic acid) over 5 minutes. MTX, MTX-D3, MTX-<sup>13</sup>C5, MTX-<sup>13</sup>C6, MTX-<sup>13</sup>C11, and MTX-<sup>13</sup>C14 were monitored using primary transitions of 455>308, 458>311, 460>308, 461>314, 466>314, and 469>317, respectively. External and internal calibrators spanned four orders of magnitude from 0.05 to 10 µM (weighted 1/x<sup>2</sup>). For single point internal calibration, MTX-D3 was present at 0.22 µM. Imprecision was assessed in duplicate using 5 plasma pools over 5 days (10 replicates per pool) and accuracy assessed with 20 patient plasma specimens previously analyzed by immunoassay then stored frozen until reanalysis.

**Results:** LC-MS/MS imprecision was similar across calibration strategies and ranged from 45-55% at 0.01 µM, 7-12% at 0.05 µM, 5-7% at both 0.10 µM and 1 µM, and 2-6% at 10 µM. The Figure demonstrates agreement between the externally calibrated MS assay and immunoassay (■), multi-point (●), and single-point (▼) internal calibration. Slope bias (Deming) between the externally calibrated LC-MS/MS assay and immunoassay or the internally calibrated LC-MS/MS assays was 10% or less.

**Conclusion:** LC-MS/MS measurement of MTX is precise and accurate without full external calibration. Internal calibration may facilitate the use of LC-MS/MS in time-sensitive applications.



## A-276

## Analytical and Clinical Evaluation of the Automated Elecsys Tacrolimus Assay on the Roche cobas e602 Analyzer

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**Background:** An increased demand for tacrolimus monitoring at the University of Chicago Medical Center (UMCC) led to the evaluation of the automated Roche Elecsys Tacrolimus electrochemiluminescence immunoassay (ECLIA) on the e602 analyzer. Tacrolimus is a first-choice immunosuppressant for solid organ transplantation due to a correlation with decreased cardiovascular risk and renal complications. Importantly, post-transplant tacrolimus target ranges are very narrow and specific. Different target ranges for the stages of kidney transplantation are within  $\pm 2$  ng/mL: 8-10 ng/mL months 0-3, 6-8 ng/mL months 3-12, 4-6 ng/mL months 12 and onward. This validation study evaluates whether the automated tacrolimus ECLIA has the required accuracy for implementation by comparison with the current UCMC laboratory developed test (LDT) for the quantitation of tacrolimus by liquid chromatography tandem mass spectrometry (LC-MS/MS).

**Methods:** The tacrolimus ECLIA assay was evaluated for precision, linearity, interference (by hemoglobin, bilirubin, triglycerides, and biotin) and clinical performance was compared to LC-MS/MS quantitation. Additionally post-extraction stability studies of patient samples (3.07-28.57 ng/mL) were performed at room temperature (21-23°C) and cold storage (4°C).

**Results:** The tacrolimus ECLIA assay is precise, exhibits a measuring range of 0.75-30 ng/mL, and is tolerant of significant interferences (plasma indices: H <2306, I <55, L <1427, and biotin <1200 ng/mL). Comparison with LC-MS/MS quantitation revealed a 21% bias in patient samples evaluated for tacrolimus concentration (n=43, range=2.2-24.4 ng/mL,  $y=1.21x + 0.52$ ,  $r^2=0.97$ ). Bland-Altman analysis revealed an absolute mean bias of 2.5 ng/mL (SD = 1.5 ng/mL). Post-extraction studies at room temperature, exposed to air, confirmed that samples were stable for up to 30 minutes, after which concentrations of tacrolimus were increased by greater than 10% of the initial measurement. Samples that were capped within 15 minutes, which allowed for an initial measurement, and stored at -4°C were stable for up to 24 hours.

**Conclusion:** The relatively high bias observed by the tacrolimus ECLIA assay compared with the LC-MS/MS reference presents major challenges to the implementation of the tacrolimus ECLIA assay routinely. Due to the very narrow concentration ranges targeted (within  $\pm 2$  ng/mL) across the stages of the transplantation process and recovery, the absolute mean bias (2.3 ng/mL) observed between the two methods led to the conclusion that the two methods gave rise to discordant clinical interpretations. In addition, post-extraction stability studies presented practical challenges to adjusting the automated laboratory workflow in order to minimize sample evaporation to prevent falsely elevated results.

## A-277

## Integration of VeriSpray and FAIMS for Quantitation of Immunosuppressant Drugs in Whole Blood

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**Background:** Immunosuppressant drugs are commonly provided to patients undergoing solid organ transplants, with drug levels closely monitored to ensure sufficient exposure while minimizing toxicity. Immunosuppressant drug concentrations can be measured using immunoassays or liquid chromatography-tandem mass spectrometry (LC-MS/MS). While commercial immunoassays suffer from poor specificity due to cross-reactivity, LC-MS/MS methods offer the advantages of improved sensitivity and specificity, but at the expense of throughput and ease of use. The VeriSpray ion source can rapidly quantify analytes in dried matrix spots in two minutes with little to no sample separation. The MS background noise can be reduced using field asymmetric ion mobility spectrometry (FAIMS). Here, we present the quantification of three immunosuppressants in whole human blood using VeriSpray paper spray ion source with and without the FAIMS Pro interface.

**Methods:** Tacrolimus, everolimus, and cyclosporine A were spiked into human whole blood to make 8 calibration levels ranging from 0.5-80 ng/mL for tacrolimus and everolimus, and 10-1600 ng/mL for cyclosporine A. The corresponding internal standards were spiked into each calibrator at a final concentration of 32 ng/mL for tacrolimus and everolimus and 640 ng/mL for cyclosporine A. Ten microliters of each calibrator were pipetted onto the VeriSpray plates and dried for 3 h at room temperature. Three to four replicates of each calibrator were analyzed using VeriSpray and VeriSpray-FAIMS interfaced to a TSQ Altis triple quadrupole mass spectrometer. All analyses were performed in the positive ion mode using a spray voltage of 3400 V, a spray solvent of methanol:chloroform 60:40 (v/v), and a rewetting solvent of methanol:chloroform 60:40 (v/v) with 0.1% sodium acetate. The FAIMS compensation voltages were optimized for each immunosuppressant.

**Results:** The VeriSpray ion source introduced analytes directly into the mass spectrometer with minimal sample preparation. Overall, the calibration curves generated using VeriSpray alone for cyclosporine A, tacrolimus, everolimus in whole human blood showed good linearity with  $R^2$  value greater than 0.95, percent RSD  $\leq 20\%$ , and percent difference < 20% above the limit of quantitation (LOQ) for the three immunosuppressant drugs. For example, good linearity ( $R^2 = 0.953$ ) was obtained for the calibration curve generated using tacrolimus whole blood calibrators, and a percent difference < 20% was achieved for concentrations above 1.25 ng/mL. For everolimus in whole blood, excellent linearity with an  $R^2$  value of 0.992, with a percent difference < 20% and a percent RSD < 15% achieved for a concentration range of 5-80 ng/mL. The FAIMS Pro interface enhanced method selectivity by adding an additional dimension of separation based on the ion mobility under asymmetric waveform. Thus, the LOQ was lowered to 25 for cyclosporine A and 2.5 for tacrolimus. Continuous effort is focused on further refining and validating this methodology using quality control samples of known concentrations.

**Conclusion:** The VeriSpray ion source allowed for the detection of three immunosuppressant drugs in whole blood with minimal sample preparation. Integration of FAIMS with the VeriSpray ion source enabled a reduction in chemical noise and matrix interferences, thereby lowering the LOQ for everolimus and cyclosporine A.

## A-278

## Loperamide: A Novel Drug of Abuse Immunoassay Interferent

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**Background:** The opioid epidemic in the United States is a public health crisis. Physicians rely on drugs of abuse testing of patient samples to identify abuse and monitor abstinence. Mass spectrometry (MS) is the preferred testing method for drugs of abuse; however, laboratories frequently utilize immunoassay screens, reflexing only presumptive positive specimens for confirmation by definitive methods. Immunoassays have important limitations, including cross-reactivity with structurally related compounds. Confirmatory MS methods are frequently designed to detect only specified analytes. As a result of this combination of cross-reactivity (immunoassay) and

targeted specificity (MS), the possibility exists that important drugs of abuse findings detected by immunoassay may be missed by confirmatory or definitive methods. We have previously experienced an event in which a patient's specimens were repeatedly immunoassay-positive for fentanyl and buprenorphine but failed to confirm via MS. A specimen from this patient was examined by untargeted high-resolution MS (HRMS) and determined to contain high concentrations of loperamide. We sought to establish whether loperamide was the cause of the false positive screens.

**Methods:** Drug-free urine was spiked with various concentrations of loperamide or its principal metabolite, N-desmethyl loperamide (dLop), and assayed on multiple fentanyl and buprenorphine assays. Fentanyl immunoassay screen-positive results were examined by HRMS for presence of loperamide and quantified by liquid chromatography tandem MS (LC-MS/MS) when positive.

**Results:** Loperamide produced positive screens on the Thermo DRI Fentanyl and Immunoassay Fentanyl assays at concentrations of 5.72 mcg/mL and 23.7 mcg/mL, respectively. dLop generated positive screens for the Thermo DRI and Immunoassay fentanyl assays at concentrations of 6.90 mcg/mL and 35.7 mcg/mL, respectively. Neither compound affected the ARK Fentanyl (I) or MEDTOX PROFILE-V fentanyl assays. dLop also produced positive buprenorphine results on the Thermo CEDIA buprenorphine assay at concentrations of 12.20 mcg/mL; MEDTOX PROFILE-V and Roche buprenorphine assays were not affected. HRMS analysis of 225 fentanyl immunoassay positive samples (Thermo DRI) yielded five specimens containing loperamide and/or dLop, four of which contained measurable quantities of fentanyl in addition to loperamide/dLop. Two specimens produced positive buprenorphine immunoassay (Thermo CEDIA) results; one contained ~210 ng/mL buprenorphine by LC-MS/MS, but the other contained no measurable buprenorphine but ample quantities of codeine, morphine, oxycodone, and noroxycodone.

**Conclusion:** Though an opioid, loperamide is deemed to have low abuse potential due to poor intestinal absorption, extensive first pass hepatic metabolism, and active efflux from the blood brain barrier. At supra-therapeutic concentrations, particularly when paired with drugs that inhibit efflux from the blood brain barrier (e.g., cimetidine), loperamide is abused as a "poor-man's methadone," and used to stave off withdrawal symptoms. Numerous case reports reflect this use, and it is important that providers who manage patients with opioid addiction be aware of its potential for abuse. Our data demonstrate that loperamide concentrations consistent with abuse are sufficient to cause false positives on select immunoassay screens. Laboratories utilizing these immunoassays should be aware of this potential limitation and be prepared to advise their clinician colleagues if loperamide interference is suspected.

## A-280

### The Prevalence of Prenatal Fentanyl Exposure and Co-Exposure to Commonly Abused Drugs in a High-Risk Population

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**Background:** Prenatal exposure to fentanyl may lead to Neonatal Abstinence Syndrome (NAS), a constellation of symptoms observed when newborns begin withdrawing from addictive substances such as opioids. The use of umbilical cord tissue segments (UC) for newborn toxicology has been increasing due to its apparent long detection window, sensitivity, and ease of collection. However, very little has been reported in the literature concerning the prevalence of *in utero* exposure to fentanyl and co-exposure with other commonly abused substances. The specific aims of this retrospective study are twofold. We will report prevalence of neonatal exposure to fentanyl for a nationwide high-risk population using UC submitted to a national reference laboratory for routine forensic toxicology analysis and the co-exposure patterns observed for these fentanyl-exposed neonates. **Methods:** A secondary analysis was performed using historical data for UC received between January 1, 2020 and December 31, 2020 for routine forensic toxicology analysis. **Results:** During the study period, our laboratory received 23,104 UC for analysis and 9667 (41.8%) of those UC were positive for at least one drug. The prevalence of fentanyl detection was 1.9% (n = 429). Of these 429 specimens there were 407 UC where both fentanyl and norfentanyl were detected. There were 14 UC where only fentanyl was detected and 8 UC where only norfentanyl was detected. When detected, the median concentrations of fentanyl and norfentanyl were 3916 pg/g (IQR: 1696, 9230 pg/g) and 10717 pg/mg (IQR: 3925, 25288 pg/g), respectively. The measured concentrations of fentanyl and norfentanyl were strongly associated (r = .575). Of the 429 positive fentanyl and/or norfentanyl UC, 33 (7.7%) were only positive for fentanyl and/or norfentanyl. Of the 396 polypositive UC, morphine was the highest co-exposure with 243 UC (56.6%) being positive for both fentanyls and morphine. The second most prevalent co-exposure observed was methamphetamine/amphetamine (n = 173; 40.3%) followed by cannabinoids (n = 113; 26.3%) and benzoylcegonine (cocaine metabolite; n = 106; 24.7%). **Conclusions:** Nonmedical use of fentanyl is an alarming trend in this country includ-

ing the maternal demographic reported here. We found that in the majority of cases included in this study, prenatal exposure to fentanyl also included exposure to other commonly abused substances.

## A-281

### Urine Drug monitoring: Stress Testing a New B-Glucuronidase Enzyme

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**Background:** Therapeutic drug monitoring of urine samples is a quick and non-invasive method for monitoring use and abuse of drug classes such as opiates, opioids, and benzodiazepines. Many of these drugs are metabolized and excreted into urine as a glucuronide conjugate form and need to be hydrolyzed before analysis by LC-MS. Urine samples are known to inhibit  $\beta$ -Glucuronidase activity, which can lead to false low results. In this study we evaluate a new pre-buffered, room temperature stable  $\beta$ -Glucuronidase and its efficacy at hydrolysis and recovery of a wide range of analytes over the course of a work week. **Method:** Drug free urine samples were spiked with different concentrations of mixtures of glucuronide derivatives of opiates/opioids (OP), opioid agonist/antagonists (OPA), benzodiazepines (BENZ), and one antipsychotic (APSY). Master mix was prepared using MS  $\beta$ -Gluc (Sigma: SRE0103), DI water and internal standard solutions in methanol and was stored at room temperature for 5 days while being tested at intervals (fresh, 24 hrs, Day 5) to ensure continued efficacy. 180 $\mu$ L of Master mix was combined with 20  $\mu$ L of urine to initiate hydrolysis reaction. Hydrolysis was carried out @ room temp. for 30 min. or 40 °C for 16 min. Post hydrolysis, samples were directly analyzed by LC-MS and recoveries were calculated.

#### Results:

	% Recovery of 11 glucuronides spiked in Urine		
	Master mix storage time = 0 Hours	Master mix storage time = 24 Hours	Master mix storage time = 120 Hours
Morphine	90 $\pm$ 5	90 $\pm$ 4	89 $\pm$ 6
Codeine	87 $\pm$ 4	95 $\pm$ 14	94 $\pm$ 12
Hydromorphone	103.9 $\pm$ 3.2	108 $\pm$ 4	108 $\pm$ 5
Oxymorphone	104 $\pm$ 5	106 $\pm$ 6	102 $\pm$ 6
Tapentadol	98 $\pm$ 6	105 $\pm$ 5	102 $\pm$ 5
Naloxone	99 $\pm$ 2.7	100.5 $\pm$ 3.4	107.5 $\pm$ 1.9
Oxazepam	94 $\pm$ 4	95 $\pm$ 4	97 $\pm$ 4
Temazepam	97 $\pm$ 6	92 $\pm$ 5	94 $\pm$ 6
Lorazepam	103 $\pm$ 5	100 $\pm$ 11	94 $\pm$ 4
Trifluoperazine	104 $\pm$ 29	95 $\pm$ 7	110 $\pm$ 20
Norbuprenorphine	84 $\pm$ 4	100.0 $\pm$ 3.3	97 $\pm$ 5

Table 1 shows that greater than 80% recoveries were obtained for all glucuronides within 30 min of hydrolysis. Standard deviation and % CV were in acceptable range for most of the compounds under study. Data also shows that this enzyme master mix is stable at room temperature with greater than 80% recoveries throughout 5 days of storage. **Conclusions:** Fast and efficient hydrolysis was obtained with high recovery of various analytes and low standard deviation. Consistent recoveries were seen throughout 5-day storage at room temperature suggest its capability for multi-day automated sample testing.

## A-282

### Evaluation of MedTox<sup>®</sup> Scan Profile<sup>®</sup>-V 13 Panel Test to Monitor Compliance with Buprenorphine Treatment for Opioid Dependence

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**Background:** Buprenorphine (Suboxone<sup>®</sup>) is a partial opioid agonist prescribed in outpatient settings to treat opioid dependence. Urine drug screens are often used to monitor compliance with treatment and to provide evidence about recent illicit drug consumption. The objective of this study was to evaluate the performance characteristics of the MedTox<sup>®</sup> Scan Profile<sup>®</sup>-V 13 Panel Test (MedTox, Burlington, NC) to accommodate the needs of a physician office laboratory within our health care system.

**Methods:**

Residual urine specimens with clinically orderable drug testing performed in our toxicology laboratory were frozen within 24 hours of testing. The MedTox Profile®-V 13 Panel test can detect: amphetamine, barbiturates, benzodiazepine, buprenorphine, cannabinoids, cocaine, methadone, methamphetamine, opiates, oxycodone, phenylcyclidine, propoxyphene, and tricyclic antidepressants. Results from two identical MedTox instruments were compared to results obtained with internal reference methods [Roche Cobas 6000 (Roche Diagnostics, Indianapolis, IN), Agilent 7890A gas chromatography-mass spectrometry (Agilent, Santa Clara, CA), or Thermo Scientific liquid chromatography-mass spectrometry/mass spectrometry (Waltham, MA)].

**Results:**

Overall percent agreement was 95% (57/60), with 100% negative (20/20) and 93% positive (37/40) agreement. Within instrument (MedTox) agreement was 98% (59/60). Discordant samples (**Table 1**) were reviewed to resolve these differences.

**Conclusion:**

The MedTox<sup>Scan</sup> Profile®-V 13 Panel Test demonstrated excellent concordance with reference, central laboratory methods. However, differences in cross-reactivity and detection thresholds resulted in rare discrepancies.

**Table 1.** Discordant results (bold) obtained during evaluation

Sample #	Reference Method Results	MedTox Results	Follow-Up and Potential Explanation
1	buprenorphine cannabinoids	<b>benzodiazepine</b> buprenorphine cannabinoids <b>*tricyclic</b> <b>antidepressants</b>	• MedTox may have detected benzodiazepine metabolite as it has a higher cross-reactivity percentage compared to reference method • <b>*Tricyclic antidepressants</b> are not currently measured by any of our reference methods
2	amphetamine benzodiazepine buprenorphine <b>cannabinoids</b>	amphetamines benzodiazepine buprenorphine	• A x5 dilution with negative residual urine was prepared to rule out an interfering substance • Upon dilution, MedTox was now also positive for cannabinoids
3	amphetamine buprenorphine <b>methadone</b> methamphetamine	amphetamine buprenorphine methamphetamine	• Dilutions (x5, x10) did not result in MedTox detection of methadone
4	amphetamine cannabinoids methamphetamine opiates oxycodone	amphetamine cannabinoids methamphetamine opiates <b>**oxycodone</b>	• Low cross-reactive metabolite detected on only one MedTox instrument • Metabolite was confirmed using reference method • <b>**Discrepancy</b> between MedTox instruments

**A-283****LC-MS/MS Analysis of PFAS Forever Chemicals in Serum**

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**Background** PFAS (per- and poly-fluoroalkyl substances) are a large class of synthesized chemicals that are used in a wide range of consumer products and industrial applications. These chemicals are referred to as forever chemicals since PFAS are extremely resistant to degradation and bioaccumulate in the environment and body. Harm from exposure is well documented and probable links to conditions such as reduced birth weights, testicular and kidney cancers, obesity, and immune dysfunction. Epidemiological studies serve to evaluate and identify the adverse effects of PFAS while allowing for the monitoring of body burdens in human and wildlife populations. An LC-MS/MS method was developed and validated for determination of six PFAS chemicals (Perfluorobutanesulfonic Acid, Perfluoroheptanoic Acid, Perfluorohexanesulfonic Acid, Perfluorononanoic Acid, Perfluorooctanoic Acid, Perfluorooctanesulfonic Acid) in human serum.

**Methods** In this method, 0.2 mL of patient samples, quality controls, and calibration standards are mixed with 0.5 mL of <sup>13</sup>C labeled internal standards. Tubes undergo centrifugation and supernatants are transferred to plastic tubes for dry down with nitrogen at 55 ± 5 degrees C. Samples are reconstituted with 0.15 mL of formic acid in methanol. The entire volume is then transferred into 0.25 mL plastic autosampler vials, using Teflon-lined snap-caps. Chromatographic separation is performed on a Shimadzu Prominence Ultra-Fast Liquid Chromatography system with a delay column (Agilent Zorbax Bonus-RP Cartridge, 4.6 x 12.5 mm, 5 micron), a guard column (Agilent Zorbax Bonus-RP Cartridge, 2.1 x 12.5 mm, 5 micron), and an analytical column (Agilent Poroshell 120 PFP, 4.6 x 50 mm, 2.7 micron). The four mobile phases

are 2.5 mM Ammonium Bicarbonate, 2.5 mM Ammonium Bicarbonate in Methanol, 0.1% Formic Acid in DI Water, and 5.0 mM Ammonium Formate in Methanol. Detection is performed on a Sciex API 4500 QTrap triple quadrupole mass spectrometer with electrospray ionization (ESI+).

**Results** Calibration curves were generated using linear regression with 1/x<sup>2</sup> weighting. AMRs were 0.050 - 10.0 ng/mL for PFBS, PFHpA, PFHxS, PFNA and 0.500 - 100 ng/mL for PFOA and PFOS. Precision and accuracy were assessed at the LLOQ, and low and high QC levels. The between day precision and accuracy (n=5 x 3 days) ranged from 1.4% to 8.2% and -7.9% to 7.2%, respectively. Dilution studies demonstrated that specimens can be diluted with DI water as needed to obtain quantitative results. Interferences were not observed but sample collection devices coated with PTFE and PVDF should be avoided due to potential specimen contamination. Stability studies show samples may be stored at RT for at least 30 days prior to testing.

**Conclusion** The LC-MS/MS method was successfully validated to perform quantitative analysis for six PFAS chemicals in human serum. Applications include one-time body burden determinations and ongoing biomonitoring evaluations to study adverse effects associated with PFAS exposure.

**A-284****A quantitative LC-MS/MS urine buprenorphine and norbuprenorphine assay with automated sample preparation**

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**Background:** Buprenorphine is used to treat substance use disorder and acute and chronic pain. In recent years, our health system has observed an increased demand for urine buprenorphine confirmation testing to monitor medication treatment compliance due to the expansion of substance use disorder services. This increase in test volume, however, has led to operational challenges caused by the combination of labor shortage and time-consuming, labor-intensive manual sample preparation. The aim of this work was to develop an automated sample preparation approach for urine buprenorphine LC-MS/MS assay that utilizes beta-glucuronidase to improve analyte detection. By incorporating automation into LC-MS/MS workflow, we would be able to scale up testing without increasing the number of full-time equivalents. **Methods:** In brief, 50 µL of urine was mixed with 170 µL of beta-glucuronidase (IMCSzyme) and internal standards (IS). 150 µL of methanol with 5% formic acid was then added after 30 minutes of incubation at 55°C followed by cooling to room temperature. To remove beta-glucuronidase, 300 µL of the sample mixture was loaded onto Phenomenex 96-well beta-Gone PLUS plate and eluted using positive pressure. This sample preparation was adapted for liquid handler (Hamilton Starlet with [MPE]<sup>2</sup>) with minimal manual intervention. For LC-MS/MS analysis, 5 µL of the eluent was injected into Sciex Triple Quad 5500+ system where analytes (buprenorphine and norbuprenorphine) and IS (d4-buprenorphine and d3-norbuprenorphine) were measured in positive multiple reaction monitoring mode. Chromatographic separation was achieved by gradient elution using Phenomenex Kinetex Biphenyl column (50 x 3 mm, 2.6 µm). To assess the impact of automation in the LC-MS/MS workflow, the labor requirement for both automated and manual sample preparations was evaluated by surveying hands-on time of 3 routine sample batches. Here, manual sample preparation involved treatment of urine with beta-glucuronidase followed by protein precipitation with methanol and centrifugation to remove beta-glucuronidase. **Results:** The assay using beta-glucuronidase removal plate was shown to be linear from 5 to 3000 ng/mL for buprenorphine and 15 to 3000 ng/mL for norbuprenorphine. Buprenorphine inter-assay imprecision was 4.8% CV and 5.1% CV for low and high quality controls (QC), respectively (n=20). Norbuprenorphine inter-assay imprecision was 5.2% CV for both low and high QC (n=20). Comparison with existing LC-MS/MS assay using manual sample preparation yielded a correlation coefficient of 0.999 for buprenorphine (slope = 0.948 and intercept = 18, n=20) and a correlation coefficient of 0.989 for norbuprenorphine (slope = 0.989 and intercept = -8, n=20) using Deming regression analysis. No significant matrix effect was observed with IS-normalized mobile phase/post extractions spiking for buprenorphine (recovery range: 95% to 114%) or norbuprenorphine (recovery range: 94% to 107%). We estimated the hands-on time for a typical batch sample preparation (n=80) was reduced from approximately 2 hours (manual sample preparation) to 30 minutes (automated sample preparation). **Conclusion:** Automated sample preparation using beta-Gone PLUS plate is a suitable sample preparation approach for urine buprenorphine LC-MS/MS assay using beta-glucuronidase and allows us to better support substance use disorder services without additional staffing.

## A-285

**Multifunctional Nanoplatform Based on Alginate Hydrogel Co-Loaded With Cisplatin and Silver Nanoparticles to Study Drug Delivery and Modulation in Cancer Cell Lines.**

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**Background:** Breast cancer is the most common invasive malignancy and a significant cause of death in women worldwide. Although several techniques, such as surgery, chemotherapy, and radiotherapy, are available for the clinical treatment of breast cancer, their efficacy may be compromised by adverse side effects and the emergence of drug resistance. The transformational power of nanotechnology is derived from its unique qualities, including the possibility of functionalization and nanoscale targeting. One of the advantages is tumor-specific targeting, which reduces or eliminates the toxicity to normal tissue. Other benefits include stability, bioavailability, and functional delivery of pharmaceuticals. There has been an increase in interest in using a single platform to administer numerous treatments to the tumor site at the same time. It is possible to achieve synergistic or cumulative toxicity by using drugs that target different cancer markers. This approach has the potential to improve therapeutic efficacy while also reducing drug resistance and other adverse effects. In this project, we studied whether silver nanoparticles coated with an alginate hydrogel embedding cisplatin as a composite platform can produce synergistic or cumulative cytotoxic effects on cancer cell lines. **Method:** The research method consists of developing a consistent preparation route for the composite alginate-based nanoparticles embedded with cisplatin. Various analytical techniques were used to characterize the Alginate/AgNPs/Cisplatin nanocomplex, including UV-vis, SEM/EDX, FTIR, DLS, and TEM. The release rate of cisplatin *in vitro* is determined by using the dialysis method and continuous analysis with HPLC. The cytotoxicity of each AgNP system (with and without Alginate and Cisplatin) is assessed using established assays such as the MTT assay to assess the effect on the viability of the MDA-MB-231 breast cancer cells.

**Results:** The UV-vis analysis demonstrates the typical surface plasmon peak of silver nanoparticles around 400 nm. High resolution TEM analysis shows black dots with diameters of ~40 nm clearly coated with alginate hydrogel. SEM images prove the expected spherical silver nanoparticles dispersed within the alginate matrix. EDX analysis further confirms the presence of silver nanoparticles and platinum. FT-IR spectroscopic analysis was used to compare the changes in the alginate functional groups before and after the conjugation. The MTT assay shows that the cytotoxicity of the Alginate/AgNPs/Cisplatin composite is lower than a mixture of AgNPs/Cisplatin. **Conclusion:** We developed and validated a new multifunctional nanoplatform drug carrier made of alginate hydrogel co-loaded with AgNPs and cisplatin. Pre-clinical studies stated that "alginate and cisplatin form a complex that increases plasma levels of cisplatin without impairing *in vitro* antitumor efficacy." In our project, we found that the presence of alginate, at least as prepared in this work, alters the pharmacokinetics of cisplatin in the presence of AgNPs. The alginate coating in the Alginate/AgNPs/Cisplatin complex may act as a modulator of cisplatin availability. These preliminary findings will enable us to study other apoptotic effects induced by the nanocomplex, such as lactate dehydrogenase leakage and the production of reactive oxygen species, and determine how the presence of alginate can be optimized to enhance the overall efficiency of cisplatin cytotoxic activity.

## A-286

**Comparison of the new Roche FEN2 and the Thermo DRI™ fentanyl immunoassays**

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**Background:** Fentanyl is a potent synthetic opioid analgesic prescribed for severe pain. It also contributes to the opioid epidemic. In clinical laboratories, fentanyl is typically detected using automated immunoassays. Roche Diagnostics recently partnered with Lin-Zhi International, Inc. and released an FDA cleared assay for fentanyl. Prior to the Roche fentanyl assay (FEN2), other fentanyl immunoassays were considered laboratory developed tests. We compared the Thermo Fisher's DRI™ (DRI) assay (which detects the parent drug but not norfentanyl) and the FEN2 (which detects both norfentanyl and fentanyl). **Methods:** The DRI and the FEN2 assay were implemented on Roche Cobas c502 analyzer according to manufacturer instructions. The DRI assay was being used clinically prior to the FEN2 assay becoming available. Detection

accuracy, intra- and inter-day precision were evaluated as part of method validation of the FEN2 assay. Accuracy was assessed by analyzing 40 positive and 40 negative patient samples with in-house LC-MS/MS quantitative method as the reference. For within-run precision, quality control (QC) samples (positive and negative) were tested ten times, while between-run precision was calculated from 5 runs of 5 specimens (N=25) for both QC samples. Over the course of three and a half months (10/14/2021-01/27/2022), 3098 patient samples were screened using the DRI assay with 367 positive results. Of these, 52 were false positive (FP) as compared with LC-MS/MS results. Twenty one of these FP specimens were tested on the new Roche FEN2 assay. In addition, of 2769 samples which screened negative for fentanyl with DRI assay, 481 were tested for fentanyl by LC-MS/MS because they screened positive for a different opiate and fentanyl was included in the confirmation assay. Among these 481 specimens, 50 were confirmed to be false negative (FN) for fentanyl on the DRI assay by LC/MS/MS. Six of the FN DRI samples were tested on the FEN2 assay. Results: The FEN2 assay met all laboratory pre-specified validation criteria. All of the LC-MS/MS confirmed fentanyl positive and negative validation samples were classified correctly. For the FEN2 assay within-run precision was 0.8% and 0.5% and between run precision was, 1.1% and 1.4% for negative and positive controls, respectively. While there was a 100% agreement between two assays on classification of positive and negative validation samples, FP and FN samples (by DRI assay) from routine patient care, were more accurately classified by FEN2 than by DRI. Specifically, of 21 tested FP samples, all were correctly classified (100%) as negative by FEN2 assay and all 6 FN DRI samples were correctly classified by FEN2. **Conclusions:** The FEN2 assay met laboratory's minimum performance criteria and correctly classified specimens that were FP and FN by the DRI method. Reduction of unnecessary treatment and/or confirmatory testing of patients with previously FP results and prompt follow up on patients with previously FN results should be possible with this new assay. This performance supports routine implementation of the FEN2 assay for improved patient care.

## A-288

**Therapeutic Drug Monitoring: Comparison of Result Accuracy and Analyte Stability in Plain Serum Tubes versus Plasma Separator Tubes**

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**Introduction:** Using serum tubes without gel separators for therapeutic drug monitoring (TDM) is traditionally considered best practice due to observed absorption of hydrophobic drugs by gel. However, this requires an extra blood draw, time allowance for clotting before processing, and serum aliquot separation from cellular components for storage. Several studies have evaluated plasma separator tubes (PSTs) for TDM testing, but these often used spiked samples or quality control material to obtain a broad concentration range. The objective of this study was to determine the bias of 10 therapeutic drugs tested in PSTs and plain serum tubes using patient samples and to determine refrigerated stability of these drugs over a 4-day period.

**Methods:** Between November 2021 and February 2022, plain BD Vacutainer serum tubes were collected for TDM testing along with corresponding PST BD Vacutainer tubes drawn simultaneously. Tubes were stored at 4°C and analyzed on the Abbott Alinity c instrument on the day of collection (within <8hr), and 24, 48, 72, and 96 hours post-collection. Drugs (number of patients) evaluated included acetaminophen (n=18), carbamazepine (n=18), digoxin (n=12), phenytoin (n=18), phenobarbital (n=16), salicylates (n=4), tobramycin (n=4), methotrexate (n=20), vancomycin (n=20), and valproic acid (n=20). Quality control coefficients of variation (CV%) closest to the average concentration were 0.56%, 6.1%, 1.5%, 6.6%, 3.5%, 4.0%, 3.4%, 1.8%, 0.6%, 3.0%, respectively. Average bias and %bias from the reported serum value were calculated at each timepoint for each tube type. Statistical analyses were performed using a paired t test, two-tailed.

**Results:**

Drug	Linear range	Range of the results	Average Concentration	Allowable Bias	Average bias (%bias) from the baseline serum result					
					Plasma Separator Tube (PST)					Plain serum
					<8 hr	24 h	48 h	72 h	96 h	96 h
Acetaminophen (µg/mL)	3.0-377.0	3.0-299.8	45.1	±1 or 10%	-4.6%	-3.6%	-4.6%	-0.9%	-0.9%	-0.8%
Carbamazepine (µg/mL)	1.9-20.0	6.8-15.7	9.8	±12%	-9.0%	-9.4%	-8.8%	-8.9%	-11.0%	-3.7%
Digoxin (ng/mL)	0.2-5.0	0.4-4.0	1.0	±0.1 or 10%	-7.7%	-10.4%	-8.5%	-9.2%	-15.4%	1.9%
Methotrexate (µmol/L)	0.04-1.10	0.07-0.91	0.32	±0.1 or 10%	-3.1%	-0.03%	0.5%	4.3%	6.1%	5.8%
Phenytoin (µg/mL)	1.8-40.0	7.8-37.3	18.6	±2 or 15%	-8.2%	-8.7%	-10.5%	-9.3%	-11.0%	4.5%
Phenobarbital (µg/mL)	2.0-80.0	11.3-29.8	21.2	±12%	-2.6%	-8.6%	-10.5%	-10.4%	-11.6%	0.7%
Salicylates (mg/dL)	5.0-100.0	9.0-24.0	14.5	±10%	4.5%	7.5%	6.8%	7.5%	5.1%	4.6%
Tobramycin (µg/mL)	0.3-10.0	2.5-11.1	5.9	±0.1 or 10%	2.5%	-0.5%	1.3%	0.3%	-1.2%	1.6%
Vancomycin (µg/mL)	1.4-100.0	11.2-51.6	26.3	±12%	10.7%	6.9%	4.2%	5.9%	8.7%	-1.0%
Valproic Acid (µg/mL)	12.5-150.0	30.8-102.3	68.7	±12%	0.6%	0.7%	1.6%	1.7%	2.1%	0.6%

The biases of 10 therapeutic drugs tested in both PSTs and plain serum tubes up to 96 hours post-collection were within allowable limits. The biases observed in PSTs were noticeably greater than those in plain serum tubes for carbamazepine, digoxin, phenytoin, phenobarbital and vancomycin. However, the degree of difference was not statistically significant (p>0.05).

**Conclusion:** PSTs can be used for measurement of acetaminophen, methotrexate, salicylates, tobramycin, and valproic acid. For the remaining drugs, test accuracy using PSTs should be evaluated.

**A-289**

**Clozapine blood levels on the Beckman Coulter DxC 700 analyzer - faster turn-around-time**

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**Background:**

Clozapine is the only drug approved by the FDA for treatment-resistant schizophrenia. US Guidelines suggest that it is useful to measure clozapine blood levels during dose titration. Measuring clozapine blood levels can also be useful when there are questions of medication adherence, efficacy, toxicity, medication interactions, or concern over other factors that may influence clozapine levels. Clozapine levels provide objective evidence for clinicians to act on and are most valuable when reported quickly. While clozapine testing by liquid-chromatography / mass spectrometry is offered by major reference laboratories, the lengthy turn-around-times associated with such testing at an outside lab reduces the ability of the mental health care provider to use clozapine blood levels to help their patients. A solution to this problem is to perform testing in-house on a clinical chemistry analyzer, to greatly reduce time-to-result. Designed for use on clinical chemistry analyzers, the MyCare Psychiatry Clozapine Assay Kit is the first FDA-approved immunoassay for the measurement of clozapine in patient serum to aid in the management of patients prescribed clozapine for the treatment of schizophrenia. To make the assay available to more users, we developed an application on the Beckman Coulter® DxC 700 and performed validation testing.

**Methods:**

The clozapine reagents have already received FDA clearance for analysis of clozapine serum samples on the Beckman Coulter AU family of analyzers including AU480, AU680, and AU5800. On the DxC 700 precision, linearity, and method comparison studies were performed. Simple precision was evaluated with three controls (low, medium, and high). Five clozapine-spiked serum samples throughout the assay range were used to assess linearity. Method comparison was performed compared to AU480 using 45 annotated specimens collected from patients taking clozapine.

**Results:**

Analysis time was < 9 minutes. Within-run precision (n=10) was 6.0% at 145 ng/mL (subtherapeutic level), 2.5% at 480 ng/mL (therapeutic level), and 3.4% at 956 ng/mL (supratherapeutic level). Repeatability was 4.5%, 2.8%, and 2.5% and within-laboratory precision coefficients of variation were 6.1%, 3.1%, and 3.8% for subtherapeutic, therapeutic, and supratherapeutic levels respectively. The assay was linear throughout the measuring range from 68 to 1500 ng/mL; with deviation from linearity of <10%. The Passing Bablok regression statistics for the method comparison were R = 0.9990, slope = 0.959, and intercept -5.8. The ninety-five percent confidence limits on the slope and intercept contained 1 and 0 respectively. Bias was -5.5%.

**Conclusion:**

The MyCare Psychiatry Clozapine Assay Kit demonstrated robust performance, allowing rapid, precise, sensitive, and specific automated measurement of clozapine in human serum using the Beckman Coulter DxC 700.

**A-292**

**Interference of Gabapentin in LC-MS/MS Analysis of Amphetamine and Proposed Solution**

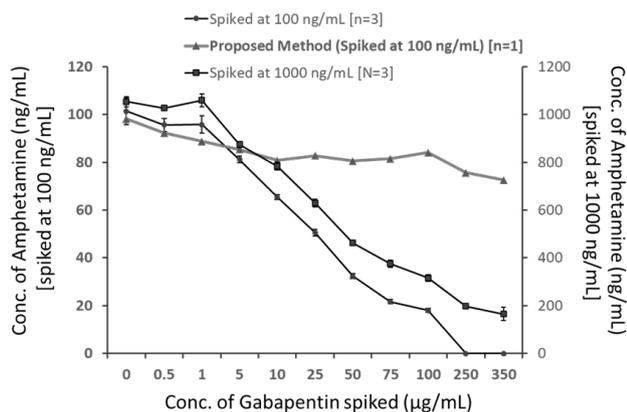
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**Background:** Gabapentin is prescribed in about 12% of patients receiving pain management treatment. In our laboratory, approximately 15-20% of samples for amphetamine confirmation are positive for gabapentin and its concentration usually exceeds 10µg/mL. Dilute and shoot LC-MS/MS method is widely used as confirmatory assay for urine drug testing and in most analytical columns, both gabapentin and amphetamine elute at similar RT leading to potential ion-suppression. We observed that intensity of amphetamine-D5-IS decreased below 65% when the gabapentin exceeds 25µg/mL, and the peak can completely disappear at 250µg/mL. In this study, we provided a systemic approach to evaluate the effect of gabapentin on LC-MS/MS analysis of amphetamine and we proposed an alternative method to resolve it.

**Methods:** 255 urine samples with gabapentin above 5µg/mL were analyzed with original and improved LC-MS/MS method. To investigate the effect of gabapentin, in negative urine(n=3), 71 drugs including amphetamine were spiked at 100 and 1000ng/mL. Gabapentin at varying range from 0-350 µg/mL were also spiked and analyzed by both methods.

**Results:** Presence of gabapentin >10µg/mL significantly decreases peak intensity of amphetamine-D5 and accuracy of amphetamine assay reduces below 75%. The negative-bias in the measurement is proportional to the concentration of gabapentin (Figure 1). Improved method for analysis of amphetamine was validated in which gabapentin were greatly separated (2.5 vs 6.5min) to resolve the interference. It utilizes same sample preparation as original method to avoid repeat sample preparation. Amphetamine was above cutoff in 53 out of 255 samples when analyzed by proposed method. In contrast, approximately 33% of the samples were false-negative by original LC-MS/MS with significant negative-bias in the concentration of amphetamine.

**Conclusion:** Laboratories need to be cautious in LC-MS/MS analysis of amphetamine from potential interference from gabapentin. We developed and validated dilute and shoot LC-MS/MS amphetamine assay that is free of interference of gabapentin.



### A-293

#### Changes in phosphatidylethanol positivity after transfusion and potential impact on interpretation of alcohol use.

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**Background:** Phosphatidylethanol (PEth) is an emerging biomarker for alcohol use with diagnostic sensitivities and specificities superior to traditional alcohol biomarkers including ethyl glucuronide, ethyl sulfate, and carbohydrate-deficient transferrin. PEth can be detected up to 28 days following alcohol use and higher PEth concentration is considered evidence of moderate to heavy alcohol consumption. This study was designed to determine the potential impact of PEth present in units of banked blood on interpretations for alcohol use in transfused patients. **Methods:** Residual frozen whole blood originally submitted for hemoglobin electrophoresis from before and after red blood cell exchange transfusions in patients with sickle cell disease were utilized. Forty-eight paired pre- and post- transfusion samples were analyzed for the presence of PEth 16:0/18:1 using a laboratory developed liquid chromatography mass-spectrometry (LC-MS/MS) method. PEth positivity was determined using a 20 ng/mL cutoff which represents the limit of quantification of our assay and is widely considered the cutoff concentration for light to moderate alcohol consumption. **Results:** The median age of the patients included in this study was 35 years (range 20-66 years) and 54% of the specimens were collected from females (n =26 of 48). Of the specimens analyzed, 75% (n = 36 of 48) tested negative for PEth prior to the transfusion. Following transfusion, 44% of the patients with pre-transfusion PEth-negative specimens tested positive for PEth with a median concentration of 41 ng/mL (range 21 - 224 mg/mL). Twelve specimens (25%) had lower PEth concentrations post-transfusion as expected from a dilution effect. However, only two (4.1%) of the initially positive specimens had concentrations below the cutoff following transfusion. **Conclusions:** Phosphatidylethanol concentrations greater than 20 ng/ml, indicating alcohol consumption can be detected following transfusion in patient specimens with negative pre-transfusion PEth results, highlighting the need for caution when interpreting results in transfused patients and particularly in transplant settings.

### A-294

#### Cutoff concentration evaluation of a fentanyl screening assay

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**Background:** With the surge of Fentanyl-related overdose deaths in recent years, screening for fentanyl has been adopted by many clinical laboratories to detect illicit drug use and monitor compliance. This study evaluated the performance of Immunalysis® SEFRIA™ Fentanyl Urine Enzyme Immunoassay on Abbott ARCHITECT chemistry analyzers, and implemented a new cutoff that was verified in our patient population.

**Material and Methods:** 20 urine samples, obtained from two reference laboratories of known fentanyl and norfentanyl concentrations were screened using the assay cutoff of 1.0 ng/mL per the package insert. Among the 20 samples, two samples have undetectable fentanyl (<1.0 ng/mL) and norfentanyl (<1.0 ng/mL), two samples have undetectable fentanyl (<1.0 ng/mL) but detectable norfentanyl (7.3 and 8.3 ng/mL),

and 16 samples have fentanyl concentrations ranging from 0.4 ng/mL to 24 ng/mL. All fentanyl and norfentanyl concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using cutoffs of 1.0 ng/mL except fentanyl from one reference lab that using 0.2 ng/mL as the cutoff.

**Results:** During initial screens using a cutoff of 1.0 ng/mL, the assay was positive for all 16 samples with detectable fentanyl (0.4-24 ng/mL). Of the two samples with undetectable fentanyl (<1.0 ng/mL) and detectable norfentanyl, one was positive (norfentanyl 7.3 ng/mL) and the other was negative (norfentanyl 8.3 ng/mL). Positive results were obtained for two samples with undetectable fentanyl (<1.0 ng/mL) and norfentanyl (< 1.0 ng/mL).

Based on these results, a retrospective review of results from July 7, 2020 to August 30, 2020, was performed to assess the impact of possible false positive rate. A total of 410 fentanyl confirmations were performed by LC-MS/MS at our laboratory, and 155 samples (38%) were identified as unconfirmed positive. In review of all the unconfirmed positive screens, 59% of the results were in the range of 1.0 - 1.3 ng/mL. For all the positive screens (unconfirmed positive plus confirmed positive), 30% of the results fall between 1.0 and 1.3 ng/mL.

**Conclusion:** The SEFRIA fentanyl screen is calibrated with fentanyl only by design and therefore has a poor cross-reactivity with norfentanyl (0.0050%), which may explain the false negative result for the sample with detectable norfentanyl. For unconfirmed positive results obtained for two samples with undetectable fentanyl and norfentanyl, the presence of designer fentanyl analogues could not be excluded.

To reduce the number of false positive screens in our patient population, the assay cutoff was increased from 1.0 ng/mL to 1.3 ng/mL. The increase in cutoff concentration may result in low-positive patients being missed during screening. However, low positive results are generally observed in patients prescribed with fentanyl, while illicit drug use typically reflects a high-positive screen result in our experience. Taking both clinical impact and laboratory workload into consideration, we conclude the new cutoff is appropriate for the detection of illicit fentanyl use in our patient population and have educated our health care providers to the new cutoff and clinical implications of the change.

### A-295

#### Potential Detection of 58 Fentanyl Analogs in Urine using Fentanyl Immunoassays

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**Background:** The ability to detect fentanyl analogs in urine aids in patient management. Little is published about the new ARK™ Fentanyl II Assay formulation's ability to detect fentanyl analogs. Norfentanyl (fentanyl metabolite) cross-reactivity with the ARK II assays is 7%, and the Immunalysis SEFRIA assay is approximately 0.005%. We hypothesize this difference in cross-reactivity extends to the ability to detect different analogs. The purpose of this study was to determine the new ARK II and SEFRIA fentanyl assays' detection of 58 fentanyl analogs. **Methods:** 58 purified fentanyl analogs were provided by the CDC and divided into four sub-classes: acryl-, butyryl-, furanyl- and thio-fentanyls. Each modification is incorporated into one of the four fentanyl molecule moieties; the piperidine ring, anilino-phenyl ring, 2-phenylethyl substitution, or carboxamide moiety. Drug-free urine was fortified with 0, 1, 10, or 100ng/mL of the fentanyl analog. Results greater than the 1.0ng/mL fentanyl calibrator on the Abbott Architect cSystem C8000 were considered positive. Based on reactivity data from the 1.0ng/mL concentration, analogs were then tested at 10 or 100ng/mL. Potential trazadone interference was assessed using the primary urine metabolite m-chlorophenylpiperazine (mCPP) at 10mcg/mL in drug-free urine. **Data Analysis:** the difference in reactivity of both immunoassay's reagents was evaluated in conjunction with the chemical structure of each analog. **Results:** Of the 58 analogs tested at ≤100 ng/mL, the ARK II and SEFRIA assays produced 51 and 57 positive results respectively. The cross-reactivity of the assay was predominantly determined by the location of the modification. Most modifications to the anilino-phenyl and/or carboximide moieties did not affect the ARK II assay, producing positive results for 36/37 and 49/51 analogs tested at ≤100ng/mL, respectively. The SEFRIA assay had similar performance for anilino-phenyl and/or carboximide moiety modifications with all analogs tested at ≤100ng/mL producing positive results. The ARK II assay was affected more by piperidine ring modifications and demonstrated cross-reactivity with 2/5, SEFRIA reacted with 4/5. 2-phenylethyl substitution modifications produced similar results, ARK II detected 4/10 and SEFRIA detected 9/10. Of the 7 compounds which were undetected by the ARK II assay, 5 were thiophene modifications to the 2-phenylethyl substitution. Norsufentanil was not detected by either assay and was the only analog not detected by the SEFRIA assay. Preliminary interference studies with m-CPP indicated it was detected by the SEFRIA assay, thus a potential source of false positives. **Conclusions:** The ARK II and Immunalysis fentanyl immunoassays can

detect a range of fentanyl analogs with acryl, butyryl, or furanyl modifications to the carboxamide moiety or anilinophenyl ring of the molecule. 2-phenylethyl substitution and piperidine ring modifications significantly affect the ARK II assay's ability to detect the analogs, while the SEFRIA assay appeared less affected and detected all analogs tested except for norsufentanil, which was also not detected by the ARK II assay. The prevalence of trazadone and the potential false positives which may result from its interference in the SEFRIA fentanyl assay are of significant clinical relevance.

## A-296

### A High Throughput LDTD-MS/MS Method to Drug Screening in Hair Samples

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**Background:** Laser diode thermal desorption (LDTD) tandem mass spectrometry has been recognized as a powerful alternative to conventional techniques. LDTD is a mass spectrometry source that delivers the sample analytes directly into the mass spectrometer system and does not require HPLC separation before detection. Typically, LDTD-MS/MS method presents reduced analytical cycle times, ~11 s per sample. This high-throughput technology has the potential to significantly decrease both analysis time and per sample cost of controlled substance and toxicology analyses in forensic laboratories. In Brazil, the amount of hair testing to detect the abuse of illicit drugs exploded in the last years, due to the legislation needed to renew a driver's license for professional drivers. Although, methods with high throughput are important to attend to the demand. **Objective:** This study aimed to develop and validate a high throughput LDTD-MS/MS as a screening method to detect 14 illicit drugs and metabolites in hair samples. **Methods:** Briefly, the extraction solvent and a labeled internal standards solution were added to 10 mg of hair sample. The samples were pulverized in a FastPrep-24™ 5G from MP-Biomedicals, sonicated at 60°C for 60 min, and 8.0 uL were pipetted into a well in a specially designed 96- or 384-well plate. The solvents were evaporated, and a laser diode heats the back of the stainless steel sheet of the well, heating the dried sample, and vaporizing the analytes. The neutral vaporized analytes were ionized through a corona discharge and they were analyzed by a mass spectrometer. An LDTD-MS/MS Luxon Ion Source Phytionix coupled with a Sciex 6500+ TripleQuad mass spectrometer was used. **Results:** The present method was validated and the drugs and metabolites monitored are morphine, codeine, 6-acetylmorphine, amphetamine, methamphetamine, amphetamine, MDA, MDMA, MDEA, fenproporex, mazindol, cocaine, PCP, and THC. The method showed a good agreement in spiked samples, few false positives and any false negatives in the 14 real samples tested, achieving 100% of sensitivity, and 94% of specificity. **Conclusion:** The LDTD-MS/MS method as a screening tool was validated for illicit drugs in hair samples. The method showed excellent sensitivity and specificity and can be used in routine analysis. The method is important to reduce the cost with analysis and allow to deliver negative samples faster.

## A-297

### A Simple Method for Opioids, Synthetic Opioids, PCP, Pregabalin, and Barbiturates Detection in hair samples by LC-MS/MS

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**Background:** Hair analysis is useful for monitoring exposure to drugs, and is a unique material for the retrospective detection of drug consumption, due to its large detection window, and it is easy to collect, store and transport. An effective and fast analytical method for the simultaneous detection of opioids, synthetic opioids, and barbiturates in hair samples was developed and validated by liquid-chromatography-tandem mass spectrometry (LC-MS/MS). **Objective:** This study aimed to develop and validate a simple, rapid, and sensitive LC-MS/MS method for the quantification of 16 opioids and metabolites, PCP, Pregabalin, and 4 barbiturates in hair samples. **Methods:** In this method, 15 mg of hair in a 2.0 mL microtube were decontaminated sequentially with dichloromethane and methanol. The sample was dried. Then, 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, 150 uL of supernatant was added to 150 uL of ultrapure water and 5.0 uL was injected directly into an LC-MS/MS system. Chromatographic separation was performed on an Agilent 1290 Infinity II equipped with HALO Biphenyl column and using 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 present method was successfully validated for carryover, linearity, preci-

sion, recovery, detection, and quantification limits, and the measurement uncertainty was calculated. The results were summarized in the table below. **Conclusion:** The LC-MS/MS method developed was simple, rapid, and sensitive to detect opioids and barbiturates in hair samples.

Analytes	Linearity	LD	LQ	Precision (%)	Recovery (%)	Uncertain
Phenobarbital, Pentobarbital, and Secobarbital	0.200 - 2.500 ng/mg	0.113; 0.095; 0.056 ng/mg	0.200 ng/mg	< 16%	90 - 96%	± 0.129; 0.130; 0.117 ng/mg

## A-298

### Analytical Method Development and Validation to Quantify Urinary Ortho-Cresol by Gas Chromatography with Flame Ionization Detector

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**Introduction:** Ortho-cresol is a toluene metabolite, which is a widely used solvent in industrial activities. This metabolite is considered one of the most specific biomarkers to monitoring the health of workers exposed to toluene. The toxic effects caused by the presence of cresols in the body occur by corrosive actions, which results in cellular membrane destruction, and the suppressive action to central nervous system. **Objective:** Develop and validate a rapid method to identify and quantify ortho-cresol in the urine of workers exposed to toluene. **Methods:** 750 µL of urine sample was submitted to a hydrolysis step with 150 µL of concentrated HCl at 100°C for 1 hour in a glass vial. After this, each vial was cooled to room temperature. 300 µL of a solution of Ethyl Acetate in Hexane (25% v/v) were added to the sample. After, the vials were agitated at 1,850 rpm for 2 minutes and centrifuged at 5,000 rpm for 7 minutes. 100 µL of the organic layer were transferred to a vial with an insert. 3 µL of the extract were injected in a splitless mode in gas chromatography with a flame ionization detector. **Results:** In a chromatographic method with a time run of 5.5 minutes plus 1.5 minutes post-run, the method was validated with a linear range of 0.025 mg/L to 4 mg/L. The detection and quantification limits were 0.004 and 0.025 mg/L, respectively, and the recovery was 98%. This method did not present a matrix effect. The precision was between 5.8 and 10.7%. **Conclusions:** A simple, rapid, sensitive, and specific method is necessary to analyze ortho-cresol in urine of workers exposed to toluene in a toxicology laboratory routine. Especially because of the complicated chromatography profile of hydrolyzed samples, a sample procedure, and a chromatography method with good separation that give the extraction specificity is required. In that regard, the method developed had good values of the validation parameters, which showed that this is adequate to use for the main purpose.

## A-299

### Determination of psychoactive substances (confirmatory method), in hair samples by LC-MS/MS

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**Background:** Detection of psychoactive substances in hair samples is of great importance in clinical and forensic investigations. Providing long-term retrospective information, helping to differentiate between single and prolonged exposure and in post-mortem investigations. The recent improvement of technologies and equipment, such as LC-MS/MS, has allowed a high sensitivity, precision and high throughput analysis in toxicology. Thus, a confirmatory method was validated for the determination of psychoactive substances in hair, through LC-MS/MS. **Objective:** Validation of an analytical method for the determination of 16 psychoactive substances (confirmatory) in hair, using LC-MS/MS analysis. **Methods:** In this method, 15 mg of hair sample were decontaminated sequentially with dichloromethane and methanol. The sample was dried and then the extraction solvent and a labeled internal standards solution were added. The hair sample was pulverized and then sonicated at 60°C for 60 min. The sample was filtered with filtration plates and 1.0 µL was injected directly into an LC-MS/MS system. The analysis was performed using an Waters UPLC - Mass Spectrometry Xevo TQS. It was evaluated the linearity, limit of detection and quantification, precision, accuracy, recovery and measurement of uncertainty values of the 16 analytes. **Results:** The method performed successfully on all required validation parameters. The results are shown in the table. The results found were satisfactory, as they presented as recovery or coefficient of variation (CV) less than or equal to the acceptance criterion, demonstrating that the method is suitable to routine. **Conclusion:**

The analytical method used proved to be adequate, since it met the criteria required. The method is suitable for routine analysis in one of the largest clinical laboratories in Brazil.

Analytes	Linearity	LD	LQ	Precision (%)	Recovery (%)	Uncertainty
AEME, Benzoylcegonine, Cocaine, Ethylene, Norcocaine, THC	0.025 – 0.250 ng/mg	0.004 – 0.023 ng/mg	0.034 ng/mg	< 19%	87 – 107%	± 0.012 – 0.015 ng/mg
Amphetamine, Methamphetamine, MDMA, Fenproporex, Amphetamine, 6Acetylmorphine, Morphine, Codeine, MDA	0.100 - 1.000 ng/mg	0.011 – 0.037 ng/mg	0.154 ng/mg	< 17%	94 – 107%	± 0.038 – 0.070 ng/mg
Mazindol, Cocaine	0.250 – 2.500 ng/mg	0.078; 0.046 ng/mg	0.360 ng/mg	< 8%	95 – 101%	± 0.164; and 0.124 ng/mg

### A-300

#### Development and validation of a highly sensitive method for detection of Synthetic Cannabinoid JWH-018, AB-CHMINACA, and their metabolites in hair samples by LC-MS/MS

B. F. Paulo, D. A. Zauli, *Pardini Group, Vespasiano, Brazil*

**Background:** Synthetic cannabinoids, originally developed for research of CB1 and CB2 cannabinoid receptors, were first identified in herbal incense in 2008. These herbal incenses, usually called 'spice', have since gained global popularity because they are sold on the Internet and in many head shops under the disguise of normal herbal products. JWH-018 was shown to have psychoactive effects similar to those of strong agonists of cannabinoid type 1 (CB1) receptor, for example,  $\Delta^9$ -tetrahydrocannabinol (THC). AB-CHMINACA, a recently introduced synthetic cannabinoid, was first reported in Japan in 2013 and has spread rapidly worldwide. It is a highly potent agonist of the CB1 receptor and can cause serious adverse health effects. Hair analysis is useful for monitoring exposure to drugs, and is a unique material for the retrospective detection of drug consumption, due to its large detection window, and it is easy to collect, store and transport. **Objective:** To aim to develop and validate a sensitive LC-MS/MS method for the quantification of JWH-018 and AB-CHMINACA and their principal metabolites in hair samples. **Methods:** In this method, hair samples were cut with scissors to fragments smaller than 3mm, and then 15 mg was weighted in 2.0 mL polypropylene microtubes. The samples were decontaminated sequentially with dichloromethane and methanol and were dried at ambient temperature. Then, 0.75 mL of methanol and a labeled internal standards solution were added. The microtubes were capped and incubated overnight at 40°C. The supernatant was transferred to a 2.0 mL glass vial and dried at 40°C with a nitrogen sample concentrator. The samples were resuspended with 0.15 mL of methanol and 10.0  $\mu$ L were injected into an LC-MS/MS system. Chromatographic separation was performed on an Agilent 1290 Infinity II coupled with a Sciex 6500+ QTrap mass spectrometer. The system was equipped with a HALO Biphenyl column and used a mobile phase constituted by water and methanol with 0.1% of formic acid. **Results:** The present method was successfully validated for carryover, linearity, precision, recovery, detection and quantification limits. The chromatographic method is suitable to analyze all of the analytes with a baseline separation, principally the isomers JWH 018 N-(4-hydroxypentyl), JWH 018 N-(5-hydroxypentyl) metabolites. The analytes JWH-018 and metabolites (JWH 018 N-(4-hydroxypentyl), JWH 018 N-(5-hydroxypentyl), and JWH 018 N-pentanoic acid) achieved the linearity of 1.25 - 50.00 pg/mg, the detection limit of 0.09 - 0.19 pg/mg, and 1.25 pg/mg of quantification limit. The precision was less than 6%, and recovery was between 91 - 113% for all analytes. The analytes AB-CHMINACA and metabolite AB-CHMINACA M2 achieved the linearity of 2.50 - 100.00 pg/mg, the detection limit of 0.44 and 0.34 pg/mg, and 2.50 pg/mg of quantification limit. The precision was less than 5%, and recovery was between 93 - 101% for all analytes. **Conclusion:** We develop and validate a sensitive and precise method to detect and

quantification synthetic cannabinoids in hair samples. The method does not need additional steps for clean-up the samples to achieve sufficient sensibility to detect these drugs at very low concentrations and can be used at routine analysis.

### A-301

#### Validation of a straightforward, robust, and rapid method for element determination in serum samples by ICPMS

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**Background:** Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has been used as the main analytical technique capable of analyzing samples with complex matrices for (quasi) simultaneous determination of multiple metals. Plasma or serum specimens can be analyzed using a "dilute and shoot" approach, where a specimen is diluted with a diluent and injected directly into the instrument. Element determination in serum has been used extensively to measure occupational exposure at harmful concentrations. Measurement of several elements in a single sample is often necessary and the ICP-MS becomes an important tool to reduce the minimum amount of serum to perform an extensive analysis of metals. **Objective:** This study aimed to validate a straightforward ICP-MS method for the quantification of Vanadium, Chromium, Manganese, Nickel, Selenium, Copper, and Zinc in serum samples for occupational evaluation. **Methods:** In this method, serum samples were diluted at a factor of 10 times with a special diluent, homogenized, and injected directly into the ICP-MS. The diluent content n-butanol, EDTA, ammonium hydroxide, Triton X-100, and rare elements used as internal standards. An Agilent 7850 ICP-MS instrument was used with a Mira Mist PTFE nebulizer, Ni sampler cone, and Ni skimmer cone. The total analysis time was 2.8 min per sample. The present method was validated for carryover, linearity, precision, recovery, and quantification limit. **Results:** The method performed successfully on all validation parameters. The results were presented in the table. The method was implemented in routine analysis with good stability during 24h routines. The diluent maintains the solubility of proteins, stabilizes the elements in solution, and helps to prevent the clogging of the nebulizer. **Conclusion:** We validate a simple, robust, and rapid method to element quantification in serum samples. The method is suitable for routine analysis of one of the largest clinical laboratories in Brazil.

Isotope - Element	Quantification Limit	Linearity	Precision (%)	Recovery (%)
51 - V	0.2 $\mu$ g/L	0.2 - 100.0 $\mu$ g/L	< 14 %	92 - 98 %
52 - Cr	0.5 $\mu$ g/L	0.5 - 100.0 $\mu$ g/L	< 20 %	92 - 103 %
55 - Mn	1.0 $\mu$ g/L	1.0 - 100.0 $\mu$ g/L	< 16 %	91 - 103 %
60 - Ni	0.5 $\mu$ g/L	0.5 - 100.0 $\mu$ g/L	< 9 %	95 - 106 %
78 - Se	20.0 $\mu$ g/L	20.0 - 1,250.0 $\mu$ g/L	< 4%	99 - 106 %
63 - Cu	20.0 $\mu$ g/dL	20.0 - 250.0 $\mu$ g/dL	< 5 %	86 - 95 %
66 - Zn	20.0 $\mu$ g/dL	20.0 - 250.0 $\mu$ g/dL	< 13 %	88 - 110 %

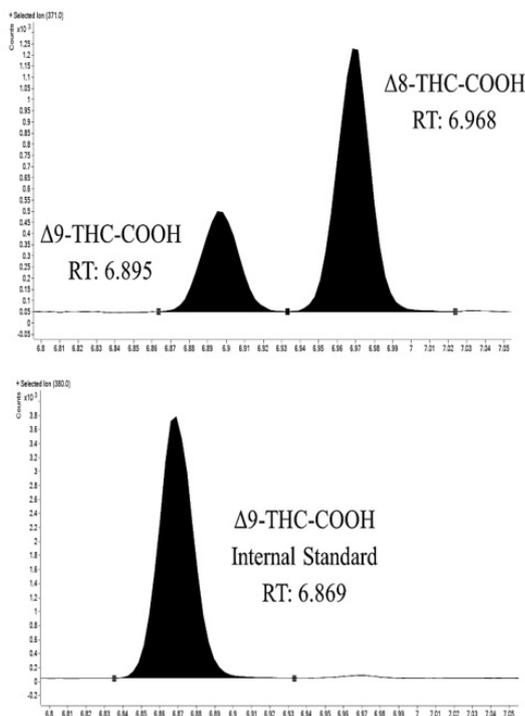
### A-302

#### Can Current Immunoassay and Gas-Chromatography Mass Spectrometry (GC-MS) methods for delta-9-tetrahydrocannabinol carboxylic acid ( $\Delta^9$ -THC-COOH) detect $\Delta^8$ -THC-COOH?

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**Background:** Delta-9 tetrahydrocannabinol ( $\Delta^9$ -THC) is the primary psychoactive ingredient of the cannabis plant (*Cannabis sativa*).  $\Delta^8$ -THC is also a naturally occurring psychoactive cannabinoid but present in very low concentrations. It is generally synthesized from cannabidiol (CBD). Unlike  $\Delta^9$ -THC, the legal status of  $\Delta^8$ -THC remains unclear. Due to the lack of federal regulation and to circumvent drug detection, the use of  $\Delta^8$ -THC is on the rise. To detect  $\Delta^9$ -THC use, its carboxy metabolite,  $\Delta^9$ -THC-COOH, is commonly analyzed. It is not well known how current immunoassays and GC-MS methods for  $\Delta^9$ -THC-COOH detect  $\Delta^8$ -THC-COOH. **Objective:** To investigate if our current  $\Delta^9$ -THC-COOH immunoassay (Siemens Emit II Plus® cannabinoid immunoassay) and GC-MS method would detect  $\Delta^8$ -THC-COOH. **Material and Methods:** Negative urine samples were spiked with various concentrations (10-50 ng/mL) of  $\Delta^8$ -THC-COOH and analyzed by Siemens Emit II Plus® cannabinoid immunoassay and GC-MS (Agilent Technologies). GC-MS analysis involved use of labeled internal standard d9- $\Delta^9$ -THC-COOH. Samples were trimethylsilyl (TMS) derivatized using BSTFA with 1% TMCS. Derivatized samples were

injected onto GC-MS installed with DB-1 column (15m x 0.25mm x 0.25 $\mu$ m). Initial oven temperature was 120°C and ramped to 280°C (30°C/min) with total run time of 10 minutes. **Results:** Siemens Emit II Plus® cannabinoid immunoassay, with a cut-off 20 ng/mL, was positive at  $\Delta$ 8-THC-COOH concentrations of 30 ng/mL and higher. GC-MS method initially developed for  $\Delta$ 9-THC-COOH was able to detect  $\Delta$ 8-THC-COOH. This was due to the difference in retention times of  $\Delta$ 9-THC-COOH and  $\Delta$ 8-THC-COOH, despite the same m/z ions used for mass spectrometry analysis (m/z 371,473 and 488 for  $\Delta$ 9-THC-COOH and  $\Delta$ 8-THC-COOH; 380, 479 and 497 for internal standard d9- $\Delta$ 9-THC-COOH). These findings were confirmed in a patient sample containing  $\Delta$ 8-THC-COOH (Figure). **Conclusions:** Siemens Emit II Plus® cannabinoid immunoassay and GC-MS method described here can detect  $\Delta$ 8-THC-COOH and thus  $\Delta$ 8-THC use.



mobile phase (5 mM ammonium formate pH3.5 in water and 100% methanol). The outlet of the column was connected to a triple quadrupole mass spectrometer with electrospray interface. Ions were detected in the positive multiple reaction monitoring mode. The concentration of analyte was calculated from the calibration curve and ion ratios between the analyte and the internal standard.

#### Results:

The linearity of clozapine and norclozapine were between 15.6 - 1500 ng/mL and 15.5-1500 ng/mL ( $r^2 > 0.999$ ). The imprecision for within-run and between-run was less than 2.3%. The accuracy was evaluated by spike recovery and the mean recovery was 98%-102%. No carryover and ion suppressant or enhancement was observed in this validated method.

#### Conclusion:

A fast and accurate UPLC-MS/MS method was developed and successfully applied for routine therapeutic drug monitoring purpose for patients treated with clozapine.

## A-303

### An UPLC-MS/MS Method for Therapeutic Drug Monitoring of Clozapine

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#### Background:

Clozapine is a tricyclic dibenzodiazepine antipsychotic drug. It is a highly effective medication for patients with schizophrenia who are refractory or intolerant to the side effects of traditional antipsychotics. Although clozapine tends not to produce extrapyramidal symptoms, other side effects of the drug (e.g., agranulocytosis, myocarditis, seizures) limit its widespread use. Clozapine is metabolized primarily by human cytochrome P450 (CYP) isozyme 1A2, yielding a pharmacologically active metabolite, norclozapine. Studies have also provided evidence that toxic side effects may be associated with clozapine metabolites. The level monitoring of clozapine and norclozapine is recommended to ensure safety and minimize toxic adverse events. This study aimed to develop a method to measure clozapine and its metabolite norclozapine by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

#### Methods:

The calibration curve samples were prepared by spiking drug free plasma with clozapine and its major metabolite norclozapine. A 20  $\mu$ L aliquot of calibrators or samples was transferred into a centrifugation tube. After addition of the stable isotope labeled internal standards (clozapine- $^{13}$ C, d3 and norclozapine-d8) and protein precipitation, the supernatant was 10-fold diluted and injected into a chromatography system consisting of ACQUITY UPLC BEH C18 (2.1x50 mm, 1.7  $\mu$ m) with gradient made of

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 Wednesday, July 27, 2022
 

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 Poster Session: 9:30 am - 5:00 pm  
**Analytical Techniques and Applications**
**B-001****No Blood, No Pain, Yes Gain**

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**BACKGROUND** A blood test is critical to assess overall health and identify possible disorders. Near infrared spectroscopy (NIRS) is a highly flexible form of analysis that can be applied to a wide range of analytical applications with a single scan. Chemometrics refers to statistical and mathematical methods and logic, to more complex data analysis. We developed a device capable of delivering clinical test using NIRS and chemometrics. The device's most significant characteristics are no sample preparation demands, speed of analysis (two minutes), non-invasive nature, suitable for practical use, universal application (availability to be point of care, bed-site instrumentation, critical and disaster alternative and more). **METHODS** The device was evaluated in 20 patients between 18-59 years old, known to be healthy, who had a routine blood draw performed at a clinical laboratory in Campinas, Brazil, within the same hour and day of each other. A method comparison was performed between NIRS and the LH 750 Beckman Coulter for red blood cells (RBC). We used Pearson's R as correlation and established the Maximum Allowable Total Error based on Biological Variation Coefficient (BVC) as the minimum analytical quality specification (AQS). Device was tested for linearity, precision and accuracy. **RESULTS** Start with linearity, then precision and then accuracy. The AQS employed as acceptance criteria was the minimum BVC (6.6%), added to the Clinical Significance Assessment (CSA). The Progenos® NIRS device has met acceptance criteria for RBC with a Deming Regression slope of 0.885. RBC was analyzed by methods LH750 BC and PROGENOS to determine whether the methods are equivalent. The Deming Regression Statistics were: Correlation Coeff (R): 0.8663. Slope<sub>Dem</sub>: 0.885. Intercept<sub>Dem</sub>: 0.657. Std Error estimate: 0.885 (0.657 to 1.114). The difference between the two methods was within allowable error for 16 of 20 specimens (80.0%). The average Error Index (Y-X)/TEa was 0.54, with a range of -0.85 to 2.54. Also important, is that patients had all normal results in the analysis of the LH 750 equipment and the same was clinically seen by the Progenos equipment. **CONCLUSION** Here we describe a novel device (Progenos®) with the potential to offer clinical actionable information on red blood cell count as a proof-of-concept technique. Data was obtained for NIRS without the need of a blood sample. While the accuracy of the results require improvements to reach a minimum level of 80-90% against a less restrictive AQS, the clear correlations between methods show potential for further development of this technology, continuous studies and application in the clinical laboratory medicine field. Such technology would significantly remove gaps in areas where the clinical laboratory cannot reach or also in clinical disasters, or areas where a blood draw is not possible.

**B-002****How Much RHIG Does This Patient Need?**

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**Background:** A 31 year old pregnant female was admitted to our facility in active labor. Patient delivered 2 healthy male infants via Cesarean section. An Rh Immune Globulin (RHIG) work up was requested. The patient's blood type was AB negative and both infants were Rh positive. **Methods:** A fetal screen was performed by the Transfusion Service (TS) and was found to be positive. The patient sample was forwarded to the hematology lab for a Kleihauer Betke (KB) or Fetal Stain test to quantify the number of fetal cells present. Testing showed that 230 cells out of 4000 were fetal cells which was 5.8% content. Calculations were performed automatically as part of the testing which recommended 11 vials of RhIG. This seemed to be an excessive amount of RHIG for a patient when there were no issues with the delivery or the infants. The high number of calculated RHIG vials was questioned by the TS staff and concerns were pushed to the TS Supervisor for further evaluation.

**Results:** It is known that patients with hemoglobinopathies may continue to make fetal hemoglobin into adulthood to compensate for a hemoglobinopathy such as Sickle Cell Disease or Thalassemia. The quantity of fetal hemoglobin that continues to be produced by these types of patients varies considerably. Sickle Cell disease patients can have a range from 1-25% fetal hemoglobin as an adult and Thalassemia adults can range from 1-10%. The diagnosis history of the postpartum patient was evaluated and found to include "D56.9 Thalassemia, unspecified." Based on the patient diagnosis and known probability of autologous fetal cells a pre-delivery specimen was located and sent for the KB testing. The pre-delivery sample was found to contain 4.3% of fetal cells with a calculated dose of 8 vials of RHIG. The TS Medical Director was consulted, and it was determined to give the patients a total of 3 vials of RHIG rather than the originally calculated 11 vials.

**Conclusion:** A questioning attitude by the Transfusion Service Team and knowing the diagnosis of this patient was key to determining the possibility that the mother's autologous fetal hemoglobin was likely causing the extremely high fetal cells being detected by the KB test. It was also helpful that our hospital currently obtains a Type and Screen (TAS) specimen when a Mom is admitted to Labor and Delivery. The fetal screen test did detect the Rh positive cells from the newborns but is not quantitative, so the KB test is needed to measure the volume of a larger postpartum bleed. The KB Test detects fetal cells but not cells that are Rh positive. Having a pre-delivery specimen enabled staff to show that the large volume of fetal hemoglobin in the postpartum KB test was partially autologous and partially the newborns. Calculating the difference between the pre and post delivery fetal cell volume saved the patient from receiving a large volume of RHIG that was not needed and saved the cost of the additional doses of RHIG.

**B-003****Evaluation of Burosumab Interference in Intact and C-terminal FGF23 Immunoassays**

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**Background:** Intact and c-terminal FGF23 immunoassays are useful in the diagnosis of hypophosphatemic diseases such as X-linked or autosomal dominant hypophosphatemic rickets (XLH, ADHR) and tumor-induced osteomalacia (TIO). Burosumab is a fibroblast growth factor 23 (FGF23)-blocking human monoclonal antibody which is FDA approved for the treatment of XLH and TIO. Burosumab therapy binds and blocks FGF23 effects on target tissues, affecting phosphate concentrations. As this treatment becomes more widely used, understanding its in vitro and in vivo effect on FGF23 immunoassays is critical to ensure the proper interpretation of measured FGF23 concentrations. In vivo, extremely high FGF23 concentrations have been reported in patients taking burosumab when tested with c-terminal FGF23 immunoassays, whereas for intact FGF23 immunoassay measurements there have been conflicting reports due to potential negative analytical assay interference by burosumab. **Objective:** To determine the in vitro (analytical) effect of CRYSVITA® (burosumab-twza) on an intact FGF23 assay and a c-terminal FGF23 immunoassay. **Methods:** FGF23 concentrations were measured using an intact serum FGF23 assay (MedFrontier, Minaris Medical Co, Ltd, Tokyo, Japan), or a c-terminal plasma FGF23 assay (Immutopics, Quidel Corporation, Athens, OH). Two serum (32 pg/mL and 181 pg/mL (reference interval ≤ 59 pg/mL) and two plasma pools (85 RU/mL and 276 RU/mL (reference interval ≤ 180 RU/mL) were created. Stock solutions of burosumab (10X higher than final target concentrations) were prepared in saline. Patient sample pools were spiked with saline or the drug to achieve final burosumab concentrations spanning the therapeutic range: 0, 1.4, 2.8, 5.6, and 11.3 ug/mL. The percent (%) FGF23 recovery was calculated by comparing the measured FGF23 concentration in the presence of burosumab to the un-spiked sample for each assay.

**Results:** For the intact FGF23 assay, increasing concentrations of burosumab resulted in a negative analytical interference. A dose-dependent decrease of the measured FGF23 concentrations was observed. FGF23 recoveries ranged from 32% at the lowest concentration of burosumab (1.4 ug/mL) to 6% at the highest assessed concentration of burosumab (11.3 ug/mL). The c-terminal FGF23 assay showed no significant negative burosumab interference with recoveries ranging from 84% to 96%.

**Conclusions:** While the exact mechanism of burosumab analytical interference in the intact FGF23 assay is not well understood, the observed reduced recovery supports a scenario where the monoclonal antibody (drug) binds the n-terminal region of FGF23 and sterically interferes with the binding of at least one of the antibodies utilized in the intact immunoassay. In contrast, the absence of a burosumab analytical interference observed in the c-terminal FGF23 assay is likely because the assay's capture

and detection antibodies target the c-terminal portion of FGF23. Potential analytical interference in the presence of burosumab should be considered in the clinical interpretation of FGF23 determinations and monitoring serum phosphate concentrations may be more appropriate in cases of analytical interference.

## B-004

### LC-MS-MS-Based Lipidomic Profiling Reveals Altered Concentrations of Bioactive Eicosanoids in Pregnant Women with HIV Exposed to Combination Antiretroviral Therapy

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**Background:** Pregnant women with human immunodeficiency virus infection (HIV+) have a higher risk for adverse obstetric outcomes. The mechanisms that underlie these remain vague. Bioactive eicosanoids and lipid mediators play crucial roles in pregnancy. Altered concentrations of bioactive eicosanoids are associated with adverse obstetric outcomes including preterm delivery and fetal growth restriction. Lipid abnormalities are common in people living with HIV and are accentuated in those receiving combination antiretroviral therapy (cART). HIV infection has been linked with altered production of eicosanoids; however, there is a paucity of evidence to associate impaired metabolism of eicosanoids to adverse obstetric outcomes in pregnant women with HIV. The adoption of eicosanoids as clinical biomarkers has been challenging partly due to the lack of a robust methodology to accurately quantify and characterize the elusive eicosanoids of clinical significance. Our objective was to quantify and characterize the concentrations of bioactive eicosanoids in maternal and cord blood of HIV+ and HIV-uninfected (-) pregnant women using an unbiased, high-throughput quantitative liquid chromatography-tandem mass spectrometry (LC-MS-MS)-based lipidomics approach.

**Methods:** Seventy-six maternal plasma samples at gestational weeks 33-38 (39 HIV+, 37 HIV-) and 55 cord plasma samples (31 HIV+, 24 HIV-) were collected from pregnant Canadian women followed prospectively throughout gestation. All women with HIV were on protease-inhibitor-based cART. We quantified the concentrations of 139 eicosanoids in maternal and cord plasma using quantitative LC-MS-MS analysis with a 1290 UHPLC System and a QTRAP5500 Mass Spectrometer. Samples were ionized by electrospray ionization. The precursors to product ion mass transitions were obtained by scheduled multiple reaction monitoring. Quantitative analysis was performed by Analyst 1.5.2 Software. The limit of quantification is 0.025ng, with values between 0.005ng and 0.025ng considered semi-quantitative. Differences between groups for each eicosanoid were assessed using the Mann-Whitney test and corrected for multiple comparisons using a false discovery rate of 0.05. Spearman correlation was used to assess the relationship between eicosanoids in maternal and cord plasma.

**Results:** A total of 53 and 58 eicosanoids and bioactive lipids were identified in maternal and cord plasma samples respectively. Differences were observed in maternal and cord eicosanoid profiles, with only 3 eicosanoids correlating between compartments among the HIV- group and none among the HIV+ group. Pregnant women with HIV had higher concentrations of arachidonic, eicosapentaenoic acid, and docosahexaenoic acid, and elevated concentrations of lipoxygenase pathway-derived bioactive lipids including different classes of clinically relevant hydroxyeicosatetraenoic acids, which are known to possess inflammatory and vasoconstrictive properties. Only 3 eicosanoids differed significantly between the two study groups in cord plasma. All were vasodilating and pro-angiogenic dihydroxyeicosatrienoic acids and were lower in the HIV+ group.

**Conclusion:** Our data suggest that exposure to HIV/cART during pregnancy is associated with altered bioactive eicosanoid profiles in maternal and cord plasma and underscore the technical capabilities of our LC-MS-MS method for identifying eicosanoids of clinical importance. Altered maternal concentrations of inflammatory eicosanoids in the context of HIV and antiretroviral exposure may contribute to placenta insufficiency and altered in utero environment that could impact fetal development and cause adverse obstetric outcomes.

## B-005

### Analytical and clinical performance of two point of care rapid antibody assays for SARS-CoV-2

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**Background:** The SARS-CoV-2 pandemic has reached to a state where populations across the world should adjust to live with it like many other diseases. Regular serosurveys are essential for disease surveillance and policy decisions. The preferred quantitative ELISA methods cannot achieve the scale and speed needed to conduct such serosurveys in low- and middle-income countries (LMICs). The qualitative point of care antibody assays can be utilized. These tests are quick and scalable to provide the primary information on seroprevalence of SARS-CoV-2. In this study, we evaluated the analytical and clinical performance of two commercially available rapid antibody (IgG and IgM) assays. We found that both assays provide the optimal level of accuracy needed to be utilized in any serosurvey.

**Methods:** SARS-CoV-2 PCR positive patients (N=104) were recruited for method evaluation study of two commercially available lateral flow Rapid IgM and IgG assays Edinburgh Genetics ActivXpress+ COVID-19 IgG/IgM Immunoassay (EGCV0092L) and Abchek COVID-19 IgM/IgG Antibody Rapid Test (NUL/COV-19/R&D/001). We evaluated the analytical and clinical performance of the two antibody kits by fingerstick of the SARS-CoV-2 PCR positive (N=104) participants after 10-15 days of a PCR positive test. We have tested all the participants for SARS-CoV-2 with a Rapid Antigen Test (Abchek) on the day of antibody testing. To evaluate clinical performance of the assays, we analyzed 100 vaccinated people (50EdGen+50Abchek) for seroprevalence of IgG after 2-3 months of full vaccination. Statistical evaluation of qualitative test performance was performed following the CLSI EP12-A guidelines.

**Results:** The IgG seropositivity after 10-15 days of PCR positivity was 97.1% (Edinburgh Genetics Assay) and 92.3% (Abchek Assay). The qualitative method comparison using EP Evaluator demonstrates 95% positive agreement and 100% negative agreement between the two antibody assays. Non-parametric McNemar test showed none of the assays had significant difference in detecting the IgG sero-positivity (p=0.063), thus they are symmetric. The Rapid Antigen Test was found to be negative for all the samples with combined IgM negativity of 93.2% (Edinburgh Genetics Assay) and 98.07% (Abchek Assay). The IgG seropositivity of vaccinated participants (N=100) was 93% using both the assays. The overall sensitivity was 100% (Edinburgh Genetics Assay) and 95% (Abchek Assay) in detecting IgG in participants either post infection or post vaccination.

**Conclusion:** The manufacturer's claim of detecting the IgM within 10-15 days of SARS-CoV-2 infection could not be verified. The caveat in the study is that in a low resource setting, people might be getting PCR test late, 5-7days after the initial infection. The window of antibody testing is probably more than three weeks after the possible initial infection. Thus, IgM is well below the detection limits of these assays while the negative antigen tests also nullify active infection. However, both the methods could detect IgG seropositivity with comparable accuracy within 2-3 weeks of infection and 2-3 months of vaccination. These qualitative assays are cost-effective, robust and scalable. Both assays can be used for serosurveys in low resourced setting with desired scale and speed when a quick observation is needed for surveillance.

## B-006

### Monitoring the Stability of Biological Samples Detected for SARS-CoV-2 RNA After 12 Months at -20°C Through Cycle Threshold (CT) Value Comparisons

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**Background:** During the SARS-CoV-2 pandemic, Molecular Biology laboratories faced two major difficulties: intense sample flow and viral load degradation in samples preserved over time. They had as important challenges to identify ways to properly store the nasopharyngeal swabs and determine the time of preservation in conditions to retest and identify possible variants. This study aims to evaluate the storage time and preservation of primary biological samples of nasopharyngeal swab from patients detected for SARS-CoV-2 after 12 months of storage at -20°C by comparing the cycle thresholds (Cts) obtained in the technique of RT-PCR. **Methods:** The threshold cycles

(Ct) of 94 patient samples detected for SARS-CoV-2 have been compared. This comparison was carried out in two distinct stages: collection day “fresh sample”, March 2021 and after 12 months, February 2022 (both stages strictly followed the same extraction, amplification and analysis processes). Viral RNA extraction was performed using the MagaPure virus DNA/RNA purification KIT (BIGFISH, Hangzhou). For amplification and analysis, the RT-PCR technique was applied through the 2019-nCoV Kit (BIGFISH, Shanghai), using fluorescence markers for the ORF1 Genes (FAM), N gene (VIC) and internal control (ROX) together with the MA-6000 amplifier (Molarray, China). All analyzes were performed following the same parameters for the interpretation of results. The Cts values were used as parameters of possible degradation of the sample and divided into 3 groups: Group 1= Ct<25, Group 2= 25<Ct<30 and Group 3= Ct>30. Statistical analysis was performed by direct positivity agreement between the results of the first and second stages and by intraclass correlation (Fleiss, J.L. (1986)). **Results:** A positive agreement of 95.74% (90/94) was obtained. For group 1 (Ct<25), 46 samples were analyzed and the positivity rate was 100% (46/46), for group 2 (25<Ct<30) 12 samples were analyzed and the positivity rate was 100% (12/12) and for group 3 (Ct>30) 36 samples were analyzed and the positivity rate was 88.89% (32/36), with 2 results considered inconclusive (I) for presenting only one gene was present in step 2. Statistical analysis through ICC intraclass correlation obtained variance between groups=0.1344, experimental error=0.0171, p-value= p<0.0001, intraclass correlation=0.7745 and conclusion of excellent reproducibility, according to Fleiss, JL (1986). The agreement between the results is highly robust and indicates a small loss of viral load for group 3, so it can be defined that samples with Cts above 30, because they have a low viral load, may have degraded. **Conclusion:** A high positive agreement has been obtained in the analyzed results, so it can be concluded that the degradation time of biological nasopharyngeal swab samples is slow when properly stored at -20° C, and can be used for retests or other purposes. For those laboratories where there's a small physical space for storage, it is recommended to adopt the criterion of storing samples only with Ct below 30.

## B-007

### Validation of a Real-Time PCR Methodology for SARS-Cov-2 Detection at Laboratory Saude, Goiania, Brasil

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**Background:** During the new Coronavirus pandemic (SARS-CoV-2), molecular biology laboratories around the world has followed their validation programs, according to the legislation of each country. These laboratories has to compare results, perform inter-laboratory tests as well as intra-laboratory tests in order to maintain data reproducibility and ensure the effectiveness of their quality processes to produce reliable and reproducible results. The aim of this work is to carry out the validation of the implementation of the RT-PCR technique with the Laboratory Saude as a Molecular Biology laboratory at biosafety level 2. **Methods:** Twelve interlaboratory samples from nasopharyngeal swab collections in sterile tubes containing 2ml of 0.9% sodium chloride were evaluated, being these: 3 inter-operator samples for two molecular analysts and another 3 samples for inter-run validation (rounds on 3 different extraction machines). All viral RNA extraction has been performed using the automated system (BIGFISH, Shanghai) using the MagaPure virus DNA/RNA purification KIT (BIGFISH, Hangzhou). For amplification and analysis, the RT-PCR technique was applied through the 2019-nCoV Kit (BIGFISH, Shanghai), using fluorescence markers for the ORF1 Genes (FAM) and N gene (VIC) in addition to the internal control of reaction evaluated by fluorescence (ROX), together with the 96 well real time fluorescent quantitative cycler PCR instrument - MA-6000 (Molarray, China). All analyzes have been performed with the same parameters for analysis of results, following the manufacturer's guidelines. Intra-run and inter-run analyzes were statistically evaluated to evaluate standard deviations (SD) and coefficient of variation (CV), in addition to qualitative analysis of the results obtained in detected and not directly detected (positivity agreement). **Results:** The results of the 12 samples provided by the partner laboratory have been evaluated, and in 100% (12/12) we've obtained the agreement of positivity, the twelfth sample that presented a negative result, was also in agreement with the result obtained, but removed for this purpose. From later statistical calculations. The mean SD (standard deviation) was equal to 2.57 and the mean CV (coefficient of variation) found was 10.38%. The inter-operator evaluation, in the evaluation between two operators, presented mean SD=0.59 and CV=2.86%. On the other hand, the inter-equipment evaluation, based on 3 equipment, 2 BFEX-32 extractors (BIGFISH, Shanghai) and 1 BFEX-96 extractor (BIGFISH, Shanghai), showed a mean SD=0.45 and CV=0.02%. **Conclusion:** The validation of the RT-PCR (qPCR) methodology with the Laboratory Saude has showed excellent accuracy of results, demonstrating that both the technique and the kit are approved for use in the laboratory routine of the institution. A more refined look shows that the

variations identified in the inter-laboratory validation are greater due to conceptually known sampling errors. When comparing the results of the inter-operator and inter-equipment validations, there is a robust precision of the machinery in the Molecular Biology department and a small variation in the inter-operator results, where operator 2 obtained 66.3% greater precision. (Cts lower values) when analyzing each region evaluated, a fact that can easily be corrected through adequate technical training.

## B-008

### The Lumipulse® G β-Amyloid Ratio (1-42/1-40): A fully automated method that combines CSF concentrations of Lumipulse G β-Amyloid 1-42 and Lumipulse G β-Amyloid 1-40 into a numerical ratio

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The Aβ1-42/Aβ1-40 ratio demonstrates high concordance with amyloid PET when distinguishing amyloid deposition due to AD from alternative causes of mild cognitive impairment (MCI). This study was conducted to analytically verify the Lumipulse G β-Amyloid Ratio (1-42/1-40) assay and evaluate performance of the assay at a set cut-off of 0.058. **METHODS** The Lumipulse G β-Amyloid Ratio (1-42/1-40) consists of two Chemiluminescent Enzyme Immunoassays for the measurement of β-Amyloid 1-42 and β-Amyloid 1-40 on the LUMIPULSE G1200 System via two-step sandwich immunoassay method using monoclonal antibodies against β-Amyloid 1-42 and β-Amyloid 1-40 respectively. The amount of β-Amyloid 1-42 and β-Amyloid 1-40 in the specimen is obtained from the luminescence signals derived from the substrate AMPDP (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt). **RESULTS** Linearity for Lumipulse G β-Amyloid 1-42 was demonstrated to be 38 pg/mL to 2645 pg/mL and for Lumipulse G β-Amyloid 1-40 was demonstrated to be 158 pg/mL to 34459 pg/mL. The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for β-Amyloid 1-42 were 2.175, 11.6 and 38.0 pg/mL, respectively. The LoB, LoD and LoQ expressed through functional sensitivity of the β-Amyloid 1-40 assay were 0.970, 33.0, and 158.0 pg/mL, respectively. No high dose hook effect was observed for samples containing 159,072 pg/mL of β-Amyloid 1-42 or 168,426 pg/mL of β-Amyloid 1-40. The Lumipulse G β-Amyloid Ratio (1-42/1-40) demonstrated imprecision ≤5.8 % (total %CV) in seven human CSF-based panels assayed in replicates of two at two separate times of day for 20 days (n=80/panel). Interference studies for β-Amyloid 1-42 and β-Amyloid 1-40 assays, showing less than ± 10% between test and control samples for potential interferences, included 12 endogenous substances and 36 commonly used therapeutic drugs which were spiked individually into CSF. Cross-reactivity of the β-Amyloid 1-42 assay with other substances, specifically additional β-Amyloid species that are similar in structure to β-Amyloid 1-42 was not observed at cross-reactant test concentrations up to 10,000 pg/mL. Although high cross-reactivity (-78.443%) was observed with β-Amyloid1-43 tested at 5,000 pg/mL, circulating levels of β-Amyloid 1-43 in the intended use population do not typically exceed ~20 pg/mL. Similarly, β-Amyloid 1-40 demonstrated insignificant cross-reactivity to other β-Amyloid species tested at concentrations up to 200,000 pg/mL. Performance of the Lumipulse G β-Amyloid Ratio (1-42/1-40) was evaluated in a subset of 292 bio-banked patient samples from ADNI. Samples included had the following clinical diagnosis: subjective complaints (SCD, n=18), early MCI (EMCI, n=111), late MCI (LMCI, n=59), or Dementia (n=104). For the Lumipulse G β-Amyloid Ratio (1-42/1-40), a PPA of 85.9% and a corresponding NPA of 93.5% were obtained when comparing the Lumipulse G β-Amyloid Ratio (1-42/1-40) cutoff of 0.058 to the corresponding PET result. **CONCLUSIONS** The Lumipulse G β-Amyloid Ratio (1-42/1-40) test combines accurate, precise, and sensitive results of Lumipulse G β-Amyloid 1-42 and Lumipulse G β-Amyloid 1-40 assays for determination of Aβ 1-42 and Aβ 1-40 in human CSF. The Lumipulse G β-Amyloid Ratio (1-42/1-40) test is highly concordant with amyloid PET in patients over 55 with cognitive complaints.

## B-009

### Validation of a Rapid and Sensitive Method for the Determination of Cotinine and Trans-3'-Hydroxycotinine in Serum by LC-MS/MS

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**Background:** Cotinine and trans-3-hydroxycotinine (HC) are the main metabolites of nicotine. Due to higher concentrations and longer elimination half-lives, these metabolites are generally preferred over nicotine as biomarkers for tobacco use or exposure to secondhand smoke. A highly sensitive method in serum is necessary to differentiate nonsmokers from those who have exposure to secondhand smoke. The ratio of HC to

cotinine is also an established biomarker of CYP2D6 activity and may be used to estimate tobacco-related disease risk. The objective of this study was to develop a rapid and sensitive assay for the determination of cotinine and HC in serum using LC-MS/MS. **Methods:** 1 mL of serum was mixed with deuterated internal standards. 100  $\mu$ L of 30% perchloric acid was added to precipitate the protein. The mixture was then centrifuged, and the supernatant was mixed with 2 mL of 50% tripotassium phosphate and 8 mL of DCM: ETAC (4:1). The organic layer was vaporized under nitrogen to dryness at 40°C. The dried extract was then reconstituted with 200  $\mu$ L of 0.05% formic acid and 5 mmol/L ammonium formate in water: methanol (1:1) and analyzed by LC-MS/MS using a 12.5 minute gradient. **Results:** The method was shown to be linear from 0.025 to 10 ng/mL for cotinine and HC ( $R^2 \geq 0.998$ ). No endogenous or exogenous interferences were observed. The LOQ was determined to be 0.025 ng/mL for cotinine and HC. The accuracy ranged from 96.1% to 104.9% for cotinine and 98.9% to 102.9% for HC. The method demonstrated no significant matrix effects. No carryover was observed up to 1000 ng/ml for both analytes. Method validation results demonstrated good reproducibility with three levels of low, medium and high quality control materials. Coefficients of variation (%CV) for intraday and interday precision for all controls ranged from 0.9% to 7.1% for cotinine, and 1.0% to 9.4% for HC. The extraction recovery was 102.4% for cotinine and 96.4% for HC. The extract was found to be stable for up to 14 days.

**Conclusion:** A quick and sensitive LC-MS/MS method was successfully developed for measuring cotinine and trans-3'-hydroxycotinine in serum samples. The application of this method has facilitated the analysis of serum samples for a large-scale biomonitoring study in Alberta.

## B-010

### Development of a standardized whole-blood interferon gamma release assay on VIDAS® to detect SARS-CoV-2 specific T-cells

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**Background:** Immunity to SARS-CoV-2 involves more than just antibodies, virus-specific T-cells being also key players in the control of the infection. Indeed, it is described that a suboptimal T-cells response contributes to COVID-19 persistence and severity, whereas early strong T cell responses may be protective. SARS-CoV-2-specific memory T cells have also been detected in exposed seronegative healthy individuals. Diagnosing individual cellular immunity response is therefore paramount. Despite its importance, existing cellular diagnostic tools are very cumbersome which hinders their broad usage in clinical settings. This study aims to describe the development of a novel, easy to use, standardized Interferon Gamma Release Assay (IGRA) solution on VIDAS® instruments to detect memory T-cells in COVID-19 patients in Whole Blood (WB). WB assays are rapid, simple and have the main advantage to mimic *in vivo* environment as they preserve all interactions between circulating immune cells. To reveal SARS-CoV-2 specific T cell response, IFN $\gamma$  secretion after stimulation with specific SARS-CoV-2 peptides was detected. **Methods:** Freshly collected blood was collected in lithium heparin tube from different cohort of patients or healthcare workers (HCWs) previously infected by SARS-CoV-2 or HCoV at 6-month post-symptom and/ or vaccinated and in SARS-CoV-2 seronegative healthy volunteers (HVs). WB was (1) stimulated with SARS-CoV-2 specific peptide pools (2) left unstimulated as negative control and (3) stimulated with mitogen as positive control. After 22-h stimulation, the IFN- $\gamma$  release on the supernatant was measured on VIDAS® automated platform in international units (IU) per milliliter. Serological measurements were performed using the bioMérieux VIDAS® IgG anti-RBD SARS-CoV-2 assay. **Results:** Because WB IGRA relies upon detecting SARS-CoV-2 antigen-specific T cells in blood via memory T cell re-activation *ex vivo*, the choice and formulation of the SARS-CoV-2 antigen itself used for the T cell recall will critically define the IGRA performances. Also, in order to develop a SARS-CoV-2 specific T cells stimulation reagent, we previously developed an overlapping pooled-peptide epitope mapping approach covering the nucleocapsid (N), membrane glycoprotein (M) and the spike receptor binding domain (RBD) to allow testing of large numbers of SARS-CoV-2 T cell epitopes. Then, based on MHC affinity, predicted immunogenicity, sequence conservation between SARS-CoV-2 vs seasonal coronavirus and coverage of high frequency HLA alleles, we identified and selected a restricted pool of peptides (RPP) from the structural SARS-CoV-2 proteins as major CD4+ and CD8+ T cell targets. Performances of WB IGRA associated with this RPP-T cells stimulation

reagent were evaluated and validated in a different cohort of patients and HCWs in compliance with a CE marking. All these measurements were correlated with serological levels of antibodies against SARS-CoV-2. **Conclusion:** We demonstrate that WB stimulation by our unique SARS-CoV-2 peptide signature allows a sensitive detection of cellular immune response to SARS-CoV-2, thus allowing in fine to identify with great accuracy individuals presenting SARS-CoV-2 specific T-cell immune memory. Given its high ease-of-use and compatibility with all automated VIDAS® platforms, themselves well-known for their robustness, this novel SARS-CoV-2 IGRA solution is no-doubt a powerful diagnostic tool for patient management in clinical routine.

## B-011

### Performance of the FibroTest-ActiTest in a Single Analytical Platform for a Simplified Testing Workflow in a Clinical Laboratory

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**Background:** Chronic liver disease affects millions of individuals worldwide and up to 40% may develop serious complications including end-stage liver disease and hepatocellular carcinoma. A higher severity of inflammation and fibrosis increases the risk of patients with chronic liver disease for fatal outcomes. Due to the limitations, risks and invasive nature of biopsies, the gold-standard for diagnosis, a number of noninvasive testing approaches have been proposed for the diagnostic assessment of liver fibrosis, cirrhosis, and necroinflammatory response. One of such tests is the FibroTest-ActiTest (Biopredictive, France) computed from six biochemical assays, adjusted for age and gender, to generate 2 scores indicating the fibrosis stage (F0-F4) and inflammation activity grade (A0-A3). The FibroTest combines alpha 2-macroglobulin (A2MG), haptoglobin (HAPT), Apolipoprotein A1 (APOA), gamma glutamyltransferase (GGT), and total bilirubin (BILT). The ActiTest adds alanine aminotransferase (ALT). The clinical utility of the FibroTest-ActiTest has been described in patients with hepatitis, and other forms of liver disease including non-alcoholic fatty liver disease. This test usually requires referral to a specialized laboratory and the generation of the scores by Biopredictive's proprietary algorithm. According to their technical recommendations, Biopredictive has verified the transferability of their algorithms across several analytical systems. We evaluated the performance of the FibroTest-ActiTest using Roche Diagnostics assays (Indianapolis, IN) in our laboratory. **Objectives:** The objective of this study was to validate the FibroTest and ActiTest using a simplified workflow in a single platform to measure the biomarkers in the panel and to compare the FibroTest and ActiTest results obtained in our laboratory to the results in a reference laboratory. **Methods:** Previously, the performance characteristics of the FDA-cleared A2MG, HAPT, APOA, GGT (IFCC standardized), BILT, and ALT with pyroxydial phosphate activation (ALTLTP) tests were individually verified in the Roche Cobas c501 instrument by performing analytical measurement range, inter and intra-assay precision, method comparison in serum and reference interval studies. We then assessed the comparability of the FibroTest stage and ActiTest grade obtained in our laboratory (sent results directly to Biopredictive) and a reference laboratory (Quest Diagnostics) using leftover samples (n=20) and the categorical agreement for stages across laboratories was determined. Specimen stability studies at different storage conditions was also evaluated. Tests were performed according to Biopredictive's technical recommendations.

**Results:** The FibroTest and ActiTest results agreed for stage/grade in 85% (17/20) and 80% (16/20) of cases, respectively. The agreement within 1 stage/grade was 100% (20/20) for the FibroTest and 95% (19/20) for the ActiTest. Based on categorical agreement, the results of the panel were reliable in samples stored 7 days at 4-8 °C and 4 weeks at -80°C, but it was not stable at -20°C due to decreases in ALTLTP.

**Conclusion:** Our study demonstrated that the FibroTest-ActiTest algorithm calculations using biochemical assays by Roche Diagnostics in the cobas 501 were acceptable for clinical use. This is a simplified workflow in clinical laboratories and also minimizing sample volume requirements.

## B-012

### Performance Evaluation of the Second Generation CALiaGold®, a New Immunoturbidimetric Test for the Quantification of Human Calprotectin in Stool

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**Background:** Calprotectin is the main protein expressed by neutrophil cells, and it is considered one of the most interesting biomarkers of inflammation. Due to its dis-

trict specificity, the measurement of calprotectin in stools is widely used to detect inflammatory process in the intestinal tract. It is mainly used to distinguish inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, from non-inflammatory disorders like irritable bowel syndrome (IBS).

**Methods:** Sentinel Diagnostics has developed the second generation CALiaGold®, a particle enhanced turbidimetric immunoassay (PETIA) for the quantification of calprotectin in fecal extracts. The aim of the study was to evaluate the performances in an external clinical lab and under routine conditions on chemistry platform SENTIFIT® 270. CLSI derived evaluation protocols and an acceptance criteria of +/- 10% bias at clinical decision level have been adopted.

**Results:** The second generation CALiaGold® showed a limit of detection of 6.1 µg/g and a limit of quantification of 18.6 µg/g. The measuring range of the assay was between 20 - 2200 µg/g; concentrations up to 22000 µg/g could be measured in automatic rerun mode. No prozone effect was found up to 6000 µg/g. Calprotectin stability in the extraction buffer after sampling was 3 days at room temperature up to 37 °C and 14 days at 2-8 °C. The method comparison between the first generation (routine method) and the second generation of CALiaGold® assays showed a bias lower than 10% on routine stool samples. The sampling procedure was done with both old and new sample collection tube on the same sample. Each tube was analyzed on SENTIFIT® 270 with their own respective reagents. A total of 306 samples were included in the study with a range of concentrations ranging between 22 and 7800 µg/g. Passing-Bablok fit gave a slope of 0.96 (0.90 - 1.02), an intercept of 1.04 (-4.84 - 7.51) and R= 0.93 (0.91-0.94).

**Conclusion:** The outcomes of the study proved that the second generation CALiaGold® met the requirements for its use as IVD-MD and offers a valid alternative solution for in clinical laboratory.

## B-013

### Assay Migration Studies on the Beckman Coulter DxI 9000 Access Immunoassay Analyzer

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**Background:** The US FDA's guidance for industry and staff titled "Assay Migration Studies for In Vitro Diagnostic Devices" provides a least burdensome approach for the transfer of previously-approved assays from an existing to a new system. This approach enables use of rigorous analytical performance data in place of full clinical data to implement a cleared product on a new platform. The Beckman Coulter DxI 9000 Access Immunoassay Analyzer\* includes numerous updates and new features designed with a goal to improve laboratory workflows and provide quality results to support patient management. Such elements include improved pipetting capabilities, updated process monitoring, increased throughput, reliability enhancements, and software features focused on the needs of the operator. The analyzer also utilizes a new alkaline phosphatase substrate reagent that provides reduced time to result for every test as well as other benefits including improved signal-to-noise and reduced sensitivity to endogenous alkaline phosphatase interference. The existing menu of Beckman Coulter Access reagents is being transferred to this system. Data herein summarize results from analytical studies described within the assay migration guidance and obtained during verification testing of assays for cardiac troponin and alpha-fetoprotein on the DxI 9000 Access Immunoassay Analyzer.

**Methods:** Analytical studies for quantitative assays were performed as directed by the assay migration guidance to compare performance of the Access hsTnI and Access AFP assays across the existing Access 2 and new DxI 9000 systems. A comparison study was performed on a panel of >180 samples across multiple sites to assess the equivalence of results from the two platforms through regression and bias analysis based on CLSI guideline EP09c, 3<sup>rd</sup> ed. and comparison to zones of allowable total difference (ATD) calculated based on each assay's variability on the existing Access 2 system. Within-laboratory precision and reproducibility were evaluated following CLSI EP05-A3. Performance at low analyte levels was evaluated following CLSI EP17-A2. Linearity was evaluated following CLSI EP06-Ed2.

**Results:** Comparison studies for the Access hsTnI assay yielded 96.8% (90% CI: 95.5% - 97.8%) of all lithium heparin plasma results within the predetermined ATD zone. Comparison studies for the Access hsTnI assay yielded 97.1% (90% CI: 96.0% - 98.1%) of all serum results within the predetermined ATD zone. Comparison stud-

ies for the Access AFP assay yielded 99.7% (90% CI: 99.2% - 100%) of all serum results within the predetermined ATD zone. All estimated results including 90% CI met expectations detailed in FDA's assay migration study guidance. Other analytical studies yielded comparable performance for both assays across the existing and new platforms that met product design requirements.

**Conclusion:** The data herein present an exercise of the US FDA assay migration guidance applied to two assays from the Beckman Coulter Access menu. Individual assay data generated for the Access hsTnI and Access AFP assays on the DxI 9000 Access Immunoassay Analyzer\* in this study met study design criteria.

\* The prototype is not cleared nor approved for commercial use in any geography.

## B-014

### Performance Evaluation & Relevance of AI 100 Artificial Intelligence System for Image Based Analysis of Peripheral Blood Smears at a Tertiary Care Oncology Centre

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**Introduction:** For hematological oncology patients, morphological examination remains the first step for rapid and accurate diagnosis and optimal follow-up. Although there have been significant improvements in hematology analyzers, no significant progress has been made in terms of automation of peripheral blood cells. Irrespective of the analyzer, approximately 15% of the blood samples require manual microscopic observation, especially in hematological malignancies. This has led to a need for automation of the cytology platform in all laboratories. On this background we evaluate the AI100 powered by Shonit™ automated digital microscope at 40X magnifications and analysed by the cloud based AI platform Shonit™ for WBC differentials under 40X magnification. AI 100 works independent of hematology analyser and staining method used. The cloud based AI platform enables the hematopathologist to access the scanned images remotely and report.

**Methods:** The study was carried out on 52 blood samples, which included samples from oncology patients taking treatment in our hospital. We compared results for White Blood Cells (WBC) differential count between AI 100 and Advia 2120i (Siemens Healthcare Diagnostic Inc, NY, USA) automated hematology analyser. In case of discrepant results, the cells were reclassified manually by experienced hematopathologist. Quantitative parameters for WBC differential values were analysed for linear regression and mean absolute difference. Ability of the software to detect blasts was compared with manual differential count results.

**Results:** The total WBC count for the samples ranged between 0.09-45.23 X10<sup>9</sup>/L. Pearson correlation coefficient (R) and 95% CI for the WBC differential count post-reclassification of cells was as follows. Neutrophil (0.92 & 15.0,-25.87), Lymphocyte (0.95 & 25.84,-11.75), Monocyte (0.22 & 18.86,-20.18), Eosinophils (0.74 & 6.11,-7.11), Basophils (0.75 & 2.53,-2.81). Average difference (Bias) for the differential WBC count was as follows: Neutrophils (-4.98), Lymphocyte (7.04), Monocyte (-0.66), Eosinophils (-0.50), Basophils (-0.14). Blasts were detected in 28 of the 52 samples by AI100 and same was confirmed during reclassification. However, blasts were not detected in one case by AI 100 which had a total WBC count of 0.34 X10<sup>9</sup>/L and the lapse can be attributed to the low count. 50% samples had a total WBC count of <2 X10<sup>9</sup>/L, which is a challenge for manual differential count. However, AI 100 counted more cells than those possible on manual screening of slide in these samples.

**Conclusion:** Good correlation was observed between AI100, Advia 2120i and Manual Differential count. AI 100 with its cloud based platform is a promising tool in the field of hematopathology which can aid the pathologist in diagnosis and classification of blood cell counts.

## B-015

### Performance Evaluation of Abbott Neuron-Specific Enolase (NSE) Assay on Alinity and ARCHITECT Systems

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**Background and Objective:** NSE is a common marker for management of patients with small cell lung cancer (SCLC). The objectives of the study include: (1) to evalu-

ate the performance of a new NSE assay on Abbott Alinity and ARCHITECT systems; (2) to compare the Alinity and ARCHITECT NSE assays with Roche Elecsys NSE assay.

**Methods:** Key performance testing including precision, limit of quantification (LoQ), linearity and method comparison was assessed at the Ramathibodi Hospital, Thailand in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines. The 5-day precision evaluation was performed using a four-member precision panel consisting of two patient specimen pools and two levels of Quality Control (QC). LoQ was verified with 4 different serum pools with 5 replicates, 2 times each day for 3 days. Linearity was assessed using 7 levels of patient sample pools ranging from 2 to 400 ng/mL. Method comparison of the NSE assays among Alinity, ARCHITECT and Roche Cobas e801 was performed using 130 patient samples with NSE concentrations spanning a measurement range of 1.6-400 ng/mL.

**Results:** Within-lab (total) imprecision for Alinity and ARCHITECT NSE assays ranged from 1.5% to 2.9% CV for the QC and patient samples. The LoQ value of 1.6 ng/mL as claimed by the manufacturer was verified on the Alinity and ARCHITECT systems. The assay was linear in the NSE range of 2 to 400 ng/mL. Both Alinity and ARCHITECT NSE assays showed a good correlation with the Roche Elecsys NSE assay.

**Conclusion:** The Abbott NSE assay showed excellent precision, linearity and sensitivity performance on both Alinity and ARCHITECT systems. The assay correlated well with the Roche Elecsys NSE assay. It demonstrates suitability for NSE analysis for the management of SCLC patients in the clinical laboratory setting.

## B-016

### Assessing Cardiac Troponin I Recovery in Blood Collections from Normal Healthy Individuals

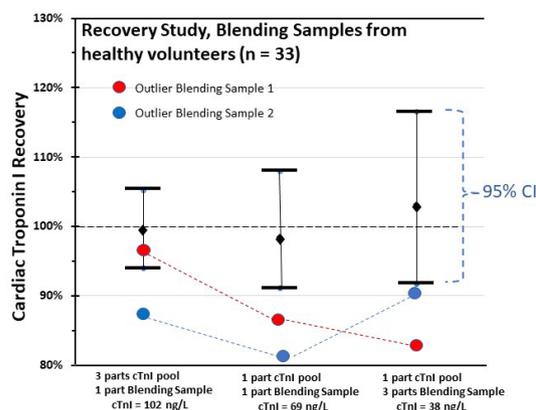
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**Background:** The IFCC Workgroup on cardiac Troponin I (cTnI) Standardization and the National Institutes for Standards and Technology are collaborating to produce a commutable cTnI reference material (RM) coined RM8121. RM8121 will be produced from pooling elevated cTnI samples from MI patients, and then blending with lithium heparin (Li-Hep) plasma from young healthy volunteers. However literature indicates that 10-20% of individuals may have autoantibodies that may negatively impact cTnI recovery and RM2181's value. We developed a simple method to identify low cTnI recovering blending plasmas from healthy volunteers.

**Methods:** RM8121 will consist of a 4-member set of Li-Hep plasma samples with cTnI values ranging from below the female 99th-percentile upper reference limit (URL) to 100-fold above the male 99th-percentile URL. To produce RM8121, samples were collected from 20 females and 39 males, age 31-89 years who had MI within the previous 72-hours. These high cTnI samples were blended with plasma from healthy females (n=16) and males (n=17), age: 23-38 years, to produce the 4-concentration RM8121 sample sets. All blending sample volunteers were non-reactive for infectious disease markers; history of cardiac disease or medications were exclusions. cTnI recovery was assessed by mixing a 135ng/L cTnI pool and each healthy blending sample in 3 proportions: 1 part cTnI pool:3 parts blending sample; 1 part cTnI pool :1 part blending sample; and 3 parts TnI pool:1 part blending sample. Recovery of each mixture for each blending sample was calculated.

**Results:** Figure shows cTnI recovery for the blending samples. Two of 33 blending samples (6%) were outliers, i.e. demonstrated decreased cTnI recovery.

**Conclusion:** Blending samples with decreased cTnI recovery may contain autoantibodies and/or other substances that inhibit cTnI recovery. Samples demonstrating cTnI under or over recovery must not be used for producing RM8121 or other materials intended for characterizing cTnI assays.



## B-017

### Confidence in your Calibrators: Metrologically Traceable Calibrators and Quality Controls for the LC-MS Analysis of Steroid Hormones

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**Background:** The routine analysis of steroid hormones is critical in understanding the function of metabolic pathways that impact sexual characteristics, inflammation and blood pressure. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) is fast becoming a sought-after technique in steroid analysis due to the advantages it provides over traditional ligand-binding techniques. These benefits include improvements in analytical sensitivity and selectivity, and the capability of multi-analyte quantitative detection in a single run. However, many LC-MS/MS methods lack harmonization or standardization. The Waters<sup>TM</sup> MassTrak<sup>TM</sup> Endocrine Steroid Calibrator and QC Sets (IVD) contain metrologically traceable materials, aiding laboratories in their compliance to ISO 15189, and provide confidence in the accuracy and harmonization of results when using validated LC-MS/MS methods. The performance of the MassTrak Endocrine Steroid Calibrator and QC Sets (IVD) was evaluated using a laboratory developed LC-MS/MS method.

**Methods:** The MassTrak Endocrine Steroid Calibrator and QC Sets (IVD) comprise the following twelve steroids in lyophilized human serum; testosterone, androstenedione, 17-OHP, 11-deoxycorticosterone, DHEAS, cortisol, 11-deoxycortisol, 21-deoxycortisol, corticosterone, DHT, DHEA, and progesterone. Internal standards were added to serum samples and precipitated with methanol, followed by dilution with water and centrifugation. An aliquot was transferred to a 96 well collection and the remaining supernatant was loaded onto an Oasis<sup>TM</sup> MAX SPE  $\mu$ Elution plate, washed and eluted with acetonitrile in water into the 96-well collection plate containing the previously transferred supernatant aliquot. The eluate was diluted with water and injected onto the LC-MS/MS system. Using an ACQUITY UPLC<sup>TM</sup> I-Class FTM system, samples were separated on a 2.1mm x 100mm CORTECS<sup>TM</sup> C8 2.6 $\mu$ m Column with an in-line filter using a water/methanol/ammonium fluoride gradient and quantified with a Xevo<sup>TM</sup> TQ-S micro mass spectrometer.

**Results:** We successfully demonstrated linearity of the Calibrator Set across the respective ranges for the steroids. Total precision and repeatability across the steroids at the three concentrations, with five replicates over five analytical runs (n = 25) was < 15% CV. Analytical sensitivity of the lowest calibrator was demonstrated with S/N > 10 (PtP) for all steroids. EQA material was evaluated to demonstrate accuracy for testosterone, androstenedione, 17-OHP, DHEAS, cortisol, progesterone and DHT. All measured values, using the commercial calibrators, were within 10% of the calculated LC-MS/MS mean concentrations for participating labs.

**Conclusion:** The Waters MassTrak Endocrine Steroids Calibrator and QC Sets (IVD), with the developed method described herein, provides linearity, analytical sensitivity, precision, and accuracy. The accuracy data demonstrates confidence in the Endocrine Steroid Calibrators, providing a key piece to the solution to steroid hormone standardization (ISO 15189) and harmonization challenges in clinical laboratories.

**B-018****Comparison of Two NT- ProBNP Assays**

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**Background:**

Heart failure is a progressive condition caused by a variety of underlying diseases such as coronary artery disease, hypertension, valve disease, myocarditis etc. Heart failure is the gradual inability of the heart ventricles to pump blood to the lungs and the extremities. Systemic immunoglobulin light chain (AL) amyloidosis is a protein misfolding disease caused by the conversion of immunoglobulin light chains from their soluble functional states into amyloid aggregates that deposit in various organs, heart and kidney showing the most damage. Scleroderma is a rare disease in which collagen accumulates in various organs, while causes are still unknown the accumulation of collagen can result in cardiac issues and chronic heart failure. An early identification is crucial to prevent irreversible organ damage. The development of sensitive biomarkers for the detection and quantification of organ involvement and damage is facilitating earlier diagnosis and improved evaluation of the efficacy of new and existing therapies. For the past 20 years natriuretic peptide biomarkers (BNP, nt-ProBNP) have become routinely used for the diagnosis and evaluation of Heart failure and to guide titration of Heart failure therapies. Using natriuretic peptides has also been associated with significant reductions in all-cause mortality compared to standard of care in patients with chronic heart failure. **Objective:** The present study aims to compare the Alere ARCHITECT NT-proBNP with the Roche NT-proBNP assay for precision, correlation, specificity, and overall concordance in non-cardiac specimens as well as populations of Heart failure, Amyloidosis, and Scleroderma patients. **Methods:** Total imprecision was evaluated per 5-day CLSI EP protocol, specificity and concordance were evaluated in a population of 300 individuals with no cardiac diagnosis, and 21 HAMA specimens, 13 Scleroderma, 16 Amyloidosis, and 100 heart failure patients. These were evaluated on the Roche Elecsys e411 as well as the Abbott ARCHITECT i2000SR platform. **Results:** The imprecision of the assays was comparable with 3.0% vs 4.5% total CV at 135 pg/mL and 3.5% vs 5.4% total CV at 5000 pg/mL on the ARCHITECT vs the Elecsys respectively. The two tests correlated well with an r value of 0.989 and a slope of 1.05. The concordance of the assays was a 100% on the symptomatic and disease populations reflecting excellent agreement. **Conclusions:** The Roche Elecsys and the Alere ARCHITECT NT-proBNP exhibit high analytical and clinical agreement.

**B-019****Method Comparison between the Glucose Isotope Dilution Gas Chromatography-Mass Spectrometry Reference Measurement Procedure and Point of Care Testing (POCT) Devices**

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**Background:** According to the CDC National Diabetes Statistics Report, 37.3 million people in the USA have diabetes. Glucose is one of the main biomarkers in diabetes management and is measured in regular clinical laboratories, as well as near patient settings in hospitals and at-home using point of care testing (POCT) devices. POCT can be a cost-effective way to screen and identify individuals potentially at risk for diabetes. Inaccurate and unreliable POCT measurements can lead to the misclassification of patients and incorrect treatment. With various studies reporting inaccurate measurements, especially in pathological glucose concentration ranges, there is a need to improve and monitor accuracy and precision of glucose measurements. An isotope dilution-gas chromatography-mass spectrometry reference measurement procedure (RMP) for glucose in serum has been previously developed and validated at CDC and can be used to assess the accuracy of POCT devices. In this study, agreement between the CDC glucose RMP and 2 blood glucose POCT devices was assessed.

**Methods:** Primary reference material NIST SRM 917c, D-Glucose (Dextrose) was used with the CDC glucose RMP to ensure traceability to the International System of Units (SI). Four levels of serum-based reference material NIST SRM 965b were analyzed as trueness controls. Analysis was performed using a 6890/5975 Agilent GCMS system. Glucose was measured in 20 single donor serum samples covering a glucose concentration range from 23.11 to 376.53 mg/dL using the CDC glucose RMP and 2 POCT devices suitable for serum testing. Samples were analyzed in 2 replicates over 2 independent runs by the CDC glucose RMP and analyzed in 3 replicates by POCT (POCT A and B). Accuracy of both POCT devices was compared to reference values obtained by the CDC glucose RMP.

**Results:** The CDC glucose RMP has a great reproducibility with inter-day and in-traday imprecision less than 0.85% with the trueness control materials, and with a total imprecision less than 1.11%. Accuracy of serum trueness control material was within  $\pm 0.67\%$ . The analytical measurement range is 1.51 mg/dL to 378.21 mg/dL ( $R^2 = 0.9999$ ). For POCT A, the mean bias was 9.45% (95% CI 6.00 to 12.90), and the sample specific imprecision ranged from 0% to 3.03%. For POCT B, the mean bias was -1.55% (95% CI -3.50 to 0.40), and the sample specific imprecision ranged from 0% to 4.18%. In POCT A, a higher positive bias was observed in hypoglycemic samples with concentrations below 50 mg/dL. The accuracy of glucose at hypoglycemic concentrations could lead to incorrect treatments. The lower ends of the analytical measurement ranges were different for both devices, making thorough assessments in the hypoglycemic range difficult.

**Conclusion:** The CDC glucose RMP allows to assess the performance of POCT devices and to identify challenges in certain concentration ranges. The data suggest a need for improving and monitoring the accuracy and reliability of POCT devices using RMPs with high accuracy and precision.

**B-020****Development of Routine Serum Free Thyroxine Assay Based on Equilibrium Dialysis ID-LC/MS/MS Procedure**

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**Background:**

Reliable free thyroxine (FT4) assay is important for diagnosis and treatment of thyroid diseases. Immunoassays (IAs) for FT4 measurements are most used in patient care. In the clinical laboratory community, 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. While the FT4 RMP is highly accurate and precise, it is not intended for routine clinical use. In the current study, we aim to develop a routine FT4 assay based on ED that can be traceable to the RMP.

**Methods:**

FT4 in serum was separated from protein-bound T4 in serum with an ED procedure following the principles described in CLSI C45-A guideline. FT4 levels could reach equilibrium after 18 hours in a commercially available micro-ED plate. The internal standard (<sup>13</sup>C<sub>6</sub>-T4) was added to collected dialysate. FT4 in dialysate was extracted by using a 96-well C18 SPE plate. FT4 in the samples was quantitated by using LC/MS/MS at positive electrospray ionization mode. The IRMM-468 certified primary reference material (JRC, Belgium) was used to prepare assay calibrators.

**Results:**

T4, Triiodothyronine (T3), reverse triiodothyronine (rT3), and other interferences in the dialysate were resolved on a C18 UPLC column using methanol gradient within 8 min. The linear range of the routine FT4 assay covered 1-100 pg/mL. There was no significant difference between the slopes of calibration curves in dialysate matrix and those in water indicating minimal matrix effect on FT4 quantification. The assay sensitivity allowed detection of 0.5 pg/mL FT4 in serum, which is sufficient for FT4 measurement covering clinically relevant ranges including hypothyroid patient samples. The mean bias of this method to the CDC FT4 RMP was within  $\pm 10\%$ . The imprecision of the routine assay, evaluated over four different days, was less than 10%. The use of a micro-ED plate in combination with an automated liquid handling system improves the throughput of the procedure. The stability of T4 calibrator stock and working solutions was confirmed at various conditions.

**Conclusion:**

Application of this FT4 routine assay based on ED will generate data traceable to the RMP, which can support the use of standardized FT4 measurements in patient care. The sensitivity, precision, accuracy and throughput of our method are suitable for routine FT4 measurements in clinical laboratories and large epidemiologic studies.

**Disclaimer:** The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry. Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service, and the US Department of Health and Human Services.

**B-021**

**Anti-SAE1 antibody in the diagnosis of paraneoplastic dermatomyositis**

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**Background:**

Dermatomyositis (DM) is an idiopathic inflammatory myopathy typically characterized by cutaneous disease followed by muscle weakness. Anti-small ubiquitin-like modifier-1 activating enzyme (anti-SAE1) antibody is the last antibody identified as DM marker which has been identified in a few patients.

**Methods:**

We performed a retrospective study to review records with suspicious of DM for anti-SAE1 antibody since its implantation in 2018. Serum samples were studied by DOT BLOT from Euroimmun® in a comprehensive profile of myositis-specific antigens, which includes 16 antigens: Mi-2 alpha, Mi-2 beta, TIF1g, MDA5, NXP2, SAE1, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ and Ro-52.

**Results:**

Of the 185 patients studied, only one patient presented anti-SAE1-positive antibodies. Patient was a 54-years-old Caucasian female with history of gastric adenocarcinoma, who started presenting after 6 months from a total gastrectomy an erythematous-decymative rash of wide distribution on arms, legs and neckline accompanied by Gottron’s papules on the hands. Her clinical presentation evolved rapidly, with a weight loss of 7 kg in one month, also reporting hyporexia and early satiety. Laboratory findings showed the next relevant results:

TUMOR MARKERS	
Carcinoembryonic antigen (ACE)	67.6 ng/mL [0.1-5]
Cancer antigen 125 (CA 125)	254.1 U/mL [0.5-35]
Cancer antigen 15.3 (CA 15.3)	27.2 U/mL [0.5-31]
Cancer antigen 19.9 (CA 19.9)	23533 U/mL [0.8-35]
Cancer antigen 72.4 (CA 72.4)	254 U/mL [0-8.2]
AUTOIMMUNITY	
Antinuclear antibodies (ELISA, quantification)	5.66 U/mL [0-0.99] (POSITIVE)
Antinuclear antibodies (title)	1/160
Antinuclear antibodies (pattern)	Nuclear speckled (AC-2,4,5,29)
Extractable anti-nuclear antigen antibodies (screening)	8.18 U/mL [0-0.99] (POSITIVE)
Extractable anti-nuclear antigen antibodies (DOT BLOT)	
Anti SSA/Ro-52 antibodies (DOT)	188 UR [0-15] (POSITIVE)
Anti SAE1 antibodies (DOT)	140 UR [0-15] (POSITIVE)

Due the constitutional syndrome and laboratory findings, a thoraco-abdominal-pelvic CT scan was performed, finding multiple hepatic, splenic and pulmonary metastases, which have been described in some investigations that shown a significantly higher frequency of malignancy in anti-SAE1-positive patients. Patient was diagnosed of paraneoplastic dermatomyositis.

**Conclusion:**

Anti-SAE1 antibody has been shown to present a very low diagnostic yield, however its appearance in a patient is diagnostic of the presence of DM. In addition, our patient presented a gastric adenocarcinoma that was accompanied by DM symptoms a few months later, what has been previously described in the literature. Unfortunately, anti-SAE1 antibody was not yet available in our laboratory at the first episode what could have been orientated the diagnosis and advanced a correct treatment.

**B-022**

**Cell counting in biological fluids: comparison of two automated methods**

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**Background:** Cytology of biological fluids is of great importance for the diagnosis of various pathologies. Traditionally, cell counting has been performed manually in Neubauer chambers with the aid of the brightfield optical microscope, which is dif-

ficult when dealing with liquids with a high number of cells. Nowadays, thanks to technological advances, new methods have been developed that allow the automation of cell counting in this type of sample. These are hematology and urine instruments that have incorporated a specific module for the analysis of biological liquids.

**Methods:** Sixty biological fluid samples were used: 20 pleural fluids, 3 pericardial fluids, 15 ascitic fluids, 12 peritoneal fluids and 10 synovial fluids. The samples were analyzed by means of two analyzers, Advia 2120i and Sysmex, whose techniques are flow cytometry without fluorescence and flow cytometry with fluorescence, respectively. The erythrocyte and leukocyte counts and, within these, the percentage of mononuclear and polymorphonuclear cells were studied. A descriptive study was carried out, the methods were compared using Passing Bablock nonparametric regression and a study of the differences was performed using the Bland-Altman plot. The statistical software used was R version 4.3.6.1. **Results:** The results of the Pasing-Bablock regression are: Red blood cells:  $R^2=0,332$ . Leukocytes:  $R^2=0,837$ . Polymorphonuclear cells:  $R^2=0,523$ . Mononuclear cells:  $R^2= 0,823$ . The study of the differences: Red blood cells:  $p=0,09$ . Leukocytes:  $p=0,33$ . Polymorphonuclear cells:  $p=0,001$ . Mononuclear cells:  $p=0,13$ . **Conclusion:** The correlation coefficient (R) differs considerably from unity, so we can interpret that there is no linear relationship between the parameters compared between the two instruments. As a main conclusion we can establish that the “gold standard” is still the cell count in Neubauer’s chamber through the optical microscope, while automated methods help us to establish an estimate of the real number of cells. Therefore, it is recommended to first make a manual evaluation under the microscope and then rely on the information provided by automated methods.

**B-023**

**Desorption Electrospray Ionization Mass Spectrometry Imaging as a Tool for Preoperative Classification of Thyroid Nodules**

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**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. To aid in preoperative thyroid nodule diagnosis, 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. Furthermore, we have applied this methodology for analysis of preoperatively collected FNA smears, with prediction accuracies greater than 80% (overall agreement per-nodule) compared to final histopathology results. Here, we present the extended validation study of the predictive performance of our classifiers on an additional independent set of clinical FNA smears, with a focus on the patient cohort with indeterminate preoperative FNA cytology.

**Methods:** FNA smears were prospectively collected from Baylor College of Medicine from patients undergoing surgical removal of thyroid nodules, frozen, and stored at -80°C until analysis. Samples were analyzed in the negative ion mode using a Q Exactive HF (Thermo Scientific) mass spectrometer coupled to a DESI imaging platform. FNA smears were H&E stained after analysis and evaluated by a pathologist. Mass spectra corresponding to clusters of thyroid cells were extracted for statistical prediction. Previously generated statistical models were used to predict on the extracted data, and the predictive performance of the models was assessed in correlation with pathology.

**Results:** Two classification models were generated from DESI-MS imaging data of thyroid tissue sections: benign thyroid versus papillary thyroid carcinoma (PTC) and benign thyroid versus follicular thyroid carcinoma (FTC). Overall, 159 FNA smears from 124 patients were analyzed using DESI-MS imaging (33 patients had two FNA smears prepared from the same nodule, and two patients had two nodules sampled). Within the 126 nodules sampled, 95 were benign, 30 were PTC, and 1 was FTC by final histopathology. The benign versus PTC model was used to predict on the benign and PTC FNAs yielding a per-nodule specificity of 93% and sensitivity of 83%. The FTC versus benign thyroid model was used to predict on the benign and FTC FNAs resulting in an 83% specificity and 100% sensitivity. Of the 126 nodules sampled for the FNA test set, 31 had an indeterminate preoperative cytology, including 24 benign, 1 FTC, and 6 PTC nodules. For this indeterminate subset of samples, the benign versus PTC model achieved an 83% sensitivity and 92% specificity while a 100% sensitivity and 83% specificity was achieved for the benign versus FTC model. We are currently working to assess additional FNA smears and transfer our DESI-MS workflow for thyroid FNA analysis to another mass spectrometer platform to expand its scope of applications.

**Conclusions:** Overall prediction accuracies of 93% and 81% were achieved for the benign vs. PTC model and benign vs. FTC model, respectively, for thyroid nodule classification. With the addition of DESI-MS, ~80% of unnecessary diagnostic surgeries could be prevented by providing improved preoperative specificity.

## B-026

### Pipeline for Rapid Autoantibody Biomarker Discovery and Development using Protein Microarray and Luminex Immunobead Platforms: Application- Companion Diagnostics for Lung Cancer Screening.

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**Background:** Lung cancer is the leading cause of cancer-related mortality and despite advancements in CT-based screening, uptake remains low in the population. Circulating biomarkers fashioned into a simple and cost-effective companion diagnostic test could revolutionize lung cancer screening. We have developed a pipeline for the discovery and development of novel candidate biomarkers to build a companion diagnostic method. This approach relies upon screening for candidate biomarkers using high-density protein microarrays, followed by translation of candidates into Luminex immunobead platforms for further testing. Specifically, we identified novel autoantibody biomarkers capable of discerning between population at “high risk” for lung cancer or with Benign lung lesions (BN) from those with pathologically-diagnosed lung cancer, including carcinoid, adenocarcinoma (AdCa) and squamous cell carcinoma (SqCC).

**Methods:** 148 serum samples were obtained from the Rush Biorepository, accrued from patients with pathologically-diagnosed lung cancer (n=35 AdCa, n=27 SqCC, and n=24 Carcinoid), pathologically-diagnosed non-malignant lesions (n=32 BN), and those at “high-risk” for lung cancer (n=30). Sample pools were created for these specimen sets, sampling each group 2-4 times, averaging 5 samples per pool. Each sample pool was submitted to CDI labs to run HuProt human protein microarrays, a platform which evaluates autoantibody reactivity to over 21,000 targets, using published protocols. Data was normalized across the sample pools and differential comparisons performed using eBayes in R. Candidate biomarkers were selected for Luminex-assay development based on significance levels and/or magnitude of application to possible relevant comparisons [i.e., AdCa vs. High-Risk, AdCa vs. BN, SqCC vs. High-Risk, SqCC vs. BN, carcinoid vs. High-Risk, carcinoid vs. BN, NSCLC (AdCa and SqCC) vs. High-Risk, and NSCLC vs. BN]. Luminex assays were developed using standard carbodiimide/NHS ester chemistry. Analytical assay performance characteristics were established for each assay using an anti-target/ PE-conjugated secondary reporter system. Validated assays were used to assess individual patient samples, defined above, with Luminex results contrasted to those from the HuProt microarray.

**Results:** 501 candidate biomarkers ( $p \leq 0.01$ ) were identified by HuProt microarray relevant to early diagnosis of lung cancer. From these findings, 8 biomarkers developed into Luminex Assays are featured: TAF10, PNMA1, GPBP1, NAP1L5, NAT9, ZNF696, HNRNPD, and RAB27A. As some of these markers showed significance for more than one comparison (described in Methods), a total of 17 comparisons were significant, which were subsequently re-assessed on the Luminex platform with individual samples. 12 of 17 comparisons that showed significant discernment via microarray, remained significant ( $p < .05$ ) when tested on the Luminex platform. In those not reaching significance via Luminex, three showed the same directionality. Interestingly, the three least significant comparisons were markers selected based on higher signal in the control. This suggests that potential markers should be filtered based on elevated levels in the disease groups. **Conclusions:** Herein, we define a convenient workflow for the discovery of candidate autoantibody biomarkers using high-density protein microarrays followed by target validation on the Luminex platform. Overall, this method shows excellent concordance between differential findings between cohorts on microarray and Luminex platforms, suggesting this discovery pipeline holds promise for a broad spectrum of potential diagnostic applications.

## B-027

### A Comparative Study Between Immunoturbidimetry & Chemiluminescence for Measuring Urinary Albumin

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**Background:** Several Immunoassays are used in medical laboratories to assay urinary Albumin. There are wide variations between the performance of these methods,

making it difficult for referral physicians to select among laboratories for diagnosing and monitoring patients. **Materials and Methods:** The present work is a comparative study between Immunoturbidimetry and Chemiluminescence methods used to assay urinary Albumin. The comparison was conducted on 108 random urinary samples. Both methods were verified first for precision, accuracy, linearity and analytical sensitivity. **Results:** The precision of both methods was tested by repeatability and reproducibility studies, using control materials and patient samples. All calculated coefficients of variation (CVs), for both methods, in all levels, were compared to Westgard desirable specifications for precision and they were all accepted. Accuracy was tested by calculating the mean percentage recovery for both methods and by calculating the bias percentage after testing a reference material. Both Immunoturbidimetry bias (7.4%), and Chemiluminescence bias (14.8%), were accepted based on the desirable bias % recommended by Westgard. The analytical measuring range of Immunoturbidimetry (0.9 - 130 mg / L) was better than that of Chemiluminescence (2.5 - 60 mg / L). One hundred and eight random urinary samples were tested using both methods, and their results were compared using Wilcoxon Signed Ranks test, Bland Altman Plot and MCNemar test, and all showed p-values > 0.05, for grouped samples and for samples divided based on a medical decision level of 30 mg / L. Regression line analysis showed a significant strong positive correlation between the two methods with 91% agreement (for 95% CI). (P value: <0.001, R Sq Linear: 0.91). **Conclusion:** Both Immunoturbidimetry and Chemiluminescence methods are comparable in measuring urinary Albumin, though Immunoturbidimetry had shown better analytical measurement range and accuracy. **Key words:** Urinary Albumin, Immunoturbidimetry, Chemiluminescence

## B-028

### Performance Evaluation of the Newly Developed N Latex aTNF $\alpha$ Reagent for Quantification of the TNF $\alpha$ Inhibitors Adalimumab, Infliximab, and Etanercept Using Automated Siemens Healthineers Immunonephelometry Systems

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**Background:** Tumor necrosis factor-alpha (TNF $\alpha$ ) antagonists like adalimumab (HUMIRA), infliximab (REMICADE) and etanercept (ENBREL) are currently quantified by time-consuming as well as labor-intensive methods like ELISA, which complicate the often needed automated testing on demand of those analytes. In contrast, the newly developed N Latex aTNF $\alpha$  reagent can be used for the quantitative, NIBSC standardized determination of adalimumab (ADA), infliximab (INF) and etanercept (ETA) in human serum and EDTA plasma using automated Siemens Healthineers Immunonephelometry Systems. The assay design is based on a competitive latex-enhanced assay principle, allowing the detection of all three TNF $\alpha$  inhibitors using a single reagent kit in combination with selective calibrators and controls.

**Methods:** Adalimumab (ADA), infliximab (INF) and etanercept (ETA) were assayed with N Latex aTNF $\alpha$  reagent in combination with dedicated calibrator sets (traceable to NIBSC standards) on the Siemens Healthineers BN<sup>TM</sup> II, BN ProSpec®, and Atellica® NEPH 630 Systems. Performance data were generated according to corresponding CLSI guidelines and include linearity, limit of quantitation, precision, interference, and method comparison to commercialized ELISA.

**Results:** Initial measuring ranges of all three assays cover the recommended therapeutic reference ranges demonstrated by LoQ and linearity studies (ADA/INF: 0.9-15 mg/L, ETA: 0.45-7.5 mg/L). Repeatability for ADA/INF/ETA ranged from 2.7 to 4.4%/5.2 to 6.5%/6.2 to 7.1% and total CV from 3.6 to 6.0%/5.9 to 6.6%/6.7 to 8.2%, depending on analyte concentration. Passing-Bablok regression results between the BN II System and ELISA were  $y = 1.080x - 0.144$  ( $r^2 = 0.933$ ) for ADA,  $y = 0.968x + 0.383$  ( $r^2 = 0.957$ ) for INF, and  $y = 1.088x - 0.056$  ( $r^2 = 0.901$ ) for ETA. No interference was observed for bilirubin, hemoglobin, and triglycerides.

**Conclusion:** The suitability of the newly developed N Latex aTNF $\alpha$  reagent for the quantification of all three TNF $\alpha$ -inhibitors - adalimumab, infliximab, and etanercept - was successfully proven by showing excellent performance data and high correlation with commercialized ELISA. Overall, this flexible 3-in-1 approach not only combines fully automated random-access testing for a streamlined workflow with fast results within minutes, but also achieves reduced handling, material consumption, and ordering complexity in the quantification of TNF $\alpha$  inhibitors.

*Product availability may vary from country to country and is subject to varying regulatory requirements.*

*Within US, the product is For Research Use Only. Not for use in diagnostic procedures.*

**B-029****Validation of a Radioimmunoassay Kit for the Specific and Reproducible Measurement of Oxytocin in Alternative Matrices**

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**Background:** Oxytocin (OT) is a nonapeptide hormone synthesized in the magnocellular neurons of the hypothalamus and released into systemic circulation or other areas of the brain. It has a variety of physiological roles such as stimulation of uterine contractions, social behaviors, and mood. Its small size and low levels in urine and saliva make it challenging to accurately measure. Measurements of OT using these alternative matrices are frequently requested due to their ease of collection and therefore we aimed to validate an assay with a focus on them. The most commonly used assay type are enzyme-immunoassay (EIA) kits due to their efficiency of measurement, however data from our lab demonstrated that reproducibility and specificity were insufficient in alternative matrices. While both LC-MS/MS and radioimmunoassays (RIA) are also available, we opted to test the Phoenix Pharmaceuticals (PP) OT RIA Kit. The goal of this study was to demonstrate specificity of antibody, sensitivity and reproducibility of the PP OT RIA kit for use in human urine and saliva.

**Methods:** Pooled material was created for urine and saliva, urine was acidified, and all pools were stored aliquoted at -80°C. Specificity of the antibody was assessed by HPLC-UV separation and assay of the fractions. Briefly, samples were extracted using 3mL C18 solid-phase extraction cartridges then dried in a centrifugal concentrator and reconstituted in 20% acetonitrile in 0.01M P04 buffer. Fractions (0.5mL) were collected for 6 minutes after injection on a 150x4.6mm C18 column then run in the kit according to the insert. Immunoreactivity was evaluated using the % bound, and the fraction times were compared to the retention time of an intact OT standard to determine which fractions would contain OT from the extracted samples. Reproducibility was assessed by running replicates of each pool over a number of assays. Sensitivity was assessed by repeated measurement of physiologically relevant low concentration specimens.

**Results:** In all tested specimens the greatest reactivity in assay corresponded to the same fraction as pure OT. Only minimal reactivity was found in the other fractions, suggesting that in an unfractionated sample the antibody reacts mostly with intact OT. In further support of this finding, the OT concentration from the fraction that contained the intact OT was similar to that of the unfractionated sample. While a slight matrix interference was found in urine samples, it did not reduce sensitivity enough to adversely affect measurement of OT. Reproducibility was acceptable for all specimens: in urine the inter-assay and intra-assay CV ranged from 9.85-11.63% and 6.71 to 8.04%, respectively. For saliva, these values were 7.89-12.8% and 4.06-5.85%. The sensitivity of the PP OT RIA was sufficient for measurement of urine pools (0.643 & 1.43 pg/mL) and saliva pools (0.485 & 4.42pg/mL).

**Conclusion:** PP oxytocin RIA is specific for measurement of intact OT in human urine and saliva. Data demonstrated that the reproducibility and sensitivity were acceptable for measurement of OT in all tested bio-specimens.

**B-030****Simultaneous Determination of Pterin Biosynthesis and Regeneration Pathway Metabolites by LC-MS/MS in Serum**

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**Introduction** Pterins are involved in essential metabolic reactions in humans, thereby their deficiency could impair the conversion of phenylalanine (Phe) to tyrosine (Tyr), the generation of nitric oxide, the hydroxylation of tyrosine and tryptophan. The cofactor tetrahydrobiopterin (BH4) is required in the chemical reaction of phenylalanine hydroxylase (PAH) transforming Phe into Tyr<sup>[1,2]</sup>. For the differential diagnosis of hyperphenylalaninemia (HPA), the analysis of pterins within the framework of the diagnostic flow charts recommended by international guidelines is of great importance<sup>[3,4]</sup>. The most broadly utilized method for BH4 quantification was still the indirect chemical oxidation (ICO) which included a pair of pH-differential iodine oxidation treatments and two corresponding HPLC-fluorometric runs<sup>[5,6]</sup>. Owing to drawbacks inherited to oxidative treatments, the tendency toward simultaneous detection of native pterins has gained increasingly attention to extend clinical validation. **Objectives** The main objective of this study was to accomplish quantitative profiling of metabolites related to biosynthesis and regeneration pathway of BH4 metabolism consisting of BH4, dihydrobiopterin (BH2), neopterin (N), sepiapterin, biopterin, primapterin, 7,8-dihydroneopterin (NH2), xanthopterin and isoxanthopterin in human serum specimens by a straightforward and cost-effective LC-MS/MS-based methodology. **Methods** According to developed analysis method, serum samples were prepared by "protein crash and shoot" approach. Sample preparation procedure included three steps;

100 µL of calibrant/sample was pipetted into a centrifuge tube. Then, 25 µL of internal standard mixture was added and vortexed for 5 sec. Next, 375 µL of reagent for de-proteinization containing antioxidant and protective cocktail was added to the tube, vortexed for 5 sec. and centrifuge at 3600 x g for 5 min. Finally, decant the supernatant into an amber HPLC vial prior to LC-MS/MS injection. Analysis of samples were conducted on Agilent HPLC system coupled with Agilent 6470 LC/MS. The total run time was 12.0 min. Quantification of the metabolites was performed using calibration curves generated with the concentrations of the synthetic serum-based calibrators with compensating for matrix effect according to yields of the assigned stable labelled isotope as internal standards. **Results** The methodological verification results showed that the method met the expected requirements and objectives in terms of sensitivity, accuracy, and precision. The calibration curves were linear over the defined concentration range and  $r^2 > 0.996$ . The values of %RSD (inter & intra-day) were within the analytical acceptable ranges. **Conclusions** In this work, a simple and rapid LC-MS/MS based bioanalytical method has been developed for quantitation of serum BH4, BH2, N, sepiapterin, biopterin (6-biopterin), primapterin (7-biopterin), 7,8-dihydroneopterin NH2, xanthopterin and isoxanthopterin in a single chromatographic run (12 min.) without employing oxidation reaction and/or derivatization. **References** [1] Qu J., et al., *British Journal of Clinical Pharmacology*, (2019), 85:893-899. [2] Valdés C., et al., *Journal of Chromatography B*, (2017), 1055-1056, 113-118. [3] Opladen T., et al., *Orphanet Journal of Rare Diseases*, (2020), 15:126. [4] Wegberg van A. M. J., et al., *Orphanet Journal of Rare Diseases*, (2017), 12:162. [5] Xiong X., et al., *Biomedical Chromatography*, (2018), e4244. [6] Yuan T-F., et al., *Free Radical Biology and Medicine*, (2018), 118, 119-125.

**B-031****Evaluation of the Buhlmann Anti-MAG (Myelin Associated Glycoprotein) Autoantibodies ELISA for Distal Acquired Demyelinating Symmetric Neuropathy**

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**Background:** Autoimmunity targeting Myelin Associated Glycoprotein (MAG) is associated with the clinical phenotype of distal acquired demyelinating symmetric (DADS) peripheral neuropathy. Distinguishing acquired demyelinating neuropathies by phenotypes can assist in evaluating responses to immunomodulating treatment. Patients typically present with slowly progressive symmetric sensory ataxia with/without distal weakness and an IgM Monoclonal Gammopathy of Undetermined Significance (MGUS). The objective of this study was to evaluate the Buhlmann MAG kit assay, a semi-quantitative ELISA-based method utilizing purified human MAG.

**Methods:** The Buhlmann assay is a semi-quantitative ELISA kit, utilizing 4 lyophilized calibrators [1000, 3000, 15,000 and 70,000 BTU- Buhlmann Titer Units] and 2 quality controls. The analytical performance characteristics were evaluated using residual serum samples covering the analytic measuring range of the assay. Studies were performed across three reagent kit lots. Studies included imprecision, stability, analytical sensitivity and specificity, linearity, accuracy, diagnostic performance.

**Results:** Intra-assay imprecision (coefficient of variation; %CV) was  $\leq 13\%$  for MAG concentrations ranging from 3000 - 50,000 BTU. Inter-assay imprecision (%CV) was 19% for a serum pool in the 3000 BTU range, 10% for a serum pool in the 30,000 BTU range and 21% for a serum pool in the 50,000 BTU range. IgM-MAG antibodies were stable for 28 days when tested at ambient, 4°C, -20°C conditions and through 4 freeze/thaw cycles. Manufacturer limit of quantitation (LOQ) was set at 900 BTU (CV <10%); our imprecision testing on serum within 20% of this value yielded 9.9% CV. Based on utilization of 4 parameter standard curve formula, approximate values for limit of detection and limit of blank were 300/200 BTU respectively. Manual dilutions of 3 high positive sera showed acceptable mean recoveries (within 80% - 120% for all samples) when samples were diluted 2-fold until they reached lowest calibrator range (1000 BTU). To assess analytical specificity, 20 samples with high concentrations of immunoglobulins (hypergammaglobulinemia [N=10] or IgG M-protein [N=10]) were tested and all were negative. Interference studies were conducted on 2 low positive MAG serum samples, spiked for highly hemolytic (1000 mg/dL), lipemic (2000 mg/dL) and icteric (60 mg/dL) conditions. All samples remained positive for MAG under high levels of a given interferent. Based on published literature a diagnostic cut-off of 1500 BTU was selected and the performance of this cut-off was assessed using 207 control samples (healthy donors [N=141], ALS [N=27], CIDP [N=11], IgM MGUS without polyneuropathy [N=28]) and 76 cases with an electrodiagnostically-confirmed DADS phenotype. Clinical specificity and sensitivity were 97.1% and 64%, respectively. Using a high diagnostic cut-off of 10,000 BTU, clinical specificity improved to 98.1% but clinical sensitivity decreased to 57.9%. A qualitative method

comparison study was performed to assess accuracy. We compared this assay to a western blot reference method [positives reflexed to titered ELISA assay] on 18 positive and 10 negative samples and found qualitative agreement of 96.4%.

**Conclusion:** The observed performance of the Buhlmann anti-MAG ELISA was adequate for clinical laboratory implementation. A cut-off of 1500 BTU was selected for clinical use.

## B-032

### Evaluation of a fully-automated random-access LC-MS/MS platform as a standalone clinical analyzer

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**Background** LC-MS/MS has proven to be a powerful platform in clinical diagnostics, yet there continues to be some challenges in bringing it to routine clinical practices. These are attributed to costs, technical difficulty and non-ideal user-experiences, especially the manual and labor-intensive nature of lab developed tests (LDT). Nonetheless, progress has been made towards the automation of LC-MS/MS into closed-system clinical analyzers. This study discusses one of the first performance evaluations of the Cascadion™ SM Clinical Analyzer - a fully-automated and random-access dual-channel-LC-MS/MS platform - at University of Rochester Central Laboratory. The clinical analyzer employs an FDA-approved assay for the quantitative analysis of 25-hydroxy vitamin D in human serum, whereby the user only needs to load sample vials to the system, since sample preparation, analysis and data-analysis are already included in the package.

**Methods** Within-day (n = 4) and between day (n = 5) precisions were evaluated on the platform with three levels of QC samples (10, 30 and 90 ng/mL). Accuracy was evaluated using blinded CDC samples (n = 120) and CAP Accuracy Based Vitamin D (ABVD) samples (n = 3). Patient samples (n = 120) were used to compare the performance between Cascadion and Roche platforms. In addition, the effect of interference was evaluated in both platforms by measuring VitD in serum (n = 120) containing abnormally elevated triglyceride levels (TG > 250 mg/dL).

**Results** Between-day (5-day, two channels) precision indicated CV ≤7% and bias within ±15% per QC level for VitD2, VitD3 and total VitD. Within-day and single channels precision were comparable. Long-term continuous monitoring of QC results (n = ~180 data points) indicated less than 10% CV for total, VitD2 and VitD3 levels. The Cascadion ABVD results met the CAP proficiency specifications. Additionally, excellent agreement was observed in the analysis of blinded CDC samples with linear 1:1 fits of 0.96\*CDC - 0.62 (r = 0.995) and 1.12\*CDC - 3.41 (r = 0.978) for the Cascadion and Roche assays respectively. However, a positive bias was observed in the Roche analysis of normal patient samples (n = 120) with an observed fit of 1.27\*Cascadion - 5.06 (r = 0.934) and a mean bias of +11% within a limit of agreement (95% CI) of -15% to 39% bias. Conversely, in the analysis of patient samples with abnormal TG levels (n = 111), a negative bias was observed with a fit of 1.07\*Cascadion - 3.8 (r = 0.928) and a mean bias of -8% within a limit of agreement (95% CI) of -38% to 22% bias.

**Conclusions** The Cascadion was successfully evaluated for 25-hydroxy vitamin D in human serum in a routine clinical laboratory. The platform compared favorably in precision, accuracy and assay comparisons, to a local IA platform. In our hands, the platform deployment is feasible as a standalone analyzer in the clinic, which holds promise to alleviate technical and staffing challenges in implementing LC-MS/MS platforms in clinical laboratories to serve patient needs.

## B-033

### Correlation between plasma sarcosine and erythrocyte folate : analysis of a retrospective cohort.

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#### Background:

In the literature, elevated plasma sarcosine levels are found in a specific inherited metabolic disease, SARDH deficiency, for which only 30 cases have been reported in the last 30 years. Recent articles suggest that plasma sarcosine may also be a marker for prostate cancer. However, the causes of its elevation are still poorly understood and require investigations. We suggest that alterations in one-carbon metabolism, and in particular folate metabolism, may be involved.

#### Methods:

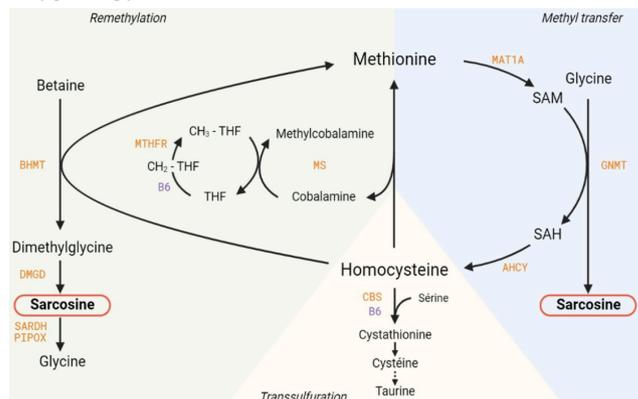
We studied erythrocyte folate compared to plasma sarcosine in patients with disrupted amino acid chromatogram. We retrospectively extracted the laboratory data of patients who were received at Lille University Hospital from 2020 to 2022 who had a disrupted aminoacidogram, and their associated erythrocyte folate. Amino acids, including sarcosine, were measured by liquid chromatography-mass spectrometry using the aTRAQ™ method. Erythrocyte folates were determined by chemiluminescence immunoassay on UNICEL DXI 800 (Beckman Coulter Inc., Brea, CA, USA).

#### Results:

We extracted biological data from 117 patients with plasma sarcosine concentration > 1µmol/L. We showed a positive correlation between plasma sarcosine and erythrocyte folate (p-value < 0.001 ; r = 0.39).

#### Conclusion:

These results suggests two hypothesis: First, high sera folate could be due to the formation of glycine from THF and serine, then metabolized to sarcosine by GNMT, related to an increased metabolic flux of the folate-dependent homocysteine remethylation pathway. Second, high folate could be related to a decrease of the folate-dependent remethylation cycle, leading to increase of BHMT-mediated homocysteine remethylation and hence, increased sarcosine production from betaine. This second hypothesis could explain sarcosine increase in prostate cancer patients, in case of decreased folate metabolism in this context. Further investigations are needed to clarify underlying mechanisms. In order to explore our hypothesis, it might be interesting to study plasma glycine and betaine.



## B-034

### Direct Quantitation of Phosphatidylethanol (PEth) in Volume-Controlled Dried Blood Spots using the Fully Automated Transcend DSX-1 System

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**Background:** Phosphatidylethanol (PEth) are specific biomarkers for repeated alcohol consumptions. The most abundant homolog of PEth, PEth 16:0/18:1, is usually quantified via liquid chromatography-tandem mass spectrometry (LC-MS/MS) in the whole blood. PEth can rapidly degrade in blood after sample collection if not stored frozen. In contrast, PEth is stable once prepared as dried blood spots (DBS), which is also minimally invasive and only requires 10 to 20 µL sample volume. Here, we describe a complete and fully automated workflow to rapidly extract and quantify PEth 16:0/18:1 in DBS using the Thermo Scientific™ Transcend™ DSX-1 system. The DBS collection at the fingertip is facilitated with HemaXis DB10™ device that allows for precise volume-control of the blood sampling.

**Methods:** Calibration and QC samples are spotted via HemaXis DB10 device, which ensures that a precise 10 µL blood is loaded on the DBS cards. PEth 16:0/18:1 is extracted with the innovative flow-through desorption technology with a heated clamp, and the internal standard (IS) is introduced via an automated IS addition module in the dried spot autosampler. The 2-dimensional TurboFlow technology allows interferences removal in the extracted samples before the analytical separation. An integrated software, Aria MX, controls every step of the sample desorption and separation. Analyte quantitation is performed on the Thermo Scientific™ TSQ Altis™ mass spectrometer, and the data is analyzed on TraceFinder™ software.

**Results:** The most abundant PEth homolog, PEth 16:0/18:1, is quantified in DBS cards using a rapid automated method on Transcend DSX-1 system. DSX-1 combines a dried spot autosampler for direct analyte extraction with Thermo Scientific™ Transcend™ UHPLC for online sample separation using the TurboFlow technology. The method only takes 8-min from analyte extraction to MS detection. Analyte carryover is minimized by the usage of a C8 analytical column and the rapid aqueous/organic washing steps. Accuracy and precision data of QC samples at two levels, 50 and 200 ng/mL, are obtained from two days with % accuracy at  $100 \pm 10\%$  and % RSD below 10%. The results are comparable with those from the manual disc-punch method of the same QC samples performed by our collaborators in Switzerland. Calibration curves are built using a weighting factor of  $1/x$  from a lower limit of quantification of 20 ng/mL to an upper limit of quantification of 2000 ng/mL with  $R^2$  values greater than 0.99, and % RSD and % Diff < 15% that meets cut-off needs of PEth monitoring in the clinical setting.

**Conclusion:** Transcend DSX-1 combines a dried spot autosampler and the TurboFlow LCMS/MS, and provides a complete workflow for fast and robust quantification of small molecules in dried matrix spots. The association of volume-controlled HemaXis blood collection device together with the current DSX-1 method provides an accurate and efficient quantification of alcohol specific biomarker PEth in blood.

## B-035

### A Clinical Research Method for Low Level Quantification of Eight Vitamin D Metabolites

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**Introduction:** While the role of vitamin D in bone metabolism is well established, comparatively little is known about its role in other diseases, although recent research suggests possible links between vitamin D deficiency and a variety of diseases. Liquid Chromatography-Mass Spectrometry (LC-MS) can provide improvements in analytical sensitivity and selectivity, and the capability of multi-analyte quantitative detection in a single run. Described here is a simple clinical research method for the extraction and analysis of eight vitamin D metabolites from human serum by LC-MS, reaching the low analytical sensitivity levels required and utilizing a Hamilton STAR Liquid Handling Robot, allowing for sample tracking from the primary tube to processed results. **Methods:** In-house vitamin D metabolite calibrator and QC samples were prepared in lyophilized human serum, comprising of 1,25-dihydroxyvitamin D3 (5-1000pg/mL), 1,25-dihydroxyvitamin D2 (10-1000pg/mL), 24,25-dihydroxyvitamin D3 (0.05-30ng/mL), 24,25-dihydroxyvitamin D2 (0.05-30ng/mL), 25-hydroxyvitamin D3 (1.3-150ng/mL), 25-hydroxyvitamin D2 (0.4-150ng/mL), C3-epi-25-hydroxyvitamin D3 (0.13-30ng/mL) and C3-epi 25-hydroxyvitamin D2 (0.05-30ng/mL). Internal standards were added to 200µL of serum samples and precipitated with zinc sulfate and methanol, followed by centrifugation. The supernatant was loaded onto an Oasis™ PRiME HLB SPE µElution™ plate, washed with methanol in water and eluted using acetonitrile into the 96-well collection plate. A simple PTAD derivatization was performed on the eluate and following incubation at room temperature, distilled water was added to stop the reaction and this was directly injected onto the LC-MS system. Using an ACQUITY™ UPLC™ I-Class system, samples were separated on a 2.1mm x 100mm CORTECS™ Phenyl 1.6µm column with an in-line filter using a buffered water/methanol/acetonitrile gradient and quantified with a Xevo™ TQ-XS mass spectrometer. Isobaric and isomeric interferences were chromatographically resolved, including the C3-epimers to allow for their independent quantification. Additional dihydroxyvitamin D metabolites, *i.e.*, those not measured in this assay, were also found to not interfere. **Results:** Calibrators were shown to be linear across the respective ranges for the vitamin D metabolites. Total precision and repeatability for all vitamin D metabolites at three concentrations, with five replicates over five analytical runs (n=25) was < 15% CV. Low level quantification for 1,25-dihydroxyvitamin D3 was achieved down to 5pg/mL. EQA samples were evaluated for 25-hydroxyvitamin D3, demonstrating good agreement with minimal bias. EQA samples were also evaluated for 1,25-dihydroxyvitamin D3, with good agreement but a negative bias was observed. One potential explanation for the negative bias is the possibility of interference from other dihydroxyvitamin D metabolites that are chromatographically resolved using this method but not by other methods. Further research will be performed to establish the cause of the bias. **Conclusions:** A semi-automated clinical research method for the analysis of vitamin D metabolites has been developed, providing good linearity, precision, and accuracy from only 200µL of human serum. Low level quantification (5pg/mL for 1,25-dihydroxyvitamin D3) required for research of vitamin D metabolites were achieved in a relatively simple extraction method, that can be easily automated for processing large volumes of research samples. For Research Use Only, Not for use in diagnostic procedures.

## B-036

### Bioanalytical Solid-Phase Microextraction (BioSPME) for sample preparation for clinically relevant hormone analytes and correlation to externally validated methods.

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#### Background:

Measurements of free hormone levels are routinely performed in clinical applications to help diagnose health concerns. There are two primary methods that have been used for quantification: immunoassays and liquid chromatography tandem mass spectrometry (LC-MS/MS) usually in conjunction with a sample preparatory technique like equilibrium dialysis (ED). Here a new and quicker sample preparatory technique is applied, BioSPME (bioanalytical solid phase microextraction), prior to analysis by LC-MS/MS for testosterone (Te), triiodothyronine (T3), and thyroxine (T4).

#### Methods:

A 96-pin BioSPME device coated with C18 sorbent was utilized for the preparation of serum samples. Calibrators were prepared fresh daily in phosphate buffered saline (PBS). Two separate methods were developed; one automated utilizing a Hamilton Starlet robot, and a manual method utilizing a heated shaker. Briefly, the analytes, Te, T3, and T4, were extracted from 200 µL of serum and 200 µL PBS simultaneously, before desorption in the presence of a stable isotopically-labelled internal standard. The analyte(s) was/were derivatized if applicable before quantitation by LC-MS/MS on an Agilent 1290 LC connected to an AB Sciex 6500+ QQQ. A qualifier and quantifier transition were used for each analyte and for each internal standard. The serum samples analyzed were pre-evaluated by a validated ED LC-MS/MS method from three external laboratories. Some of the serum samples were collected in collaboration with the Clinical & Translation Science Institute at Penn State University.

#### Results:

Following optimization of analyte transitions, the instrumental LOD and LLOQ were determined by injection of serial diluted samples. Testosterone was derivatized with hydroxylamine hydrochloride, Te-NHOH, to improve sensitivity. Te-NHOH was determined to have a LOD of 0.2 pg/mL and LLOQ of 1 pg/mL and RSD=8.0%, S/N=30. The analytes, T3 and T4 were analyzed without derivatization. The LLOQ's of the T3 and T4 quantifier transitions were both 0.3 pg/mL, RSD 18.3% and 17.0%, S/N of 12 and 14 with LOD of 0.2 pg/mL respectively. The extracted calibration curve for free testosterone quantification was linear in the range of 10-200 pg/mL with  $R^2$  at 0.9928 and 0.9872 when using  $1/(x*x)$  regression. A correlation plot of free testosterone concentration values for samples prepared using automated BioSPME versus ED methods yielded linear correlations of  $y=0.9982x+11.8$ ,  $R^2=0.9214$  (n=28) and one month later  $y=1.021x+2.1$ ,  $R^2=0.9601$  (n=21). A manual BioSPME sample preparation (n=14) using serum after multiple freeze thaw cycles yielded a linear correlation with ED results of  $y=0.9166x+15.8$ ,  $R^2=0.8528$ . Extraction of T3 and T4 hormones from serum sample followed a similar BioSPME extraction procedure. The extracted calibration curves for T3 and T4 were linear in the range of 0.5-10 pg/mL and 5-25 pg/mL, respectively with  $R^2 > 0.987$ . The comparison of the free hormones value using BioSPME extraction to these obtained for serum samples using ED will be reported in the final presentation.

#### Conclusion:

A BioSPME extraction method prior to analysis by LC-MS/MS was developed, and the evaluation results showed strong correlation against externally validated equilibrium dialysis LC-MS/MS for determination of free hormones from serum samples. The time to process one 96-well plate was less than an hour.

## B-037

### Measurement of HbA1c in Packed Red Blood Cells using the Abbott Hemoglobin A1c assay

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**Background:** The importance of Biorepositories in medical research is increasingly being recognised. These unique and/or hard to obtain samples have proved invaluable for large scale research studies. Being able to use samples from Biorepositories for the measurement of haemoglobin A1c (HbA1c) would be valuable; but these small

volume samples tend to be stored frozen as packed red blood cells (RBC), limiting their usefulness. Thus this study evaluated the utility of determining HbA1c values from packed RBC using a commercial HbA1c assay.

**Methods:** A comprehensive evaluation of the Abbott Enzymatic HbA1c method was undertaken using Clinical and Laboratory Standards Institute-based protocols to assess imprecision (EP05), accuracy (EP09), and linearity (EP06). 50 whole blood EDTA samples, with HbA1c ranging from about 20-120 mmol/mol, were measured on both the Abbott Alinity c Analyzer and the A.Menarini Diagnostics Premier Hb9210 analyser (boronate affinity high performance liquid chromatography). To assess imprecision, three patient pools (low, medium, and high) were run in duplicate twice per day for 20 non-consecutive days. To evaluate linearity, two patient samples (one low and one high HbA1c level) were mixed together incrementally to produce a set of panels at five distinct levels. The Abbott Enzymatic HbA1c method was adapted to use a manual pretreatment procedure to measure HbA1c on packed RBC samples before and following storage at -80°C.

**Results:** The Abbott Enzymatic HbA1c method correlated well with the A.Menarini Hb9210 analyser with a regression slope of 1.00 and an  $r$  of 0.99. Total imprecision for each patient pool: mean (mmol/mol) was: 35.68; 68.60; 102.65; respective total CV (%): 3.1; 2.1; 2.5. The results obtained for the EP06 linearity study showed acceptable linear performance [acceptable if  $p \geq 0.05$ ]. The modified Abbott enzymatic assay measured 10uL of packed red blood cells when diluted in the assay diluent. No significant difference was found in HbA1c results when frozen packed red cell samples were compared to the original fresh whole blood samples.

**Conclusion:** HbA1c can be measured in small packed red cell samples (typically stored in Biorepositories) and the results obtained accurately reflect the results found in fresh whole blood samples.

## B-038

### Development of the hCG+ $\beta$ immunoassay standardized to the 6<sup>th</sup> WHO International Standard for hCG for Abbott's Alinity i<sup>®</sup> analyzer

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**Introduction:** Human chorionic gonadotropin (hCG) is an important biomarker for the early detection and monitoring of pregnancy as well as in the diagnosis and management of gestational trophoblastic disease (GTD) and germ cell tumors of testicular, ovarian, and extragonadal origin. The relative proportions of hCG and its metabolic variants can change during pregnancy or the progression of hCG producing cancers. Next generation hCG assays must be standardized to the newest WHO International Standard (IS) for hCG and be able to detect each of the hCG isoforms at physiologically relevant levels (Berger et al 2016). Here we report that the Research Use Only (RUO) Alinity i<sup>®</sup> hCG+ $\beta$  immunoassay has been developed for the quantitative determination of the sum of hCG plus the free hCG  $\beta$ -subunit in human serum and plasma. Development of this RUO assay included critical standardization with the newest WHO 6<sup>th</sup> International Standard to suitably detect hCG+ $\beta$  and its variants.

**Methods:** This newly formulated Alinity i<sup>®</sup> hCG+ $\beta$  RUO assay is a two-step sandwich chemiluminescent microparticle immunoassay. The assay is standardized against the newest WHO 6<sup>th</sup> IS for hCG (18/244) and reports the concentration of hCG in pmol/L and in IU/L in parallel. The 6<sup>th</sup> WHO IS has higher purity and reduced hCG cross contaminants compared to the 3<sup>rd</sup> and 4<sup>th</sup> IS (Berger et al 2016). Analytical performance of this RUO assay was assessed on the Alinity i<sup>®</sup> and included evaluation of hCG isoform recovery, precision, sensitivity, and linearity.

**Results:** An assessment between the WHO 4<sup>th</sup> IS and the newest WHO 6<sup>th</sup> IS for hCG on this RUO assay reported significant differences in hCG concentration (up to 40%) due to the higher purity of hCG in the WHO 6<sup>th</sup> Standard. Isoform testing using International Reference Reagents on this RUO assay demonstrated variable recovery for each of the isoforms. Testing showed over recovery for hCG $\beta$  at 143% relative to total hCG. hCGn and hCG $\beta$ n were within 10% of the hCG isoform at 94.7% and 108.1% respectively. The core fragment, hCG $\beta$ cf, reported a recovery of 67.2% relative to hCG. In addition, a 20-Day precision study completed using validity controls and panels with spiked analyte in normal human serum resulted in a within-run precision of 2.3-4.8 %CV and with a total imprecision of 2.7-6.2 %CV. The sensitivity was calculated using an acceptance criterion of  $\leq 25\%$  TEa for the lowest measurable concentration with a resulting LOQ of 0.67 mIU/mL. Linearity was evaluated and met the acceptance criteria (10% or  $\leq 0.1$  mIU/mL for low end samples) for our analytical measuring interval (LOQ - 10,000 mIU/mL).

**Conclusions:** The hCG+ $\beta$  RUO immunoassay developed for the Abbott Alinity i<sup>®</sup> instrument system has been standardized using the newest 6<sup>th</sup> WHO International Standard giving the assay the following benefits: •Standardization with the purest and most recent Human chorionic gonadotropin (hCG) WHO International Standard

•Detection of hCG isoforms at physiologically relevant levels •Results reported in industry standard IU/L units and new pmol/L units •Increased sensitivity at the low end of the calibration curve to accurately read hCG producing oncology samples

## B-039

### Validation of the ProteinSimple Ella Serum Mesothelin Assay

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**Background:** Mesothelin is a membrane-bound protein expressed on normal mesothelial cells and overexpressed in several cancers including mesothelioma, ovarian cancer, pancreatic cancer, and triple-negative breast cancer. The upregulation of mesothelin on tumor cells promotes their proliferation and invasiveness and correlates with increased tumor burden and decreased overall survival in multiple cancer types. Overexpression of mesothelin also causes it to be shed into the blood, where it is known as soluble mesothelin-related protein (SMRP). This soluble mesothelin is useful for determining disease stage and prognosis and monitoring patients' response to therapy in mesothelioma and potentially other cancers. Our laboratory currently employs the manual MESOMARK SMRP assay (Fujirebio Diagnostics) to measure serum mesothelin; however, this assay will be discontinued later this year. An alternative mesothelin assay is available on the ProteinSimple Ella<sup>™</sup>, a fully automated, cartridge-based immunoassay platform. Ella performs sandwich immunoassays with fluorescent detection entirely on microfluidic Simple Plex cartridges. Analyte concentrations are measured in triplicate for each specimen using a factory calibrated, on-cartridge standard curve. The purpose of this study was to validate the ProteinSimple mesothelin assay on Ella for clinical use.

**Methods:** All assay components including diluent, wash buffer, quality control (QC) material, and mesothelin SimplePlex cartridges were obtained from ProteinSimple. Residual waste serum and plasma specimens were obtained from the clinical laboratory and diluted before loading on a cartridge. Matched serum and plasma samples from 15 patients were used to compare mesothelin levels in each matrix. Intra- and inter-day imprecision were determined using 2 levels of QC material. Method comparison with the MESOMARK SMRP assay was performed using 57 patient samples. Linearity was evaluated by measuring 5 replicates of QC material that was serially diluted to span the assay's given reportable range. A reference range was established by measuring mesothelin levels in serum from 120 healthy donors. Mesothelin stability was determined by spiking patient samples with QC material and incubating at ambient temperature, 4°C, or -20°C for times ranging from 2 hours to 30 days. Interference studies were performed by spiking patient samples with varying concentrations of hemoglobin, triglycerides, and bilirubin (Assurance Interference Test Kit, Sun Diagnostics).

**Results:** There was no significant difference between mesothelin values measured in matched serum and plasma samples, so we proceeded with validation in serum. Intra-day imprecision ranged from 4.3 – 8.6% and inter-day imprecision ranged from 3.9 – 5.4%. The mesothelin assay was linear from 13.1 – 50,000 pg/mL. Measuring mesothelin values in healthy donor serum yielded a reference range of 10,649 – 47,363 pg/mL. Preliminary data suggest acceptable stability of mesothelin at different temperatures. Comparison with the MESOMARK SMRP assay indicated that the assays are not analytically equivalent; Passing-Bablok linear regression resulted in a slope of 0.28, an intercept of 0.26, and an  $R^2$  value of 0.639.

**Conclusions:** The analytical performance of the ProteinSimple mesothelin assay in serum is reproducible, linear, and robust. However, the assay is not analytically equivalent to the MESOMARK SMRP assay. Additional studies to evaluate the accuracy and clinical utility of the ProteinSimple mesothelin assay are underway.

## B-040

### Development of Liquid Chromatography-Tandem Mass Spectrometry Method for Quantification of Free 25-Hydroxyvitamin D<sub>3</sub>

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[Background] Serum 25-hydroxyvitamin D (25(OH)D) have been widely used as a surrogate marker for the assessment of vitamin D sufficiency and bone health. 25(OH)D exist in three forms: vitamin D binding protein (DBP) bound, serum albumin (Alb) bound, and unbound (free). At this point, most clinical laboratories use fully automated

assays based on immunochemical technology to quantitate 25(OH)D to use as a vitamin D status marker. However, an earlier study showed that free fraction of 25(OH)D showed a better correlation with bone health than total 25(OH)D<sup>1</sup>. Due to its low concentration, improvement of extraction and detection techniques have been still needed. In this study, we developed a practical free-25(OH)D<sub>3</sub> quantitation method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) in combination with ultrafiltration. We validated the method and evaluated the distribution of free-25(OH)D<sub>3</sub> in disorder groups. [Methods]JeoQuant™ Kit for LC-MS/MS analysis of vitamin D metabolites (JEOL, Akishima, Tokyo, Japan) was modified to quantitate free-25(OH)D<sub>3</sub>. Calibration was performed using diluent of JeoQuant™ calibrator (resulting 25(OH)D<sub>3</sub> concentration of 43.9, 22.0, 11.0, 1.1 pg/mL). JeoQuant™ internal standard (IS) was diluted with 30 v/v % acetonitrile to obtain 100.0 pg/mL of 25(OH)D<sub>3</sub>-<sup>13</sup>C<sub>5</sub> (IS solution). To obtain free fraction of serum 25(OH)D<sub>3</sub>, 700 μL of serum samples were loaded onto 30k ultrafiltration device, and centrifuged 2,000g 30min at 7°C. 300 μL of calibrators or filtrated serum was mixed with 80 μL of IS solution, followed by solid liquid extraction (ISOLUTE SLE+; Biotage, Uppsala, Sweden) and derivatized as manufacturers instruction. The residue was dissolved in 20 μL of 50 v/v % acetonitrile, injected 5 μL into the LC-MS/MS system. The prepared calibrators or samples were analyzed using an Eksigent ekspert™ microLC 200 System coupled to a TripleTOF™ 4600 System (AB SCIEX, Framingham, MA, USA). HPLC separation was conducted using an CAPCELL CORE C18 column (1.0mmI.D.×100-mm) (OSAKA SODA, Osaka, JAPAN) with a binary mobile phase (A: 0.1 v/v % formic acid in water; B: 0.1 v/v % formic acid in acetonitrile). The Extracted ion chromatogram (m/z) were monitored as followed: 619.3→341.1 for 25(OH)D<sub>3</sub>, 624.5→341.1 for 25(OH)D<sub>3</sub>-<sup>13</sup>C<sub>5</sub>. Method validation was performed including repeatability, reproducibility, the lower limit of quantification (LLOQ). Serum samples of patients who visited Shinshu University Hospital were used to quantitate free 25(OH)D<sub>3</sub>. The patients had the following disorders (n=5 each): chronic kidney disease, hepatic cirrhosis, pregnancy. Serum samples were obtained via centrifugation at room temperature and were held at -80 °C until further analysis. The study protocol was approved by the ethics committees of Shinshu University (approved number: 4928). [Result] Repeatability: 3.4-4.0% CV, reproducibility: 4.8-6.0% CV, LLOQ: 0.97 pg/mL. Free 25(OH)D<sub>3</sub> concentration in individuals: chronic kidney disease (5.0-24.4, median 9.1 pg/mL), hepatic cirrhosis (4.4-28.3, median 14.9 pg/mL), pregnancy (8.0-31.0, median 15.4 pg/mL). [Conclusion] We developed a practical LC-MS/MS based method for quantification of free-25(OH)D<sub>3</sub> using JeoQuant™ Kit for LC-MS/MS analysis of vitamin D metabolites. The method validation results were satisfactory and can be used in studies as a valuable method to study clinical relevance of free-25(OH)D<sub>3</sub>. 1.Scand. J. Clin. Lab. Invest. 2014, 74, 177-183.

**B-041**

**Comparison of two recently formulated low-density lipoprotein-cholesterol calculations with direct measurement in patients with moderate to severe hypertriglyceridemia**

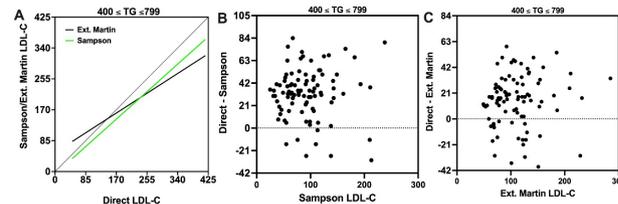
S. Islam, B. Osa-Andrews, I. Hashim, P. M. Jones, A. R. Muthukumar, J. Cao. *UT Southwestern Medical Center, Dallas, TX*

**BACKGROUND** The widely used Friedewald calculation of low-density lipoprotein-cholesterol (LDL-C) is not valid when triglyceride (TG) is over 400 mg/dL, and thus a common practice is to reflex to direct LDL-C measurement, which has been reported to yield inaccuracies in dyslipidemic patients. Among the alternative calculations of LDL-C, the formula-based LDL Sampson (Sampson M et al., JAMA Cardiology 2020) was validated against the beta-quantitation reference method. More recently, extended LDL Martin/Hopkins (Sajja A et al., JAMA Network Open 2021) was validated against vertical density gradient ultracentrifugation for LDL-C up to 800 mg/dL. To date, no studies have evaluated the performance of LDL Sampson or extended LDL Martin/Hopkins in relation to direct LDL-C measurement in patients with 400 TG 799 mg/dL. **OBJECTIVES** The study objective is to examine the differences in LDL-C using LDL Sampson or LDL Martin/Hopkins calculations and direct LDL-C measurement in patients with 400 TG 799 mg/dL. **METHODS** This study retrieved paired standard lipid panel and direct LDL-C results (tested on Atellica, Siemens Healthineers, Malvern, PA) from a pediatric patient population over a 2-year period. LDL Sampson and extended LDL Martin/Hopkins calculations were compared with direct LDL-C measurements when 400 TG 799 mg/dL (n=101).

**RESULTS** LDL Sampson and extended LDL Martin/Hopkins LDL-C calculations exhibited a strong and modest correlation, respectively, with the direct measurements (Pearson r=0.99 and 0.69, respectively) in patients with 400 TG 800 mg/dL (Figure 1 A). Average % bias of 48% and 25%, respectively, were found between direct LDL-C and LDL Sampson or extended LDL Martin/Hopkins (Figure 1B-C).

**CONCLUSION** Friedewald remains the dominant LDL-C calculation at TG<400 mg/dL while reflex to direct LDL-C when TG 400 mg/dL is a common practice in

clinical laboratories. Our study demonstrated a substantial bias of direct measurements compared to LDL Sampson or extended LDL Martin/Hopkins when 400 TG 900 mg/dL, both offer more accurate estimation of LDL-C in moderate to severe hypertriglyceridemia at no extra cost to patients.



**B-042**

**Bias Implications When Performing Anti-dsDNA Method Comparisons**

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**Background:**

Anti-dsDNA autoantibodies are linked to the diagnosis and monitoring of systemic lupus erythematosus (SLE). These autoantibodies are notoriously heterogenous, and evidence suggests high avidity anti-dsDNA autoantibodies are more closely associated with disease activity in SLE patients. Commercially available analytic tests used to detect anti-dsDNA antibodies within suspected SLE patients are also heterogenous, so test positivity does not always indicate clinical disease. A recent systematic literature review and meta-analysis highlighted the variability of anti-dsDNA test specificity across methods. In addition, standard laboratory method evaluations used to compare anti-dsDNA tests often introduce verification bias and spectrum bias. This practice not only misrepresents the accuracy of the index test being evaluated, but also minimizes potential accuracy issues within the reference test. We evaluated a real world set of method comparison results for anti-dsDNA tests, with accompanying clinical information, in order to illustrate the clinical implications of test bias when detecting heterogenous autoantibodies.

**Methods:**

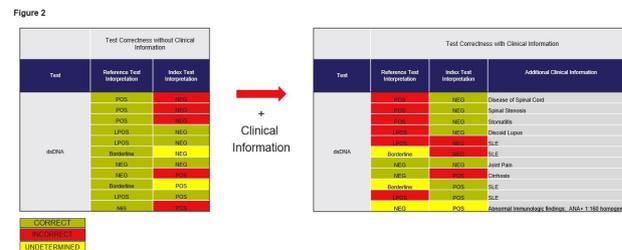
An anti-dsDNA method comparison set from a US laboratory was pulled. Accompanying clinical information was used as inclusion criteria. Test ‘correctness’ was assessed with the reference test as the gold standard. Test ‘correctness’ was re-assessed with clinical information as the reference point. (Figure 1)

**Results:**

When the reference test was considered the gold standard, 100% of interpreted values were correct. Error was only identified on the side of the index test, with a test ‘correctness’ rate of 36%. When clinical information was used as the reference point, the ‘correctness’ of the reference test dropped to 18%, while ‘correctness’ of the index test increased dramatically.

**Conclusion:**

When detecting heterogenous autoantibodies, depending on the test method, analyte detection does not always translate to clinical disease. In addition, traditional method evaluations often introduce verification and spectrum bias. These combined challenges translate to limited value in predicting test performance within the clinical setting.



**B-044****Method Development and Validation for Simultaneous Determination of Glucocorticoids on Liquid Chromatography Tandem Mass Spectrometry**

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**Background:** Glucocorticoids are widely used as highly effective drugs for the treatment of inflammatory diseases and adrenal disorders. Cross-reactivity with endogenous steroids leads to grossly inaccurate results of immunoassay measurements. Mass spectrometry, being a structural technique, has high specificity and decreased inter laboratory variation. The scarcity of available methods of determination of glucocorticoids on high end instruments like liquid chromatography- mass spectrometry warrants its development and validation. There is a great suspicion of presence of glucocorticoids in many quack formulations causing deleterious effects on health in humans. This study was aimed to develop a simple liquid chromatography tandem mass spectrometry method to simultaneously determine four glucocorticoids: betamethasone, dexamethasone, hydrocortisone and prednisolone in quacks formulating powders with simplified sample preparation and good sensitivity to allow quantification in low volume. **Methods:** A method was developed and validated on Liquid chromatography tandem mass spectrometry (LC-MS/MS) to simultaneously determine four glucocorticoids, including betamethasone, dexamethasone, hydrocortisone, and prednisolone. Method development was initiated to detect these glucocorticoids using commercially available standards to achieve analytes signal. Liquid phase extraction was performed to extract the glucocorticoids from samples by using methanol as an extracting solvent. Using two mobile phases (0.1% formic acid in water and 0.1% formic acid in acetonitrile in a gradient flow). Chromatographic separation of compounds was achieved with Agilent Poroshel 120 EC-C18 column (2.1mm x 75mm x 2.7m). For detection and quantification, a 6460 Triple quadrupole LC-MS/MS system having an ESI source with jet steam technology along with software Mass hunter was used. **Results:** The validated Analytical Measuring Range (AMR) of betamethasone, dexamethasone, hydrocortisone, and prednisolone was 7.8-1000 ng/mL. The method showed an excellent correlation (> 0.99) for these glucocorticoids. Accuracy and within/between day precision of these glucocorticoids were within  $\pm 15\%$ . For each dilution factor, the integrity of samples was maintained after dilution. Storage and freeze-thaw cycles verified the stability of the method. **Conclusion:** The developed method is sensitive and useful for detecting, quantifying, and confirming glucocorticoids betamethasone, dexamethasone, hydrocortisone and prednisolone. This method can be used in routine for accurate estimation of these glucocorticoids with good time management and less financial burden.

**B-045****Method Development and Validation of Methylmalonic Acid by Liquid Chromatography Tandem Mass Spectrometry**

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**Background:** Serum methylmalonic acid (MMA) is a useful biomarker for early detection of cobalamin deficiency and methylmalonic academia, a rare inborn error of metabolism. In vivo, methylmalonyl-CoA is converted to succinyl-CoA by an enzyme, methylmalonyl-CoA mutase (MUT), that requires cobalamin as a cofactor. Deficient activity of MUT causes accumulation of methylmalonyl-CoA and MMA. Quantitative determination of MMA helps clinicians in identifying and monitoring methylmalonic academia and cobalamin deficiency. Recently, gas chromatography / mass spectrometry (GC/MS) has been replaced by Liquid chromatography- tandem mass spectrometry (LC-MS/MS) as a method of choice for MMA estimation with less time-consuming sample preparation and better sensitivity. In present study, we have developed and validated an in-house method on LC-MS/MS with simple sample preparation protocols, shorter run time, and cutting down the price to less than half. The objective was to develop and validate an accurate and cost-effective method for analyzing methylmalonic acid in serum on liquid chromatography/mass spectrometry. **Methods:** Method development was initiated for detection of MMA using commercially available standards diluted in methanol to achieve analyte signal. For sample preparation, 300 $\mu$ l of extracting solution (Methanol:Acetonitrile:Formic acid in 2:2:1) along with 100  $\mu$ l of d3-methylmalonic acid as internal standard were added to 100  $\mu$ l of serum in a sample preparation vial. The mixture was mixed for 30 seconds on vortex mixer & subsequently centrifuged for 5 minutes at 11500 RPM. Centrifuged supernatant was filtered into a sample vial through 0.2  $\mu$ m filter and injected in LC-MS/MS for analysis. Separation of compound was achieved with Agilent SB-C18 column (4.6 x150mm, 1.8  $\mu$ m). For detection and quantification, a 6460 Triple quadrupole LC-MS/MS system having an ESI source with jet steam technology along with soft-

ware Mass hunter was used. Method validation studies were carried out on all these parameters with respect to linearity (calibration) with recovery, Limit of detection (LOD), Lower limit of quantitation (LLOQ), Analytical measurement range (AMR) by calibration curve analysis, sensitivity, precision, accuracy, stability after short/long term storage and freeze thaw cycle and dilution effects. **Results:** The analytical measurement range of MMA was 33-4227 nmol/L with limit of detection at 15 nmol/L. The lower limit of quantification was validated at 33 nmol/L. The calculated bias was -12.7%. The within and between days imprecision at four levels of concentrations were 0.7-7.5%. The method was found stable after the storage & freeze-thaw cycle. The integrity of the diluted sample was maintained for each dilution factor. **Conclusion:** The presented method for MMA determination is accurate, cost-effective, specific, and has a good clinical correlation. This method can be used in routine for accurate estimation of MMA levels with good time management and less financial burden.

**B-046****Metabolic characterization of Barth Syndrome Cellular Model**

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**Background:** Barth Syndrome is an X-linked disease caused by the mutation in the TAZ gene that encodes for acyltransferase tafazzin. The absence of tafazzin activity results in an increased level of monolysocardiolipin (MLCL), a decrease in total cardiolipin (CL), and as a result increase in MLCL: CL ratio.<sup>1</sup> Barth syndrome is also manifested by growth delay, neutropenia, skeletal myopathy, muscle weakness, elevated levels of plasma and urine 3-methylglutaconic acid (3-MGC), hypocholesterolemia, impaired electron transport chain (ETC), elevated reactive oxygen species (ROS), increased basal oxygen consumption, and a depleted ATP pool.<sup>2</sup> To facilitate new therapies developments and to study pathological effects of TAZ mutation to facilitate therapies development, several mammalian and cell models are currently available. Here we report key TAZ metabolic phenotype features in control human-induced pluripotent stem cells (hiPsc) line and a genome-edited isogenic hiPsc-TAZ<sup>C517delG</sup> cellular model. **Method:** TAZ<sup>C517delG</sup> and isogenic healthy control hiPscs were incubated under the standard stem cells culture conditions. MLCL: CL ratio and 3-methylglutaconic acid (3-MGC) were analyzed by laboratory-developed tandem mass spectrometry-based methods. *De-novo* cholesterol biosynthesis rate was measured by stable isotope labeling techniques with heavy water (D<sub>2</sub>O) followed by GCMS analysis. ATP and total glutathione levels were analyzed by commercially available kits. **Results:** In agreement with Barth syndrome phenotype, TAZ<sup>C517delG</sup>-iPSC exhibited a significant increase in both MLCL: CL ratio and a cellular 3-MGC level. Heavy water labeling study revealed that TAZ is associated with a decrease in *de novo* cholesterol biosynthesis in agreement with observed hypocholesterolemia in Barth syndrome-affected individuals. We also found that TAZ<sup>C517delG</sup> exhibited a significant decrease in ATP and total GSH which is consistent with exercise intolerance and increased ROS production respectively. **Conclusion:** TAZ<sup>C517delG</sup> iPSC cellular model demonstrates key metabolic phenotype similarities to the human Barth Syndrome metabolic phenotype and thus represents a powerful cellular model to test therapeutic interventions.

**References** 1. Hauff, K. D. & Hatch, G. M. Cardiolipin metabolism and Barth Syndrome. *Progress in Lipid Research* vol. 45 (2006) 2. Dudek, J. & Maack, C. Barth syndrome cardiomyopathy. *Cardiovascular Research* vol. 113 (2017).

**B-047****Serum MAGEA3 Is a Potential Prognostic Marker for Prostate Cancer**

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**Background:** Melanoma-associated antigen 3 (MAGE-A3) is broadly expressed in a variety of malignancies such as breast cancer, melanoma, head and neck cancer, lung cancer, gastric cancer, colorectal cancer, and prostate cancer. It has been shown that MAGE-A3 expression is associated with cancer progression and metastasis. In addition, increased expression of MAGE-A3 in cancer cells is associated with poor prognosis of cancer patients.

**Methods:** To detect MAGE-A3 in serum sample from cancer patients, a high sensitivity Sandwich ELISA was developed using antibody pair that recognizes epitopes on human MAGE-A3, and the assay sensitivity and specificity were analyzed. MAGE-A3 level in serum samples from 31 prostate cancer patients and 24 non-cancer patient controls were measured using this assay.

**Results:** The assay range is from 78-5000pg/mL, and assay sensitivity is less than 13pg/mL. This assay recognizes recombinant human MAGE-A3 and MAGE-A6. The cross-reactivity with recombinant MAGE-A6 is about 11%. No significant cross-reactivity was observed in other MAGEA family proteins. Serum MAGE-A3 levels were detectable in all 31 prostate cancer patients (median: 247pg/mL; range: 36-2532 pg/mL; mean: 447pg/mL; SD: 616pg/mL). The levels were significantly elevated compared with those of the controls (median: 136pg/mL; range: 84-337pg/mL; mean: 168pg/mL; SD: 76pg/mL  $p=0.018$ ), and the levels were significantly elevated in late-stage prostate cancer patients (median: 270pg/mL; range: 130-2532 pg/mL; mean: 850pg/mL; SD: 909pg/mL) compared with those of the early-stage prostate cancer patients (median: 205pg/mL; range: 36-444 pg/mL; mean: 225pg/mL; SD: 137pg/mL;  $p=0.046$ ). However, the serum MAGE-A3 levels in early-stage prostate cancer patients have no significant change compared to those of the controls.

**Conclusion:** Our results suggest that serum MAGE-A3 is a promising prognostic biomarker for prostate cancer. Future studies should investigate whether serum MAGE-A3 could be used to monitor therapeutic effects.

## B-048

### Analytical and clinical performance of plasma p-tau181 assay on the high-sensitivity Simoa HD-X platform

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**Background:** The feasibility of detecting, tau phosphorylated at threonine-181 (p-tau181) in CSF makes it a valuable biomarker for diagnosis in the field of Alzheimer's Disease (AD). Recently, the novel technologies accurately measuring biomarkers directly in blood offer a unique advantage for use in clinical testing and trials. This study describes the promising analytical and clinical performance of the plasma p-tau181 assay on Simoa HD-X platform.

**Methods:** The analytical performance (intra- and inter-assay precision, specificity, detection limits, linearity) and the analyte stability was evaluated using ADx Neurosciences developed p-tau181 specific Simoa assay on HD-X platform. The clinical performance was studied in 254 AD patients ((female = 125), 80 non-AD cases (female=59)) and 25 healthy controls (female = 18) with normal cognition.

**Results:** The p-tau181 assays showed robust clinical performance. The average of plasma p-tau181 was  $73.2 \pm 36.6$  ng/L in AD patients,  $24.2 \pm 24.1$  ng/L in non-AD cases and  $35.2 \pm 16$  ng/L in controls. The concentrations of plasma p-tau181 in patients with AD was significantly increased with at-least 3-fold versus non-AD ( $p < 0.001$ ). ROC analysis demonstrated an AUC of 0.92 (0.79-0.98). Furthermore, p-tau181 assays showed good analytical performance, including the sensitivity, specificity, and intra- and inter-assay precision. The intra- and inter-assay variability was 10.3% CV and 10.7% CV, respectively. The p-tau181 levels were stable up to 3 freeze/thaw cycle and up to 4 days at 4°C.

**Conclusion:** The plasma p-tau181 concentrations in AD cases were significantly higher than non-AD and controls with limited overlap. The specific analytic findings of plasma p-tau181 quantification shows good promise in offering a non-biased measurement and help in the clinical assessment of AD patients and clinical trials.

## B-049

### Comparison of diagnostic performance of combined CSF biomarkers with plasma p-tau181 concentrations in predicting clinically diagnosed Alzheimer's disease

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**Background:** The feasibility of detecting, amyloid beta (A $\beta$ ), Ttau and tau phosphorylated at threonine-181 (p-tau181) in CSF makes them valuable biomarkers for diagnosis in the field of Alzheimer's Disease (AD). Recently, the novel technologies accurately measuring biomarkers directly in blood offer a unique advantage for use

in clinical testing and trials. This study compares the performance of a novel plasma p-tau181 assay with combined and separate CSF biomarkers in predicting clinical diagnosis of AD.

**Methods:** Data were obtained after analysis of EDTA plasma and CSF samples from cases with clinical AD who had been referred to the UBC Hospital Clinic and were assessed for dementia and AD between 2008 - 2018. The plasma samples were assayed by using an ADx developed p-tau181 specific Simoa assay and the CSF samples were analysed for p-tau181, Ttau and A $\beta$ 1-42 by INNOTEST FUJIREBIO immunoassay.

**Results:** 54 cases including AD (n= 35), Non - AD (n = 19) evaluated. The average of plasma p-tau181, CSF p-tau181 and CSF Ttau were about three-fold and twice higher in AD patients respectively, but the average of CSF A $\beta$  was about one and half lower in AD cases. There was a positive correlation between elevation of plasma p-tau181, CSF p-tau 181 and CSF Ttau and a negative correlation between increasing of plasma p-tau181 and CSF A $\beta$ 1-42. The sensitivity and specificity and the receiver operating characteristic (ROC) analysis demonstrated an area under the curve (AUC) of 0.92, 0.76, 0.73 and 0.76 for plasma p-tau181, CSF p-tau181, CSF Ttau and CSF A $\beta$ 1-42 respectively. The AUC of combined CSF biomarkers was 0.8.

**Conclusion:** The CSF biomarkers and plasma p-tau181 concentrations in AD cases were significantly higher than non - AD cases. The specific analytic results of plasma p-tau181 showed superior performance in the clinical assessment of AD patients. These findings should be further verified by prospective longitudinal collected samples with more heterogeneous participants.

## B-050

### Alanine-glycine Ratio is a Novel Predictive Biomarker for Type 2 Diabetes Mellitus: The Korean Genome and Epidemiologic Cohort Study

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**Background:** Type 2 diabetes mellitus has an increasing burden among metabolic disorders that cause various micro- and macro-vascular diseases. It has been reported that 50% of patients present with microvascular complications, whereas 27% present with macrovascular complications. As not all people with obesity develop diabetes, the classification of patients with a healthy-obese and unhealthy-obese status is needed for identifying populations requiring early interventions. In the past decade, numerous studies have attempted to identify metabolites that can act as biomarkers for diabetes. Traditionally, the levels of branched-chain amino acids (e.g., isoleucine, leucine, valine) were found to be significantly elevated in diabetes patients. Here, using data from a larger cohort in Korea, we suggest a novel, simple biomarker—the alanine-glycine ratio (Ala/Gly)—for predicting type 2 diabetes. **Methods:** Initially, 2,356 individuals with available metabolomic data were enrolled from the Korean Genome and Epidemiologic Cohort Study. The diagnostic ability of the new marker was evaluated by generating a receiver operating characteristic (ROC) curve. We fitted a Cox proportional hazards regression model with 1,880 individuals without diabetes to test the predictive value for type 2 diabetes. Survival analyses were performed to compare four subgroups defined by baseline Ala/Gly levels and obesity status.

**Results:** The Ala/Gly showed better diagnostic performance for type 2 diabetes than the homeostatic model assessment of insulin resistance (HOMA-IR) in ROC analysis using cross-sectional data (optimal Ala/Gly cutoff: 1.6). Among 1,880 individuals without diabetes, 406 were diagnosed with type 2 diabetes during the 12 years of follow-up. A multivariate Cox regression model adjusted for age, sex, and BMI showed that alanine (hazard ratio [HR], 1.41; 95% CI, 1.27-1.57) and glycine (HR, 0.78; 95% CI, 0.60-0.87) were associated with new-onset type 2 diabetes. Classification of the nondiabetic population with the Ala/Gly and obesity status showed a significant different risk of incident type 2 diabetes. We assessed the HR of incident type 2 diabetes in each group. Participants with obesity with an Ala/Gly  $\geq 1.6$  showed the highest HR (3.29; 95% CI, 2.57-4.21;  $p < 0.001$ ). Participants with obesity with an Ala/Gly  $< 1.6$  showed a lower HR (1.44; 95% CI, 1.08-1.94;  $p = 0.015$ ) than lean participants with an Ala/Gly  $\geq 1.6$  (2.10; 95% CI, 1.58-2.79;  $p < 0.001$ ).

**Conclusion:** The Ala/Gly can be used to classify individuals without diabetes using a stable cutoff. Amino acid metabolism changes early in the pathogenesis of type 2 diabetes, and the Ala/Gly could be used to evaluate the risk of diabetes, especially in lean individuals.

**B-051****Performance evaluation of hematology parameters in 3D quantitative phase imaging-based individual blood cell analysis**

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**Background:** Image-based automated hematology analysis is an emerging method which has numerous benefits for point-of-care testing (POCT) devices. It shows better usability with easier maintenance than conventional flow cytometry-based methods. Three-dimensional quantitative phase imaging (3D QPI) is a label-free method reconstructing the volume and the refractive index of transparent specimens. 3D QPI has been widely applied to investigate the feasibility of label-free cell classification such as lymphocyte subtypes. As the refractive index of an erythrocyte correlates to the hemoglobin concentration, 3D QPI can be an exclusive method for achieving image-based automated analysis without any staining procedure, while the other methods require additional optics such as spectroscopy, brightfield microscopy, or fluorescence microscopy. **Method:** Whole blood collected by a K2EDTA tube was loaded onto a cell counting chip after dilution. The blood cells in the images acquired by a 3D QPI device were classified by a size-based filtering algorithm. RBC indices (MCV, MCH, MCHC) were calculated based on the 3D volume and the mean refractive index of individual erythrocytes. Cell counts and the related parameters (RBC, PLT, HGB, HCT) were calculated from the cell numbers in the images. The test group (n=273) included patients (n=182) that at least an RBC index was not in the clinical reference range. Each sample was measured three times for evaluating test consistency. The results were compared to the complete blood count (CBC) results from predicate devices (DxH 800 and LH 780, Beckman Coulter). All data were analyzed by R packages, including Intraclass correlation coefficient (ICC), Passing-Bablok regression, Bland-Altman plot, and residual box plot. Interference study was conducted to investigate whether any substance may affect the test accuracy. **Results:** The ICC of all measurements except MCHC were higher than 0.75, respectively, which represents the excellent precision of the performance (n=247,  $p < 0.001$ ). MCV (0.852, CI: 0.820-0.879) and MCH (0.842, CI: 0.808-0.871) which have direct relevance to the principle of 3D QPI optics showed the highest performance. The mean CV% of MCV and MCH were 3.71 and 4.69, respectively. In the method-comparison study (n=260), MCV ( $R^2=0.912$ ), MCH ( $R^2=0.885$ ) showed acceptable correlation to the reference device. PLT count also showed great precision (ICC=0.961, CI: 0.952-0.969) and accuracy ( $R^2=0.956$ ), however, the mean CV% was relatively large (11.46). The RBC count and the related parameters (HGB, HCT) which are not relevant to the optics showed less precision than the other measurements. It showed that further study is required for accurate measurement of confluent erythrocytes. No significant interference was observed in representative substances: glucose (900 mg/dL), bilirubin (30 mg/dL), triglycerides (400 mg/dL). **Conclusion:** This study demonstrates that the 3D QPI-based analysis method can accurately measure numerous hematology parameters with high precision. The results imply that 3D QPI can be applicable for developing an image-based automated hematology analyzer. This method can provide additional information about the distribution of hemoglobin concentration and the volume of individual erythrocytes. Further studies about designing robust sample preparation protocol and the image analysis algorithms are required to improve the analytical performance of all hematology parameters.

**B-052****Performance Evaluation of an Urine Dipstick Analyzer CYBOW R-600S**

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**Background:** We evaluated the performance of a Korean manufacturer's new semi-automated urine analyzer CYBOW R-600S, which can additionally obtain the ratio of albumin or protein to urine creatinineS in conventional urinalysis.

**Methods:** The precision was measured using two commercially available quality control materials. A total of 300 urine samples were used to compare the results of CYBOW R-600S (DFI Co, Korea) with UC-3500 (Sysmex, Japan) and AU5800 (Beckman Coulter, USA). uACR and uPCR were also calculated for the evaluation of CYBOW R-600S's analytical performance.

**Results:** All of  $\pm 1$  grade agreement of within-run and within-day precision for both low and high level control were 100%.  $\pm 1$  grade included concordance rate were 100% except for urine leukocyte (99.3%), microalbumin (99.7%), protein (96.7%), pH (99.3%), SG (99.7%). Comparing the CYBOW R-600S with the two reference analyzers, UC-3500 and AU5800, Concordance rate of uACR and uPCR were 95.0%,

96.3%, respectively. Kappa coefficient of those were 0.914 (95% CI, 0.872-0.956) and 0.819 (95% CI, 0.724-0.915). CYBOW R-600S showed excellent diagnostic performance for uACR and uPCR. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of uACR were measured as 99.4%, 95.8%, 96.3%, and 99.3%. In uPCR, sensitivity, specificity, PPV, NPV were 91.2%, 99.2%, 93.9% and 98.9%, respectively.

**Conclusion:** The CYBOW R-600S showed good performance of the precision and  $\pm 1$  group concordance rate when compared with the two reference analyzer, UC-3500, AU5800. Therefore, we could conclude CYBOW R-600S are suitable for the screening test and clinical application.

**B-053****Deep Single-cell Type Proteome Profiling of Mouse Brain from Alzheimer's Disease Model by Nano-scale Tandem Mass Tag Mass Spectrometry**

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**Background:** Alzheimer's disease (AD) is an irreversible and progressive neurologic disorder that affects estimated 6.2 million Americans in 2021. The pathology of AD is associated with the accumulation of extracellular amyloid beta ( $A\beta$ ) as neuritic plaques and intracellular accumulation of hyperphosphorylated tau as neurofibrillary tangles. However, the cause of AD is still not fully understood, which limits the development of therapeutic strategy for this disease. Multiplexed isobaric labeling-based quantitative proteomics, such as tandem mass tag (TMT), has become a powerful approach for directly profiling more than 10,000 proteins in complex protein samples with the combination of high resolution two-dimensional liquid chromatography and tandem mass spectrometry (LC/LC-MS/MS) for the protein discovery. However, there are still challenges in analyzing samples at single-cell or single-cell-type levels. We recently established a novel TMT-based LC/LC-MS/MS method for deep analysis of samples at the nanogram level including single-tube sample processing, eliminating a peptide desalting step, increasing the protease amount to act as a carrier, adapting basic pH LC fractionation and finally optimizing acidic pH LC-MS/MS for maximum identification. Here we combined this pipeline with the Fluorescence-Activated Cell Sorting (FACS) strategy for cell-type-specific proteome profiling of brain samples from the AD mouse model.

**Method:** Microglia, astrocyte, oligodendrocyte, and other brain cells including neuron from APP knock in (APP KI) and wild type (WT) mice were sorted by flow cytometry with different cell markers. The protein amount at 500 ng (~25000 cells) per sample was processed in a single tube with one pipette tip for each sample. Each sample was lysed in 10  $\mu$ L of 8 M Urea buffer and enzymatically digested with 10 ng Lys-C and 2.5  $\mu$ g Trypsin at room temperature. Digested samples were labeled with 16-plex TMTpro (TMT: protein (w/w) = 30:1) at room temperature for 30 min and quenched with 0.5%  $NH_3OH$ . The 16 samples were then mixed and fractionated by an offline basic pH LC into 40 concatenated fractions. Each fraction was then separated by a high sensitivity LC column (30  $\mu$ m ID x 20 cm) and analyzed by Q Exactive HF Orbitrap MS.

**Results:** A total of 7,852 unique proteins were identified and quantified from APP KI mice, with a protein false discovery rate (FDR) less than 5%. A cell-type specific differentially expressed (DE) proteins analysis was performed to investigate protein dysregulation in APP KI mice. The proteins were filtered with a previously reported statistical method based on probability value and fold change, and 674 DE proteins were identified. Interestingly, our DE list contains many reported AD-related genes such as GFAP, APOE, MAPT, PTK2B, HTRA1, ADAMs, and ANK3 that are highly expressed in different cell types respectively.

**Conclusion:** Overall, this methodology is simple, quick, and easily applicable for proteomic profiling of scarce clinical samples such as specimens captured by laser capture microdissection (LCM) and micro-biopsy samples. Our data from APP KI mice revealed cell-type specific biological alterations in AD, and provided new insights into the pathogenesis of AD, which may be used as the potential therapeutic targets for treating this disease.

**B-054****Neuron Specific Enolase in Serum and CSF using the BRAHMS KRYPTOR Analyzer**

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**Background:** Neuron Specific Enolase (NSE) is a glycolytic enzyme present in neurons, peripheral nerve tissues and neuroendocrine tissues. Serum NSE is utilized as a tumor marker while NSE in cerebrospinal fluid (CSF) can serve as a nonspecific marker for neuronal damage. The objectives were to evaluate NSE in serum and CSF using the BRAHMS NSE immunoassay on the Thermo Scientific BRAHMS KRYPTOR compact PLUS analyzer.

**Methods:** The KRYPTOR measurement principle is based on TRACE Technology, which measures the signal that is emitted from an immunocomplex with time delay. Studies included accuracy, linearity, imprecision, analytical sensitivity, hemolysis interference, freeze-and-thaw stability, and carryover. Method comparison was performed between the KRYPTOR and the CanAg NSE ELISA using 24 serum and 20 CSF specimens, spanning the AMR. A subset of CSF samples (n=14) was sent for comparison to another KRYPTOR analyzer. Linearity was determined for serum and CSF by diluting a high pool with kit diluent to create a set of 6 samples, tested in duplicate. Imprecision was determined by testing 2 levels of KRYPTOR NSE serum controls (10.0 and 49.4 ng/mL) and a CSF pool (20.3 ng/mL), once daily, for 5 days, 3 replicates per level. Limit of detection (LOD) was assessed by measuring 10 replicates of saline and 3 replicates of a low serum patient pool. Hemolysis interference was studied first by determining the [NSE]/[H-index] ratio in 80 individuals to establish an expected population distribution; then determining the increase in NSE at H-index around 25, 50, 100 and 300 (n=10 at each level). Carryover was evaluated by testing a high and a low patient serum pool (184.7 and 3.7 ng/mL). The manufacturer's serum reference interval was verified using 59 fresh samples from self-reported healthy volunteers while the CSF reference interval was established using 122 CSF specimens previously tested for oligoclonal banding. All data were analyzed using EP Evaluator.

**Results:** Method comparison between KRYPTOR and CanAg ELISA yielded  $y=1.28x+7.76$ ,  $R^2=0.97$  for serum and  $y=2.82x+8.82$ ,  $R^2=0.96$  for CSF. Due to these observations, a set of CSF samples were compared to the KRYPTOR at another laboratory, and yielded  $y=1.01x+2.77$ ,  $R^2=0.97$ . The manufacturer's linearity claim up to 200 ng/mL was verified using serum and CSF samples. The maximum within-run imprecision was 2.6 %CV and the maximum total imprecision was 3.9 %CV. KRYPTOR's LOD claim of 3.1 ng/mL was confirmed. Hemolysis contributed NSE activity at variable amounts across individuals. The increase in NSE was linearly related to the H-index. However, median NSE/[H-index] ratios did not remain constant as a function of hemolysis. Serum and CSF NSE concentrations changed <15% for 3 freeze-thaw cycles. Carryover was not observed. The manufacturer's reference interval for serum at 12.7 ng/mL was verified. A reference interval for CSF of  $\leq 21.5$  ng/mL was established.

**Conclusion:** The performance of the BRAHMS KRYPTOR NSE assay in serum and CSF was acceptable for clinical use. Caution is recommended for hemolytic samples since the KRYPTOR NSE assay is impacted by both the NSE released from red blood cells and the susceptibility to interference from hemoglobin.

**B-055****Effect of Automated Lot Calibration of Selected Clinical Chemistry Assays on cobas pure and cobas pro integrated solutions**

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**Background:** The novel cobas® pure and cobas pro integrated solutions (both Roche Diagnostics International Ltd, Rotkreuz, Switzerland) are seamlessly designed clinical laboratory analyzers with shared reagent packs, methodologies, assay menus, operations, and user interfaces. The instruments are comprised of multiple analytical units for clinical chemistry, immunochemistry, and ion selective electrode assays. One feature of the clinical chemistry analytical units is automated lot calibration (cobas AutoCal): subsequent calibrations of new reagent lots are calculated by downloading reagent parameters and information provided by the instruments. This single center

study evaluated the performance of cobas pure and cobas pro integrated solutions by comparing the precision between the analyzers, automated lot calibration performance, and calibration time efficiency.

**Methods:** Commercially available quality control (QC) materials were measured on both cobas pure and cobas pro integrated solutions using 19 selected clinical chemistry assays at two (urine applications) and three (serum applications) analyte concentrations, based on the Clinical and Laboratory Standards Institute EP05-A3 precision protocol over five consecutive days. Different reagent lots were used in each round of testing. For selected analytes, the automated lot calibration feature was used after Lot 1 and before the second reagent lot (Lot 2) was placed in use. Coefficients of variation (CVs) were calculated for precision. The performance of the automated lot calibration feature was measured by calculating the median CV of the lot-to-lot and instrument-to-instrument variance component. Automated lot calibration efficiency was measured by recording hands-on and system operation time.

**Results:** Across all 19 selected assays, the median CV (range) for intermediate precision was 1.67% (0.76–10.40%) for cobas pure and 1.80% (0.64–7.29%) for cobas pro integrated solutions. The median CV (range) of the lot-to-lot variance across all assays was 0.41% (0.00–5.25%) for cobas pure and 1.45% (0.00–5.37%) for cobas pro integrated solutions. The median CV (range) of the instrument-to-instrument variance across all assays was 1.11% (0.00–4.10%). Using the cobas AutoCal function, the automated lot calibration operating time between Lots 1 and 2 took less than five seconds and did not involve an actual measurement.

**Conclusion:** cobas pure and cobas pro integrated solutions demonstrated minimal lot-to-lot and instrument-to-instrument variation, as well as a short hands-on and system operation time with automated lot calibration using cobas AutoCal.

**B-056****NOVEOST™ Mechanical Reliability, Precision and Clinical Performance vs ImmunoCAP™ 250 and IMMULITE™ 2000**

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**Objective:** Allergen-specific IgE (sIgE) measurement in the clinical laboratory aids in the evaluation and diagnosis of allergic disorders. The NOVEOST™ Immunoassay Analyzer is a high throughput automated platform that utilizes paramagnetic microspheres in combination with fluorescent and chemiluminescent signals to quantify allergen-sIgE present in human serum. The objective of this study was measuring the functionality and usability of the NOVEOST™ by looking at its mechanical reliability, precision, and clinical performance.

**Methods:** The mechanical reliability of the NOVEOS platform was tested over a one-month period where the instrument ran for 6 hours each working day; over the course of the study, daily runs of approximately 400 tests were completed. Within-Day and Total Precision were performed by identifying any sample that was tested against the same allergen for two or more days. Any result that was <0.17 kU/L was removed from the analysis. A total of 121 sample/allergen combinations verifying from 4 to 10 replicates were used to calculate %CV. A precision profile relationship was obtained by analyzing %CV vs specific allergen concentration. Clinical performance was obtained by comparing more than 6000 results from the ImmunoCAP™ and the NOVEOST™ systems using D001 (house dust mite; *D. pteronyssinus*), D002 (American dust mite; *D. farina*), E001 (cat dander; *F. domesticus*), G006 (timothy grass or hay; *P. pratense*), G004 (grass; *M. fescue*) and G008 (grass; *P. pratensis*). A subset of specimens that demonstrated discordant results between the ImmunoCAP™ 250 and the NOVEOST™ were treated with a cross-reactive carbohydrate determinant (CCD) inhibitor and then retested with each assay.

**Results:** Although an initial attempt to record mechanical reliability was interrupted by an unscheduled maintenance event, a subsequent run was successfully completed after testing 400 tests/day for 20 days. Precision was below 10% and 15% for the Within-Day and Total precision respectively. The regression line comparing the NOVEOST™ to the ImmunoCAP™ and to the IMMULITE 2000™ were  $y = 0.8688x - 2.0986$  and  $y = 1.012x + 7.7695$  respectively. Results from CCD inhibitor treated specimens were within 95%-105% of values from untreated specimens tested on the NOVEOST™. Surprisingly, ImmunoCAP™ demonstrated CCD interference on these same samples where multiple results were positive when untreated but negative in the presence of the inhibitor.

**Conclusions:** The NOVEOST™ system has satisfactorily met the study requirements for mechanical reliability. The NOVEOST™ precision and clinical performance are comparable to the ImmunoCAP™ 250 and IMMULITE™ 2000 but are not affected by CCD interference.

**B-057****The Impact of Circulating Hydrogen Sulfide on the Risk of Major Adverse Cardiovascular Events**

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**Background:** Hydrogen sulfide (H<sub>2</sub>S) is a gas transmitter that is endogenously produced through both enzymatic and non-enzymatic pathways. H<sub>2</sub>S is also produced via gut microbiota through dissimilatory pathway. H<sub>2</sub>S is proposed to have many physiologic roles including the regulation vascular tone and blood pressure. Abnormal H<sub>2</sub>S levels have been linked to an array of pathologies. However, it is still not clear of the precise effect of circulating levels of H<sub>2</sub>S have on the risk major adverse cardiac events (MACE; myocardial infarction, stroke and death).

**Methods:** H<sub>2</sub>S levels were measured in two large cohorts. A discovery cohort from individuals (n = 2,088) undergoing elective diagnostic cardiac evaluation with longitudinal follow up at the Cleveland Clinic, USA; and a validation cohort (n = 836) sequential subjects undergoing elective cardiac diagnostic coronary angiography at the Charité University Hospital, Campus Benjamin Franklin, Germany. Hydrogen sulfide was quantified with a robust, sensitive, and accurate stable isotope-dilution electrospray ionization liquid chromatography with on-line tandem mass spectrometry (HPLC-ESI-MS/MS) method. The correlation between H<sub>2</sub>S levels and clinical phenotypes were determined by hazard ratios (HRs) with 95% confidence intervals (CI) and Kaplan-Meier plots where *p*-values less than 0.05 was considered statistically significant.

**Results:** In the discovery cohort, KM plots in the whole cohort showed significance (*p* = 0.005). While in H<sub>2</sub>S levels in the primary prevention group did not show significance (*p* = 0.07) the levels were significantly increased when comparing the first tertile to the third tertile in the secondary prevention group (*p* = 0.02). H<sub>2</sub>S is significantly associated with 3-years MACE risk in the stable angiographic cohort (HR=2.26 [95%CI 1.63-3.15]). Following adjustments for traditional cardiovascular risk factors including age, sex, smoking, hypertension, diabetes, HDL, LDL, high-sensitivity C-reactive protein and EGFR, elevated H<sub>2</sub>S predicted increased MACE risk in both primary prevention (HR=3.89 [95%CI 1.40-10.81]) and secondary prevention groups (HR=1.96 [95%CI 1.37-2.82]).

**Conclusion:** Hydrogen sulfide was identified as being associated with 3 year MACE risk in both primary and secondary prevention as well as in overall 3 year mortality. Further analyses are needed to investigate the underlying biological mechanisms for these observations.

**B-058****Analytical Characterization of Diagnostic Assays for Atypical Respiratory Infections Caused by Mycoplasma pneumoniae and Chlamydia pneumoniae with the use of LAMP technology**

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**Background:** Mycoplasma pneumoniae and Chlamydia pneumoniae are, just after Streptococcus pneumoniae, the most common etiological factor of Community-Acquired Pneumonia (CAP), which is the worldwide cause of mortality and morbidity. The prevalence of atypical pneumonia differs in various countries, but on average they account for about 25% of all CAP cases, with the Chlamydia pneumoniae being the leading etiological factor. Several diagnostic methods have been implicated, including isolation, serological testing, and molecular-based methods. The latest are characterized by the highest specificity and sensitivity, however very demanding in terms of qualified personnel and equipment. Due to numerous extrapulmonary complications like heart or liver damage, it is an extreme need for fast diagnosis and accurate treatment. Therefore we have developed a point-of-care diagnostic method for detecting Mycoplasma pneumoniae and Chlamydia pneumoniae using the isothermal amplification method, in particular, the loop-mediated amplification technique (LAMP). Isothermal amplification techniques are rapidly developing alternatives to PCR that have comparable or even better diagnostic parameters such as higher sensitivity and shorter time to results (TTR). What is more, there is no need for highly specialized equipment. **Methods:** We have developed and optimized a diagnostic method for atypical bacteria detection using LAMP technology suitable for usage in point-of-care medicine in the real-time approach. The fluorescence signal was detected every minute monitoring the product's growth over time. The LAMP was realized using WarmStart® LAMP Kit (DNA&RNA) NEB, fluorescence dye used in the assay was EvaGreen® Dye, 20x in Water Biotium. Self-designed sets of six primers and five primers were used for Chlamydia pneumoniae and Mycoplasma pneumoniae, respectively. The sensitivity of the assays was determined using Genomic

DNA from Chlamydia pneumoniae Strain: CM-1 (VR135DQ™, ATCC) and Amplirun® Mycoplasma Pneumoniae DNA Control (Viracell), respectively. Specificity was established by numerous reactions with DNA obtained from possible pathogens that might give similar symptoms, increase a chance of co-infection, or stand for the physiological microbiome. **Results:** The obtained limit of detection (LOD) of the Chlamydia pneumoniae assay was established as 1 copy per reaction, the Mycoplasma pneumoniae assay was established as 5 copies of standard DNA per reaction. The time to results (TTR) was as follows: MP 1000 copies – 8.05 minutes, 5 copies – 13.97 minutes; ChP 1000 copies 11.87 minutes, 1 copy – 18.54 minutes. The cross-reactivity studies showed 100% specificity of both of our assays. We have not shown any non-specific cross-reactivity amplification for any of the analyzed pathogens. **Conclusion:** Developed diagnostic assays utilizing LAMP technology are very sensitive (detect as low as one copy per reaction) and highly specific. The time to results in highly positive samples is shorter than 10 minutes, for low positive samples they do not exceed 20 minutes. Thanks to its high sensitivity and short TTR optimized assays stand good resolution for point-of-care diagnostic methods available at the point-of-care medical facilities, preferably with our Genomtec ID® System.

**B-059****Performance of a C2 Assay for EDTA plasma and serum on the Binding Site Oplitite Analyser**

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**Background:** Here we describe the performance testing of a C2 assay for use on the Binding Site's Oplitite® analyser. Decreased C2 concentrations are a result of immune complex mediated classical complement pathway activation. C2 deficiency is the most common inherited complement component deficiency and is associated with a variety of autoimmune diseases including systemic lupus erythematosus (SLE), glomerulonephritis and vasculitis. C2 is a β1-glycoprotein cleaved by activated C1s into two fragments, C2a and C2b. The larger fragment of C2 then combines with C4b in the presence of Mg<sup>2+</sup> to produce C3 and C5 convertases. The C2 assay for the Oplitite analyser is programmed to produce a multi-point calibration curve from a single calibrator, with a measuring range of 2.29 - 25.78mg/L for both EDTA plasma and serum at a standard 1+9 dilution. High samples are automatically re-measured at a dilution of 1+19, with an upper limit of 51.57mg/L. **Methods & Results:** A matrix comparison using 45 paired serum and EDTA plasma samples demonstrated good agreement between the sample types with a Passing Bablok slope of *y*=1.012 - 0.362. A linearity study was performed following EP06-A using EDTA plasma. The assay was demonstrated to be linear over a range of 1.16mg/L - 34.45mg/L at the standard dilution of 1+9. Sensitivity was tested according to EP17-A2 and demonstrated a limit of quantitation (LoQ) of 2.29mg/L at a 1+9 dilution. A precision study was performed (EP05-A2), by testing 3 EDTA plasma levels on a single analyser over 20 days, across the three levels tested this gave a maximum within run coefficient of variation (CV) of 5.1%, a maximum between instrument CV of 3.2% and a maximum total CV of 5.8%. Comparison was made to a commercially available C2 assay on the Binding Site SPAPLUS analyser, using EDTA plasma samples (n=122, value range 2.82 - 48.95mg/L). Good agreement was observed when the data was analysed by Passing-Bablok regression; *y*=1.052 - 0.1893. A reference range was constructed using 120 samples from healthy donors, this gave a 95<sup>th</sup> percentile range of 10.12 - 32.37 mg/L. This matches closely with reference ranges produced using C2 radial immunodiffusion (RID) kits of 10-30mg/L (Sheffield Protein Reference Unit & Immunology Department, UK). Interference testing was performed (EP07-A2) at 3 EDTA plasma concentrations, challenging with hemoglobin (200mg/dL), triglyceride (300mg/dL), intralipid (125mg/dL) and bilirubin (20mg/dL). All results were  $\pm$ 10% when compared to equivalent negative controls. **Conclusion:** In conclusion, the C2 assay for use on the Oplitite® provides a reliable and precise method for quantifying C2 in EDTA plasma and serum samples and correlates well with existing methods.

**B-060****Performance Evaluation of the High-sensitivity ADVIA Centaur and Atellica IM Serum Neurofilament Light Chain (sNfL) Assay**

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**Background:** Neurofilament light chains (NfL) are neuronal-specific intermediate proteins that are released from neurons and axons upon injury. In cerebrospinal fluid (CSF), NfL was discovered to be a biomarker of neurodegeneration with high cor-

relation to clinical and MRI disease activity in multiple sclerosis (MS) patients. NFL concentrations in CSF are approximately 50 to 100 times higher than in blood, making it a more robust biomarker. However, the invasiveness of lumbar puncture limits the practicality of using CSF-NfL for routine monitoring. The continuous advancement of acridinium ester (AE) chemiluminescence technology by Siemens Healthineers led to the development of a novel, reliable IVD assay for the quantification of NfL in blood.

**Methods:** The ADVIA Centaur® and Atellica® IM serum Neurofilament Light Chain (sNFL) assay is a fully automated two-step sandwich immunoassay using acridinium ester chemiluminescent technology for the detection of neurofilament light chains in blood. This assay uses two highly specific noncompeting monoclonal antibodies against the conserved rod domain of NfL, without cross-reactivity for neurofilament medium chain (NfM), neurofilament heavy chain (NfH), or glial fibrillary acid protein (GFAP). In the assay's Lite Reagent, the detection antibody is labeled with a high-yield acridinium ester. In the Solid Phase, the capture antibody is biotinylated and preformed to streptavidin-coated paramagnetic particles. The assay uses both serum and plasma specimens. Analytical performance was evaluated for precision, detection capability (LoB, LoD, and LoQ), interferences, linearity, specimen equivalency, and hook effect. A method comparison against a previously published Siemens Healthcare Laboratory Neurofilament Light Chain assay is shown. **Results:** Precision was tested on eight native samples ranging from 2.84 to 376.01 pg/mL. Repeatability ranged from 6.4% at 2.84 pg/mL to 0.8% at 376.01 pg/mL. Within-run precision ranged from 12.4% at 3.00 pg/mL to 2.1% at 376.01 pg/mL. On the Atellica IM Analyzer, the assay has a LoD of 0.66 pg/mL and a LoQ of 1.00 pg/mL, with a linear measuring range  $\geq 500$  pg/mL. An onboard dilution extends the reportable range up to 3000 pg/mL. Specimen equivalency was determined using 104 serum/EDTA plasma paired samples ranging from 3.4 to 421.1 pg/mL. A Passing-Bablok regression analysis resulted in a slope of 1.00 (95% CI: 0.96-1.04) and an intercept of -0.4 pg/mL (95% CI: -0.8 to 1.1). Interference testing showed all biases to be  $<10\%$ , including biotin at 3500 ng/mL. There was no hook effect up to 1,000,000 pg/mL. A method comparison to the (LDT) assay was performed using Passing-Bablok regression analysis, resulting in a slope of 0.93 (95% CI: 0.83-0.98) and an intercept of -0.3 (95% CI: -0.87 to 0.83) pg/mL. **Conclusion:** The ADVIA Centaur and Atellica IM serum Neurofilament Light Chain (sNFL) assay is a high sensitivity, high-throughput, in vitro diagnostic assay with appropriate performance for measurement of NfL in blood. Specimen type studies show no statistical difference in NfL levels between serum and plasma collections. \*Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

## B-061

### Spectroscopic and Computational Investigation of Fe-Bound and Co-Substituted C93G/Y157F Cysteine Dioxygenase

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**Background:** Free cysteine (Cys) is vital for many biological processes in mammals, including the production of glutathione, taurine, pyruvate, and protein synthesis. However, a high concentration of Cys is associated with rheumatoid arthritis, Parkinson's disease, Alzheimer's disease, cystinosis and an increased risk of cardiovascular disease. Therefore, the concentration of Cys in tissues must be tightly regulated to satisfy an organism's metabolic needs without causing harm. This regulation is partially performed by a mononuclear non-heme Fe(II) dependent thiol dioxygenase, cysteine dioxygenase (CDO). CDO, a cytosolic enzyme largely produced in the liver, performs the first step in cysteine catabolism by incorporating both oxygen atoms of molecular oxygen into the thiol group of free cysteine to produce cysteine sulfenic acid (CSA). Although numerous X-ray crystal structures of resting and Cys-bound CDO have been reported, the role of secondary sphere residues in the catalytic mechanism and the identities of on-pathway intermediates remain incompletely understood. Most notable is a covalent crosslink between the thiol of C93 and an ortho-carbon of Y157 (*Mus musculus* CDO numbering). Removal of this crosslink via site-directed mutagenesis creates variable kinetic effects dependent on the specific amino acid substitution; the C93G variant remains nearly as active as fully crosslinked WT CDO, while the Y157F variant is completely inactive. To examine the hypothesis of untethered C93 inhibiting CDO activity, a novel C93G/Y157F variant has been created which readily incorporates non-native metals, facilitating the investigation of catalytic intermediates.

**Methods:** Ultraperformance liquid chromatography (UPLC) and a quantum mechanics/molecular mechanics (QMMM) computational analysis of the Fe-bound C93G/Y157F CDO variant was performed. UV-vis absorption (Abs) and electron paramagnetic resonance (EPR) spectroscopic data of Co-substituted, Cys-bound C93G/Y157F CDO were also collected.

**Results:** UPLC data reveals that this Fe-bound variant can oxidize Cys while QMMM computations reveal how specific amino acid substitutions of the crosslink control water coordination to the metal center. Abs data of the Co-substituted variant exhibit weak Co(II) d-d transitions and charge transfer bands that shift upon the addition of Cys, indicating that C93G/Y157F CDO can incorporate Co and bind Cys. X-band EPR data evince an axial radical signal centered at  $g=2$  that exhibits Co-hyperfine structure, consistent with the formation of a Co(III)-superoxo intermediate.

**Conclusion:** Collectively, these data add evidence that an Fe(III)-superoxo intermediate is a likely catalytic intermediate for CDO and that the crosslink increases the enzyme's catalytic activity by preventing the stabilization of a water molecule at the metal center of substrate-bound CDO. These data further advance the understanding of CDO's structure-function relationships, providing a foundation to better probe malfunctioning CDO in various disease states.

## B-062

### Evaluation of a Lipase Open Channel Assay Using the Abbott Alinity c Analyzers

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**Background:** Measurement of lipase may aid in diagnosis and management of pancreatic disorders such as acute and chronic pancreatitis. The objective of this study was to evaluate the analytical performance of the Sentinel Lipase New Generation (NG) Open Channel assay configured on the Abbott Alinity c analyzers.

**Methods:** Method comparison was conducted using residual patient plasma samples collected in lithium heparin plasma tubes (n = 49). They were analyzed using the Sentinel Diagnostics Lipase NG reagent on the Abbott Alinity c analyzers. The previously validated Architect c8000 using Abbott's Lipase reagent was the comparator method. Further, the selected Alinity c analyzers were compared to each other. Linearity and analytical measurement range (AMR) verification were performed by using Maine Standards' Validate GC3 Test Set and diluting with saline solution. Precision was tested using Bio-Rad Liquid Unassayed Multiquant Controls (QC). All levels of QC were assayed one run per day, five replicates per run, over five days. Reagent carryover was verified using Bio-Rad QC materials and following Abbott's reagent carryover evaluation protocol. Sample carryover was performed by analyzing a patient sample (high concentration, H) and level 1 of Maine Standards (low concentration, L) according to the protocol described by EP Evaluator. Onboard and manual dilution studies were performed using diluted Maine Standards, Abbott's Lipase calibrator and patient sample pool.

#### Results:

Method comparison of the Sentinel Lipase NG Open Channel to the Abbott Lipase exhibited a negative bias with average slope of 0.76, intercept of 2.98, r (correlation coefficient) of 0.99, and %bias of -21.5%. However, this comparison met the expected criteria of observed total error allowable (TEA)  $\leq 30\%$ . The assay was linear over a measured range of 4.0 to 296.3 U/L with maximum deviations from target recovery  $\leq 2.8\%$ , average intercept of -1.05, and slope of 1.02. Precision studies demonstrated average %CVs  $\leq 2.2\%$  for within run and  $\leq 2.8\%$  for total. No clinically significant carryover was observed for either reagent and sample carryover studies (%bias  $< 10\%$  and error limit  $\leq 0.6$  accordingly). The observed results for onboard and manual dilution studies were found to be acceptable, with %difference  $< 10\%$ .

#### Conclusion:

The Sentinel Lipase NG assay, configured for the Abbott Alinity c analyzers, demonstrated a negative bias when compared to the Abbott Lipase reagent using the Architect c8000. However, all Alinity c analyzers agreed with each other well. The Sentinel Lipase NG assay had favorable linearity and precision. There was no clinically significant reagent and sample carryover detected. Overall, the Sentinel Lipase NG Open Channel is acceptable for patient testing on the Abbott Alinity c analyzers.

## B-063

### The OnSite® Plus. A new concept of portable mini laboratory

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**Background** In February 2021, amid the COVID pandemic, it was decided to rehabilitate the old Military Hospital of Sevilla to become the Covid Emergency Hospital

for the entire city and thus avoid the saturation of the rest of the hospitals. It was decided to reopen this hospital with 125 hospitalization beds, 25 ICU beds, and a series of central services to provide support, including a hospital response laboratory for COVID patients. **Objective** To describe the results of the one-year operation of a portable mini-laboratory called OneSite® Plus (AWE Medical...), implanted in a short period of time in a hospital that did not have one and that had to treat COVID-19 patients, delivering results in real time. **Methods** Instruments. It was decided to use POCT technology combining different devices that are integrated into two similar platforms, the OneSite® Plus, a mini-laboratory that allows specific combinations of POCT units according to customer needs. The systems used in our study consisted of 5 analyzers: two Chemistry Analyzer Pointcare®cM4 (MNCHIP Technologies Co, Tianjin, China) for biochemical parameters, one ADVIA 360 for absolute counts of the three hematological cells and the differential leukocyte counting, and one RP 500 for blood-gases and electrolytes, metabolites, and Co-Oximetry, both from Siemens Healthineers (Erlangen, Germany) and, finally, an AFIAS-6 (Boditech, Gangwon-do, Korea) for immunochemistry. An outstanding feature of OneSite® Plus is its proprietary software plus Siemens middleware (Servolab), which allows all the POCT units to be interrelated and permits their connection to the HIS and LIS. Implementation site. The laboratory that would respond to COVID-19 patients had to be implemented in a space of 21 square meters and working in a network with the central laboratory located 3 km away. Portfolio. In addition to the many laboratory variables that OneSite® Plus can measure (over 600), a specific COVID-19 profile including hepatic and kidney function, acid-base balance and electrolytes, D-dimer, three population counts Hematology and WBC formula, CRP, ferritin, procalcitonin and cardiac troponin I, were offered to clinicians. Assays were available 24/7 and performed by laboratory technicians. **Results** After 1-year of implementation, the OneSite® Plus platform had produced 168,817 tests results of which 43,892 came from the ICU; the mean number of parameters per request was of 13.92. The most requested tests (>9,500/year) were renal markers, hepatic and muscle enzymes, CRP, hematological counts, D-Dimer, and ferritin.

**Conclusion** The OneSite® Plus mini-laboratory was pivotal in solving the urgent need for a clinical laboratory in a hospital that lacked one amid the COVID-19 pandemic. After 1 year of daily use, the system proved to be robust enough for processing thousands of different biochemical, hematological, and immunochemical tests with adequate TAT. Our results show that OneSite® Plus could be a reliable alternative to central laboratories when needed. This laboratory allows providing a solution to COVID-19 patients in a limited space using 100% POCT technology.

## B-064

### Comparison of the same free light chain assay on the Optilite versus cobas Instrument

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**Introduction:** Serum free light chain (sFLC) assays are used clinically to diagnose and monitor monoclonal gammopathies including multiple myeloma, lymphocytic neoplasms and primary amyloidosis. The Freelite assay is the most commonly used reagent for assessing sFLC concentrations, comprising 91% of labs on a recent, 2021 CAP survey. Although this assay is available on multiple instrument platforms, data comparing their performance is limited. Thus, we evaluated the Freelite sFLC assay on The Binding Site Optilite and Roche cobas instruments and compared analytical performance, assay workflows, and reagent usage. **Method:** sFLC concentrations were measured in 300 fresh serum specimens with physician ordered sFLC testing, 48 previously frozen specimens with documented sFLC > 20 mg/dL (28 kappa and 22 lambda) were also assessed. Specimens were analyzed using open channel parameters on the Roche cobas 8000 and the Optilite analyzers within 8 hours using the Freelite Lambda and Kappa assays (The Binding Site) according to the manufacturer's recommendations. Assay performance was compared using Deming regression. Median turnaround time (TAT) was calculated for each method and the number of tests required was assessed using a chi-squared test. **Results:** Deming regression of the observed concentrations from each instrument revealed a slope of 1.042 (95% CI 0.88 to 1.21) and an intercept of -0.767 (95% CI -2.31 to 0.77) for sFLCκ and a slope of 0.896 (95% CI -0.035 to 1.83) and intercept of 1.593 (95% CI -3.14 to 6.33) for sFLCλ. The median (IQR) TAT for the Optilite was 15.3 mins (15.3-15.6) for sFLCκ and 15.3 mins (15.2-15.7) for sFLCλ. The median TAT for the cobas was 17.0 min (12.0-29.0) for sFLCκ and 16 mins (12.0-29.0) for sFLCλ. The proportion of specimens with sFLCκ or sFLCλ with a TAT >60 minutes was 0.33% for the Optilite and 8% for the cobas (p<0.0001). The Optilite instrument required 49 fewer tests to analyze 300

specimens for sFLCκ (p=0.0004,) and 12 fewer tests to analyze the 299 specimens for sFLCλ (p=0.016) relative to the cobas. For the additional specimens with sFLC > 20 mg/dL, the median (IQR) TAT on the Optilite for

sFLCκ (n=28) was 46.1 mins (15.3 - 46.1) and for sFLCλ (n=22) was 46.0 mins (30.6-46.2). On the cobas, the median TAT for sFLCκ was 255 mins (103-322) and for sFLCλ was 123 mins (102.0-242.0). **Conclusion:** Analytic performance of the FLC assay on the Optilite was comparable to the cobas. However, the Optilite had reduced TAT, required less reagent, and reduced manual dilutions for samples. This effect was enhanced in specimens with sFLC concentrations greater than 20 mg/dL.

## B-066

### Analytical performance comparison of COVID-19 diagnostic methods during the acute phase

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#### Background:

Since the report of the first cases of acute respiratory syndrome in Wuhan, China (December 2019), the health sciences authorities and researchers focused their resources on identifying the cause and manners to diagnose COVID-19. During the following months a wide range of diagnostic methodologies was presented, mostly focused on the detection of epitopes of the SARS-CoV-2. Those methodologies were based on sequencing the virus genetic code which is very effective but costly in both time and monetarily. An agile option was the polymerase chain reaction in real-time associated with the reverse transcriptase (RT-qPCR) that detects multiple targets in trivial amounts, which has rapidly been determined as the gold standard method for diagnosing COVID-19. As the pandemic goes on, many efforts have been put to offer other kinds of tests in a short period of time. One of the tests that arouse is the protein (antigen) detection by lateral flow immunoassay (LFIA). Due to differences in sensibility each test is indicated at different infection stages. The RT-PCR methodology applied at our laboratory has been used abroad as a gold standard (SeeGene®) as various targets (gene N, gene S, and RDRP) can be detected by the same filter simultaneously increasing the detection probability. Although its advantageous role, the RT-PCR needs specialized professionals, and it is time-consuming. As a faster and more affordable alternative, the LFIA can be utilized. Since 2020, many kits and reagents from different manufactures have been tested at Associação Fundo de Incentivo à Pesquisa, São Paulo (Brazil). In this study we present a comparison of all reagents for both RT-PCR and LFIA according to their analytical performance.

#### Methods:

Since 2020, the laboratory professionals tested 7 different RT-PCR detection kits (MicoBio, Roche, HybriBio, LGC, LGC - fast protocol, Seegene, and Om Mico Rox) and 11 antigen detection kits (Acro, Bioscience, Panbio, ECO -Rapid, Fluorescence-based, and hybrid test - Labtest, PelkinElmer, Roche, Wama, and Siemens) to evaluate their analytical performance. All tests were performed according to their manufacturer's instructions. Sensitivity, specificity, and agreement (Cohen's Kappa index) were calculated for each detection kit.

#### Results:

Over 260 samples were tested by RT-PCR methods. Sensitivity ranged from 88.2% to 100%, Specificity from 96.7% to 100%, and Agreement from 92.3% to 100%. For LFIA tests results, a total of 293 samples were tested. Sensitivity ranged from 60% to 100%, Specificity of 100%, and Agreement from 80% to 100%.

#### Conclusion:

According to the World Health Organization, LFIA tests for antigen detection should have sensitivity of 80% and specificity of 97% when compared with a molecular test in order to qualify for WHO Emergency Use Authorization listing. Most of the kits tested by us have accomplished that recommendation. Panbio kit have not reached that mark, however it was due to the selection of samples with high CTs in the RT-PCR. These data show that rapid antigen detection present appropriate performance when compared to RT-PCR. They can be a faster and applicable alternative, especially when used in the recommended detection window to reduce false-negative rates.

**B-067****Analytical Performance Evaluation of the Next Generation Clinical Chemistry assays on the Architect System**

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**Background:** This study aimed to evaluate the analytical performance of 6 representative newly developed Strong Six Sigma chemistry assays on the Abbott ARCHITECT system. In addition to evaluating precision, linearity, method comparison, and accuracy, sigma metrics were determined to assess the method performance in relation to allowable performance limits.

**Methods:** Imprecision was assessed by measuring 2 levels of quality control (QC) material (Biorad Chemistry UA) and 3 pooled patient samples 5x twice per day, for five days. Acceptable imprecision and bias were determined based on the Accreditation Canada Diagnostics (ACD) recommendations. Linearity testing consisted of 5-6 levels of commercially available linearity materials, with 3-4 replicates per level. Method comparison between the new methods and the current Architect methods were evaluated using 120 serum/plasma specimens, measured in duplicate. To determine accuracy, we measured 10 replicates of NIST 470 and 456 materials for albumin and amylase, respectively, or calibrators for cholesterol, total protein, and urea. The imprecision and bias from the target value were used to calculate the sigma value. Total allowable error (TAE) limits were obtained from the Clinical Laboratory Improvement Amendments (CLIA) and ACD guidelines. Statistical analysis was performed using EP Evaluator. Method comparison analysis was performed using Passing Bablok regression.

**Results:** The observed results for precision, accuracy, linearity, and method comparison for representative clinical chemistry (Serum/Plasma) assays are shown in **Table 1**. The 6 next generation ARCHITECT assays demonstrated  $\geq 6$  Sigma performance.

**Conclusion:** Representative clinical chemistry assays utilizing photometric technology on the ARCHITECT c8000 system demonstrated acceptable performance for precision, accuracy, and linearity. Method comparison data showed good agreement with on-market ARCHITECT clinical chemistry assays.

Analytical performance evaluation results for 6 Strong Six Sigma chemistry assays				
Assay	Precision (Range of total %CV across 5 concentrations)	Accuracy (% bias from target value)	Linearity (Range of % recovery)	Method comparison to ARCHITECT (Correlation)
Albumin (Bromocresol green)	0.5 – 2.1%	0.0%	99.1 – 109.4%	0.998
Albumin (Bromocresol purple)	1.0 – 4.0%	-1.4%	96.3 – 107.7%	0.996
Amylase	0.4 – 3.7%	-2.0%	98.4 – 106.8%	1.000
Cholesterol	0.8 – 1.3%	0.7%	89.6 – 105.7%	1.000
Total Protein	0.7 – 3.6%	-0.2%	96.5 – 98.6%	0.999
Urea Nitrogen	0.7 – 2.4%	-1.4%	98.5 – 104.8%	1.000

**B-068****Reproducibility of a Volumetric Absorption Device for Both Drugs and Endogenous Compounds in Whole Blood**

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**Background:** Dried blood techniques are of significant interest for a variety of reasons but particularly because of their potential to allow for sample collection outside of typical collection environments used for venipuncture. Different techniques have been around for quite some time but full adoption in the laboratory space has been slow for different reasons including concerns over reproducibility being among the most common. Here we tackle the question of reproducibility using a Volumetric Absorptive Microsampling (VAMS)® device called Mitra® from Neoteryx. We performed repeat sampling using the tips and analyzed them using LC/MS/MS to determine how reproducible our results would be from the same sample. We analyzed a range of compounds including 10 cannabinoid drugs, 4 endogenous hormones, and caffeine.

**Methods:** For Cannabinoids, spiked whole blood was used to dip the 30 uL tips for analysis. For hormones and caffeine samples both from spiked blood and finger-prick samples were evaluated for reproducibility. The samples were then extracted and analyzed on a Shimadzu LCMS-8060 mass spectrometer with a nexera autosampler.

**Results:** All analytes were reproducible across repeated sampling with CV's under 20% and many were under 10% CV for tip to tip variance. This proved true for both tips collected from spiked venipuncture blood as well as tips collected from finger prick samples.

**Conclusion:** This data shows when instructions are followed correctly, the Mitra® device can be a reliable tool to measure small molecules in whole blood. CV's were in an acceptable range for many clinical and research applications. Future work will build on these results to compare venipuncture samples to finger prick samples across the analytes.

**B-069****Quantitative analysis of ethanolamine plasmalogen species in red blood cells using liquid chromatography tandem mass spectrometry**

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**Background:** Plasmalogens are glycerophospholipids characterized by a vinyl-ether bond with a fatty alcohol at the *sn*-1 position, a polyunsaturated fatty acid at the *sn*-2 position, and a polar head group at the *sn*-3 position, ethanolamine in most tissues. Although still not well-defined, they play a critical role in several cellular processes. The first two steps of plasmalogen biosynthesis occur in peroxisomes; hence, patients with defects in peroxisome biogenesis (PBD), including Zellweger spectrum disorders (ZSD) and Rhizomelic chondrodysplasia punctata (RCDP), exhibit markedly reduced plasmalogens. We have developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to quantify 18 intact ethanolamine plasmalogens (pl-PEs) in packed red blood cells (RBCs) to aid the diagnosis of PBD patients, particularly RCDP.

**Methods:** RBCs were washed with normal saline and lysed by freezing. Plasmalogens were extracted with methanol containing two labeled internal standards, shaking for one hour at room temperature. Chromatographic separation was performed using an Acquity Premier BEH C<sub>18</sub> UPLC column and a binary gradient of 5 mM ammonium acetate in water:methanol (15:85) and 5 mM ammonium acetate in methanol. Analysis was performed using a XEVO TQ-XS Mass Spectrometer with Ultra-High Performance Liquid Chromatography (Waters) in multiple reaction monitoring mode. Eighteen pl-PEs were quantified using four commercially available standards. Cumulative values were also calculated for the six C16:0 (palmitoyl), C18:0 (stearoyl) or C18:1 (oleoyl) species, and for total plasmalogens. Preliminary reference intervals were established with a focus on the pediatric population (n=185, 99 males, 86 females; mean age= 8.3 years, range 0 to 57 years) using RBCs from self-reported healthy volunteers (n = 118) and de-identified clinical samples referred for unrelated testing. The study was approved by the Institutional Review Board of the University of Utah.

**Results:** The method was linear throughout the calibration curve ( $r^2 > 0.99$ ), with mean accuracy 95.5-102% of target and coefficient of variation (CV) ranging 2.4% to 6.4% (n=20). Intra-assay (n=6 replicates on 2 runs) and inter-assay variability (n=10 runs) in three donor RBCs was <10%. Tubes containing sodium heparin or EDTA were both appropriate for blood collection. Neat standard stock solutions in chloroform (tested up to 2 years), calibrators prepared in methanol (tested up to 6 months) and packed RBCs (tested up to 15 months) were found to be stable long-term at  $\leq -65^\circ\text{C}$ , exceeding previously reported data. RBCs were also stable up to 10 freeze/thaw cycles. Plasmalogens were stable in refrigerated (2 - 8°C) whole blood for up to 7 days after draw. Total plasmalogens showed a modest increase with age ( $r_s = 0.394$ ,  $p < 0.0001$ ). The levels in neonates ( $\leq 4$ -week-old; n=46;  $368 \pm 37 \text{ nmol/mL}$ ) were significantly lower than in children and adults (n=139;  $431 \pm 56 \text{ nmol/mL}$ ;  $p < 0.0001$ ).

**Conclusion:** We have developed a robust LC-MS/MS method to quantify intact ethanolamine plasmalogen species in packed RBCs to aid in the diagnosis of peroxisome biogenesis disorders. Age-specific reference ranges will be used for the clinical interpretation of the results.

**B-070****Comparison of Three Automated Immunoassays for the Detection of Anti-cardiolipin and Anti-beta 2 Glycoprotein I Antibodies according to the Different Cut-off Values**

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**Background:** Anti-cardiolipin antibodies (aCL) and anti-β2 glycoprotein I (aβ2GPI) antibodies are laboratory criteria for the classification of antiphospholipid syndrome (APS) and systemic lupus erythematosus (SLE). Although immunoassays for aCL and aβ2GPI have been widely used, they differ in assay performance and cut-off values. We aimed to assess and compare the diagnostic performance of three automated immunoassays according to the different cut-off values.

**Methods:** A total of 368 serum samples from 10 patients with primary APS, 25 with APS secondary to SLE (SAPS), 63 with SLE, 150 with other autoimmune diseases (non-SLE), and 120 healthy controls (HC) were tested. Serum aCL IgG and aβ2GPI IgG levels were measured with three automated immunoassays (QUANTA Flash (QF), BioPlex, and ImmunoCapEliA (EliA)). We investigated diagnostic performance and concordance between assays according to the assay-specific cut-off values recommended by the manufacturer and obtained at 95<sup>th</sup> or 99<sup>th</sup> percentiles of 120 healthy controls.

**Results:** Three aCL IgG and aβ2GPI IgG assays results showed great differences in each group (Figure 1). Three aCL IgG and aβ2GPI IgG assays had good performances in APS/SAPS diagnosis from the non-SLE/HC group (QF; 0.936 and 0.939 (AUC), BioPlex; 0.871 and 0.857, EliA; 0.818 and 0.854) with QF assays having the higher AUC values than EliA ( $P < 0.05$ ). However, for the diagnosis of SLE from the non-SLE/HC group, all three assays had similar performances with AUCs of 0.711-0.753 for aCL IgG assays and AUCs of 0.658-0.790 for aβ2GPI IgG assays. Based on the manufacturer's recommended cut-off, the concordance in 248 patients' sera for detection of aCL IgG was best between the BioPlex and EliA assay (84.7%) [QF vs. BioPlex; 72.2%, QF vs. EliA; 68.1%]. The concordance for detection of aβ2GPI IgG was similar in three assays (83.9-85.1%). All three aCL IgG assays and QF aβ2GPI IgG assays differentiated APS from HC ( $P < 0.05$ ). The 95<sup>th</sup> percentile or 99<sup>th</sup> percentile cut-off exhibited concordance and diagnostic performance similar to the manufacturer's cut-off, except for reduced sensitivity in the QF aβ2GPI IgG assay.

**Conclusion:** Three assays showed good performance in APS/SAPS diagnosis, but a moderate agreement between results was found. Further standardization and harmonization are required for the aCL IgG and aβ2GPI IgG immunoassays.

**B-071****Performance Evaluation of the Abbott BinaxNOW® G6PD Test**

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**Background:** Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconolactone with reduction of nicotinamide adenine dinucleotide phosphate (NADP) to reduced nicotinamide-adenine dinucleotide phosphate (NADPH), which is essential to protect cells from oxidative stress. G6PD deficiency is a common human enzyme disorder, caused by one of many possible gene mutations, leading to reduction of enzyme stability and expression in red blood cells. G6PD deficient erythrocytes have difficulty handling oxidative stress and, subsequently, are more susceptible to lysis. Because many commonly used drugs are strong oxidizing agents, individuals with G6PD deficiency are at risk for developing severe clinical complications such as acute spherocytic hemolytic anemia, if prescribed one of these medications. The reference method used to diagnose G6PD deficiency is the quantitative spectrophotometric analysis of NADPH production. However, this method is not routinely available in many hospital labs. In order to provide acutely actionable results, we verified the performance of the BinaxNOW® G6PD test, a rapid enzyme chromatographic test for the qualitative detection of G6PD enzyme activity in whole blood. **Methods:** Prior to implementing rapid G6PD testing using the BinaxNOW G6PD assay, a verification study was performed. In addition to running positive and negative control samples provided with the testing kit, 8 patient samples were also evaluated. Parallel testing of these samples was performed by Mayo Clinic Laboratories using the spectrophotometric reference method to quantify G6PD enzyme activity. After implementation of the rapid assay, we also per-

formed a prospective assessment of the assay's performance. For this study, parallel testing was performed on all samples submitted to the lab for rapid G6PD testing. Remnant samples were sent to Mayo for assessment using their reference method.

**Results:** Initial verification of the BinaxNOW G6PD test revealed 100% (8/8) agreement with the reference method. After implementation, prospective assessment of the BinaxNOW performance was evaluated in 35 patients by comparing the rapid results to the reference method. In the quantitative assay, the median G6PD activity was 9.4 U/g hemoglobin (range = 0.8 - 17.2) with 6 (17.1%) patients identified as deficient and 29 identified as normal. The sensitivity of the BinaxNOW test to detect deficient subjects was 50% (3/6), and specificity was 96.5% (31/35). The overall % agreement for the prospective study was 88.6% (31/35). **Conclusion:** Accurate diagnosis of G6PD deficiency is critical to avoid the risk of acute hemolysis in susceptible patients. Initial evaluation of the BinaxNOW G6PD test suggested excellent performance of this assay for the rapid and reliable detection of G6PD deficiency. However, the prospective study revealed sub-optimal performance as 50% of patients with deficient G6PD enzyme activity based on quantitative results from Mayo, were reported as normal based on results of the rapid assay. It is possible that the subtlety of color change in the chromatographic assay made result interpretation difficult and contributed to the low sensitivity. Based on results of this study, our institution has discontinued the use of the BinaxNOW G6PD test as the sub-optimal sensitivity requires confirmation by the reference method.

**B-074****A fully automated quantification method of microRNA using Bioluminescent Enzyme Immunoassay**

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**Background:** In recent years, microRNAs (miRNAs) have been reported to be associated with various diseases including cancers. However, despite its importance as a clinical biomarker, the miRNA measurement assay has not yet been implemented in clinical practice, due to inconsistency issues inherent to the variable recovery rate of miRNAs in sample preparation and the different accuracy in quantification depending on the methodologies. Thus, a fully automated reliable quantification system should be established to serve as a routine clinical diagnostic assay.

**Methods:**

For a fully automated miRNAs quantification, the bioluminescent enzyme immunoassay (BLEIA) technology was applied. In this study, we used magnetic particles conjugated with monoclonal antibodies that target DNA/RNA hybrids, a biotinylated DNA probe specific to the target sequence of the miRNA, and firefly luciferase-labeled streptavidin. This method enables direct use of the diluted serum samples and all the process was performed automatically within 1 hour. For comparison, a conventional qRT-PCR for miRNAs purified manually from the serum samples was used as a gold standard protocol for laboratory testing.

**Results:** In the analytical performance for synthetic miRNA quantification, this assay showed good linearity in a wide dynamic range from 10 fmol/L to 1 nmol/L, high sensitivity with the detection limit of 1.6 fmol/L to 6.3 fmol/L, high specificity with a false positive rate under 2.3%, and high reproducibility in intra and inter experiments (under coefficient of variation 10% and between-days correlation  $r = 0.9965$ , respectively). Furthermore, an excellent correlation with qRT-PCR was demonstrated for quantification of the synthetic miRNA-21-5p spiked in a pooled healthy serum ( $r = 0.9993$ ,  $n = 5$  points), and a good correlation with qRT-PCR for the endogenous miRNA-21-5p in healthy volunteer serum ( $r = 0.823$ ,  $n = 8$  sera).

**Conclusion:** We newly developed a fully automated miRNA quantification assay based on the BLEIA technology, which showed good linearity in a wide range, high sensitivity, high specificity, and high reproducibility. Our system can use directly clinical samples, indicating the potential to accurately reflect the miRNA amount in clinical samples. The turnaround time for all the processes is less than 1 hour. Taken together, this assay could offer an avenue for the field of miRNA measurements to converge on a standardized assay in a clinical setting, although further study is needed for the clinical performance of this assay.

**B-075****Diagnostic Markers of Ectopic Pregnancy**

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**Background:** An Ectopic Pregnancy (EP) is the implantation of a fertilized ovum outside the normal uterine cavity. As it is the leading causes of maternal morbidity and

mortality worldwide, an accurate and expeditious diagnosis is pertinent. Numerous biomarkers of implantation, angiogenesis and placentation are being evaluated. The aim of this study was to investigate these potential biochemical markers in the diagnosis of EP, particularly parameters which can reliably identify the condition at the early gestational age of 4 to 7 weeks where other diagnostic modalities are indecisive. The ability to do so would not only reduce the need for follow-up but also decrease the time needed for surgery for both diagnostic and therapeutic indications. This would allow the clinician to counsel patients appropriately and is likely to improve decision-making in management, by both clinicians and patients.

#### Methods:

The objectives of the study were estimation of total  $\beta$ -hCG, Progesterone and sFLT-1 in normal and EP, and to study their clinical utility in EP; gene expression of micro RNAs (miRs)141,218, 519d, 873, 223, 517a, 523 and 323-3p in normal and EP, and to study their clinical utility in EP. Methods: This was a prospective case control study conducted over two years from 2015 to 2017 in 280 women between the age group of 19 - 38 years at Sri Ramachandra Medical College & Research Institute, Chennai, India. The control group comprised of 140 normal pregnant women and study group comprised of 140 cases of confirmed EP. Total Beta-hCG and Progesterone were assayed by chemiluminescence, soluble fms-like tyrosine kinase - 1 by ELISA and relative quantification of miRs by Reverse Transcription PCR analysis. Statistical analysis of protein markers was performed by SPSS software version 16.0. Relative gene expression has been estimated by using the fold change calculation by LIVAK method.

#### Results:

Total  $\beta$ -hCG has a sensitivity of 31.4% and specificity of 100% at a cut-off value of 1616 mIU/mL, which is near the clinically used discriminatory zone. Progesterone had a better sensitivity of 94.3% and specificity of 93.6 at a cut-off value 14.4 ng/mL but its usage as a single biomarker has however, been negated by the recent NICE guidelines of 2019. Of the 8 miRs evaluated in sera of women with EP in this study, Four miRs were differentially down regulated: hsa-miRs-141,218, 519d and 873 and four miRs were differentially up regulated: hsa-miRs-223, 517a, 523 and 323-3p. As an individual marker sFLT-1 at a cut-off value 634 pg/mL has a very good sensitivity of 98.6% and specificity of 90%.

**Conclusion:** MiR 519d appears to be a promising potential biomarker of EP with highest specificity of 97.1%, sensitivity of 47.1%, and expression fold change of 0.46. Furthermore, the combination of sFLT-1 with miR 519d also improved the sensitivity to 100% with a good specificity of 87.1%. Increasing the number of women in each gestational age group can help in establishing reference values for normal pregnancy and gestational age-specific cut-off values for EP. This may further increase the usage of this marker in a clinical setup.

## B-076

### Fixing Activated Basophils for Prolonged Novel Basophil Activation Test (BAT) read-out in Allergy Diagnostics

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#### Background:

Basophil Activation Tests (BAT) have gained increasing importance in the field of allergy diagnostics, with increasing scientific evidence showing higher accuracy and clinical relevance amongst other allergy tests. Basophil activation is a valuable biomarker in immune therapy and in food allergy BAT has the concrete potential to significantly reduce the necessity of Oral Food Challenges. BAT is a flow-cytometry-based functional assay that assesses the degree of basophil activation after exposure to allergens directly in the whole blood. It requires live cells, and it is crucial for the standardization of the assay to assess the appropriate storage conditions and times that preserve the basophil integrity, with variability in the assay results in an acceptable range, thus allowing shipment of blood from collection centers to the performing laboratory.

**Methods:** The study was performed according to CLSI approved guideline EP25-A and measurements were performed on Attune™ NxT Flow Cytometer in combination with the plate handler. EDTA-blood from four (4) normal blood donors (A-D), provided by the blood donation center in Basel (CH, Blutspendezentrum Basel), were used. The donors were assessed for their background activation (PB) and for the stimulation by the controls included in the kit. The main positive control in BAT testing addressing the IgE-mediated signaling pathway is a monoclonal antibody - anti-FcεRI (Positive control 1, PC1). Basophils were gated according to side scattering and anti-CCR3 staining intensity and the basophil activation was assessed by CD63 activation marker. All BAT tests were performed according to the instructions for use with a new version of the BÜHLMANN's Flow CAST® kit which include a stabilizing agent in

the buffer to reconstitute the cells after the red blood lysis. The specimen and sample stability and storage were assessed storing the EDTA whole blood at different temperatures (2-8°C or 28°C) and measuring at different time points (0 to 4 days after blood donation). For this experiment basophil were acquired immediately after the stimulation. The stability of the processed samples were assessed on blood stimulated and processed for Flow CAST® testing at day 0 and the processed samples were stored at different temperatures (2-8°C or 28°C) and measured at different time points (0 to 10 days) after cell stimulation and fixation. **Results:** Stability of basophils in the EDTA blood samples at 2-8°C was optimal for all donors up to 2 days, with a minimal decay of the signal. Blood stored at 28°C was stable for BAT testing only within 24 hours as sample stability significantly decreased afterwards. The stabilization of the basophils with the new reconstitution buffer, after stimulation, allowed for long-term storage of processed basophils at 2-8°C, as the percentage of activated basophils remained stable for 10 days, for each of the four donor basophils. Storage of stimulated and fixed basophils at 28°C showed a high variability amongst donors, as only one donor was stable over the whole observation period of 7 days. **Conclusion:** All together this data offers an improvement on the feasibility and logistics of BAT testing from health care professionals to the laboratory.

## B-077

### Serum Levels of Infliximab and Adalimumab Biosimilars Can Be Measured Equivalently to Original Drugs By Quantum Blue Rapid Testing As Tool For Therapeutic Drug Monitoring.

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#### Background:

Therapeutic drug monitoring (TDM) of inflammatory bowel disease (IBD) patients under anti-TNF therapy is based on trough level determination of the drug. The Quantum Blue Infliximab and Adalimumab rapid tests were validated for the originator biologics. It has been previously shown that the Quantum Blue® Infliximab can be equally used to measure drug levels for the infliximab biosimilar CT-P13 (1) and SB2 (2). The aim of this study was to demonstrate the equivalent quantification of the biosimilars of infliximab GP1111 and the adalimumab biosimilar adalimumab-adaz as for their originators with the Quantum Blue Infliximab and Adalimumab rapid tests in combination with the Quantum Blue Reader, respectively.

#### Methods:

Formulated infliximab (originator or biosimilar) and adalimumab (originator or biosimilar) as kindly provided by the manufacturer company were quantified by means of human IgG analysis on a clinical chemistry analyzer (Mindray BS-380) and used to spike pooled filtered human serum (normal donor serum). The same amount of infliximab or adalimumab originator and biosimilar were used. These specimen with known concentrations were analyzed with the Quantum Blue Infliximab and Adalimumab assays, respectively. The result of the biosimilar specimen was compared to the expected values. A recovery  $< \pm 15\%$  was used as acceptance criteria.

#### Results:

For each spiked sample the expected concentration was calculated and compared to the obtained concentration (recovery). A mean relative bias of -1.9% was found for infliximab originator. For the infliximab biosimilar a mean relative bias of -4.0% was found. For adalimumab a mean relative bias of -6.7% was found for originator, while for the biosimilar a mean relative bias of 1.0% was found. The mean bias between both analyzed biosimilar and originator pairs were within acceptance criteria. In a method comparison Bland-Altman analysis was used to calculate the mean relative bias of the concentration ( $\mu\text{g/mL}$ ) levels of the analyzed samples. For infliximab biosimilar and originator, the bias was -7.6%, while the mean relative bias between adalimumab biosimilar and originator was 12.0%. The mean bias between both analyzed biosimilar and originator pairs were well below  $\pm 15\%$ .

#### Conclusion:

The results support the conclusion that the Quantum Blue Infliximab and Adalimumab rapid trough level tests can be used to measure the originator as well as their GP1111 and adalimumab-adaz biosimilars, as previously shown for SB2 and CT-P13 biosimilars. Obtained results are equivalent and the assay can be used for both infliximab and adalimumab biologics as part of the therapeutic drug monitoring.

**B-078****Standardization of Quantum Blue® Rapid TDM Assays with WHO International Standards For Adalimumab and Infliximab**

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**Background:**

Therapeutic drug monitoring of inflammatory bowel disease (IBD) patients under anti-TNF therapy is based on trough level determination of the drug. Rapid assays and multiple ELISAs are available that measure anti-TNF biologics. Recently, WHO introduced anti-TNF standards for adalimumab (ADL) and infliximab (IFX). A WHO international reference material (IRM) based standardization is crucial for the harmonization of assays available on the market.

**Methods:**

The aim of the study was to standardize the BÜHLMANN Quantum Blue® ADL and BÜHLMANN Quantum Blue® IFX based on the WHO IRM for ADL and IFX, respectively. A value transfer from the WHO reference material to the internal calibrator sets for both assays was based on a protocol previously described by Blirup-Jensen et al. (Clin Chem Lab Med 2001) and by means of a commercially available ELISA. A method comparison of the ELISA and the rapid test was carried out before the value transfer to guarantee comparability of both assays. Additionally, the correlation of the WHO IRM with the currently used calibrator material was determined for ADL. The correlation of the WHO IFX standard (NIBSC 16/170) with the currently used calibrator material was presented recently (Keller et al. 2020, UEGW 2020). Calibration curves were generated with BÜHLMANN ADL calibrators and with calibrators made from WHO IRM for ADL (NIBSC 17/236). Serum samples, covering a concentration range from 1.0 to 35 µg/mL, were analyzed with both calibration curves and compared by Bland-Altman and Passing-Bablok analysis.

**Results:**

A preliminary value transfer study revealed a relative uncertainty of 12.3% for both drugs. A good comparison of the ADL and IFX rapid test and the ELISA is given: Passing-Bablok correlation coefficient (R) of 0.953 (ADL) and 0.942 (IFX), and a mean bias determined by Bland-Altman of 1.59 µg/mL (ADL) and -0.5 µg/mL (IFX), respectively. The sample values gained with BÜHLMANN calibrators showed an excellent correlation with values gained with the WHO international standard for ADL as calibrator. Passing-Bablok regression analysis revealed a slope of 1.3 and correlation coefficient (R) of 1.0.

**Conclusion:**

The Quantum Blue® ADL and Quantum Blue® IFX, are one of the first commercially available quantitative lateral flow assays comprising a WHO based standardization. Additionally, it was demonstrated that the current standardizations of Quantum Blue® ADL correlates very well with the WHO international standard for ADL.

**B-079****Laboratory Validation of a Neurofilament Light Chain (NfL) Clinical Trial Assay for Applications in Neurological Diseases Testing Services**

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**Background**

The identification and quantification of axonal damage in neurological diseases management could be crucial for improved prognosis and diagnostic accuracy. Neurofilament light chain (NfL) is a neuron specific structural protein which can be detected in blood and Cerebrospinal fluid (CSF) as a biomarker associated with axonal injury or degeneration.<sup>1</sup>

The role of NfL as a biomarker has been reported in Alzheimer's disease (AD), traumatic brain injury (TBI), and multiple sclerosis (MS).<sup>2</sup> Siemens Healthcare Laboratory (SHL) validated an in-house NfL assay that runs on a Siemens Healthineers high throughput platform.

**Methods**

The Siemens Healthcare Laboratory NfL Clinical Trial Assay (CTA) is an automated 2-site sandwich immunoassay using direct chemiluminometric technology. This NfL assay uses two specific non-competing monoclonal antibodies against the conserved rod domain of NfL, without cross-reactivity for neurofilament medium chain (NfM), neurofilament heavy chain (NfH), or glial fibrillary acid protein (GFAP). In the assay Lite Reagent, the detection antibody is labeled with a high yield acridinium ester.

The SHL NfL CTA was analytically validated in a CLIA-CAP accredited laboratory for use in clinical trials. Validation studies were performed using serum, plasma, and cerebrospinal fluid.

**Results**

Assay	Reportable Ranges	Reproducibility <sup>1</sup>	Method Comparison <sup>2</sup>
Serum	3.9 to 500 pg/mL	4.9 to 8.4%	Avg Quantitation Difference: -8% Pearson correlation: R=0.995
K2EDTA Plasma	4.9 to 544 pg/mL	3.2 to 18.1%	Not tested
LiH Plasma	2.4 to 549 pg/mL	3.5 to 16.3%	Not tested
CSF	85.5 to 25,700 pg/mL	4.0 to 16.5%	Avg Quantitation Difference: -29% Pearson correlation: R=0.994

<sup>1</sup>Percent CVs in reportable ranges. <sup>2</sup> Comparison of Simoa vs SHL NfL CTA

**Conclusion**

NfL in CSF, serum, and plasma samples were detected reproducibly across the reporting range. SHL NfL CTA exhibited good correlation to Simoa NfL assay. The stability of NfL in samples exposed to different storage conditions was shown to be practical for the use of NfL detection in clinical trials.

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**B-080****Middle-up Approach for Therapeutic Monoclonal Antibodies (t-mAbs) Monitoring in Human Serum using LC-HRAM-MS**

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**Background:** Each year, more therapeutic monoclonal antibodies (t-mAbs) are approved by regulatory agencies to treat a wide range of diseases. For effective treatment, it is important to monitor t-mAbs efficacy and maintain drug concentrations in an optimal therapeutic range. Also, understanding loss of response is necessary when patients fail treatment. Several laboratory-developed tests are available for t-mAbs monitoring in clinical laboratories, including mass spectrometry-based methods. Bottom-up approach has been used to quantify signature peptides specific to t-mAbs. However, as more humanized t-mAbs are produced, signature peptides become limited, differentiating from the endogenous antibodies or other t-mAbs. Additionally, sample preparation is often laborious to generate final peptides in a desirable purity or concentration. To overcome such challenges, the middle-up approach has emerged as an alternative by targeting subunits from the light and heavy chains of t-mAbs. Here, we present the middle-up approach to accurately quantify the light chain of t-mAbs in human serum using HRAM MS.

**Methods:** T-mAbs used in this study include adalimumab, bevacizumab, camrelizumab, daratumumab, golimumab, nivolumab, and vedolizumab. The calibration curve was generated with different concentrations of standards ranging from 1 µg/mL to 200 µg/mL. Serial dilutions were performed using a normal pool of human serum (Fisher BioReagents). Thermo Scientific™ Melon™ Gel Monoclonal IgG Purification Kit was used for the sample purification. The purified samples were subjected to IdeS digestion following the protocol provided by the vendor (FabRICATOR® Ides, Genovis). The samples were then reduced using Thermo Scientific™ Bond-Breaker™ TCEP Solution, Neutral pH at a final concentration of 10 mM and Thermo Scientific™ Pierce™ 8 M Guanidine-HCl Solution at a final concentration of 4 M. LC separation was performed by Thermo Scientific™ Vanquish™ HPLC system using Thermo Scientific MAbPac Reversed Phase HPLC column (2.1 x 50 mm, 4 µm). MS analysis was performed by Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer (OE240).

**Results:** The method generated three subunits including light chain (LC), a fragment of the heavy chain constant regions (Fc/2), and a fragment containing the heavy chain variable region (Fd') with all sizes around 25k Da. A stringent reduction condition with 4 M Guanidine-HCl was necessary to ensure fully reduced three subunits across chromatograms. Without using 4 M Guanidine-HCl, samples showed multiple peaks with non-reduced or semi-reduced disulfide bonds, which complicated data profiles and data processing. The OE240 MS fully resolved isotopic clusters of different charge states of three subunits. It was key to use a resolution higher than 120k which

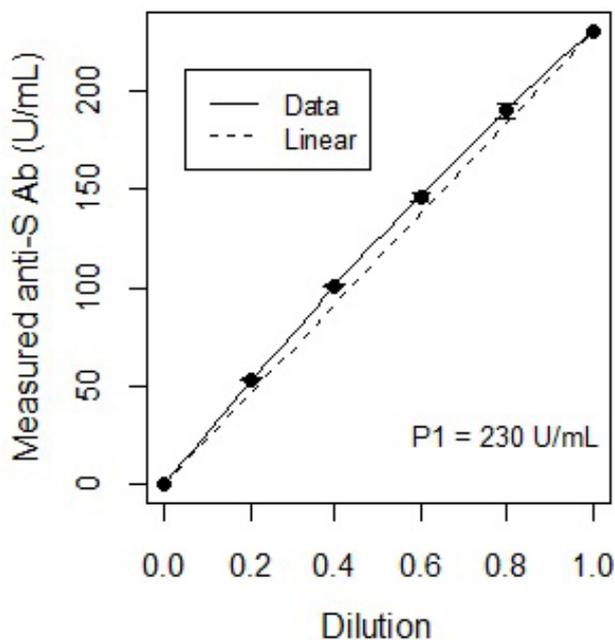
significantly benefits accurate identification and quantification of the target t-mAbs in a complex matrix. Confirmation of three subunits was performed using Thermo Scientific™ BioPharma Finder™ 4.1 software. Three subunits were accurately assigned with a mass accuracy of less than 10 ppm. For the targeted quantification, only the LC was monitored using targeted selected ion monitoring (tSIM) mode. The assessment of different analytical parameters and column lot-to-lot reproducibility is in progress. **Conclusion:** Sample preparation and LC-MS conditions were optimized and HRAM MS generated fully resolved isotope clusters of three subunits with accurate confirmation of their masses.

## B-081

### Notes regarding dilutions and quantitative results for the Roche Elecsys Anti-SARS-CoV-2 S antibody assay

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**Background.** Our institution began use of the Roche Elecsys Anti-SARS-CoV-2 S assay in December 2020, under FDA's Emergency Use Authorization (EUA). The assay allows for reporting of both qualitative and quantitative results (positive = >0.8 U/mL). We routinely report quantitative results only within the direct measurement range of the assay (0.4-250 U/mL). However, researchers requested us to report quantitation for samples reported as >250 U/mL. According to the Roche method sheet for the assay, dilution of samples for quantitation is allowable, but with potential for nonlinearity due to antibody heterogeneity. Our objective was to investigate properties of dilutions for quantitative assay results. **Methods.** A pooled plasma (PP) specimen was created from among routine patient plasma specimens having high values (>250) for S antigen. PP was diluted using Roche universal diluent to have a value between 200 and 250 U/mL (specimen P1, 230 U/mL). Subsequent dilutions of P1 (0.8, 0.6, 0.4, 0.2) were prepared in parallel, and measured for anti-S Ab by the automated assay. Dilutions were prepared in triplicate. Data analyses were performed using R programming. **Results.** Dilution measurements were consistently greater than those predicted for a linear response (Figure). The slope of the dilution curve increased progressively as dilution was increased. For this reason, dilution-corrected results progressively overestimated the anti-S Ab concentration of P1 as the dilution was increased. Overestimation was >+10% for dilution factors less than 0.4. This non-linear behavior for dilutions was observed consistently among individual patient samples as well. **Conclusions.** Sample dilutions producing quantitative measurements within the reportable range of the Roche Elecsys Anti-SARS-CoV-2 S antibody assay were non-linear. Dilution-corrected results produced dilution-dependent overestimates of the undiluted anti-S Ab sample concentrations. Researchers utilizing sample dilutions for anti-S Ab quantitation should be aware of these properties. Importantly, there are no adverse clinical consequences of nonlinearity.



## B-082

### Development and Validation of an ICPMS Method for Trace Metal Analysis of Urine on NexION2000 ICPMS

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**Background:** Determination of trace metal concentrations in human urine is useful for numerous clinical indications including diagnosis of diseases, monitoring toxic metal exposures and assessment of nutritional status. Inductively coupled plasma-mass spectrometry (ICP-MS) is currently the gold standard for trace metal analysis, providing a highly sensitive method for simultaneous detection of multiple metals. Based on Clinical and Laboratory Standards Institute (CLSI) guidelines, we developed and validated a robust method for analysis of 11 metals in human urine on PerkinElmer NexION ICP-MS. As our laboratory shifts from existing PerkinElmer Elan to NexION ICP-MS, we also assessed the comparability of results between these two platforms.

**Methods:** Cell gas optimizations were performed with ammonia and oxygen dynamic reaction cell modes to achieve lowest background equivalent concentrations. Matrix-matched calibration was established from automated dilution of TruQms PerkinElmer certified standards traceable to NIST Standard Reference Materials. 20x dilution was performed for urine samples using acidic diluent containing 1% nitric acid, 1.5% isopropanol and 200 ppb gold, matched to unspiked samples containing Sigmatrix urine diluent. Rhodium and Iridium were used as internal standards for low and high mass metals. Analytical performance parameters included imprecision (Coefficient of variation CV%), limits of blank (LoB), detection (LoD), quantitation (LoQ), linearity, carryover and method correlation studies against PerkinElmer Elan ICP-MS. Accuracy was assessed with Proficiency Testing Materials from various External Quality Assurance (EQA) providers.

**Results:** Linear calibrations were established with correlation coefficient more than 0.999 and internal standard recoveries within 80 to 120%. Within-run and total imprecision CV for all metals achieved between 0.4 to 1.8% and 1.0 to 4.9% respectively. LoB and LoD established using non-parametric ranking and precision profile approach respectively recorded between 0.00 to 0.14 µg/L and 0.02 to 1.28 µg/L across all metals. Root-mean-square deviation model was employed to determine LoQ, with ranges from 0.25 to 3.00 µg/L. Analytical measuring ranges were verified to be linear using least squares polynomial regression and lack-of-fit ANOVA analysis, with no significant carryover. Comparison studies demonstrated acceptable differences within Royal College of Pathologist Australasia (RCPA) analytical performance specifications. Urinary copper measurement, which is pivotal in the management of Wilson's disease and most frequently ordered in our service, demonstrates good comparability between Elan and NexION; with mean difference of 5.37% (3.760 to 6.975%, 95% CI) on Bland-Altman plot, Passing-Bablok regression of  $y = 3.182 + 1.026x$  and a Spearman's Rank Correlation of 0.997 (0.994 to 0.998, 95% CI). For accuracy assessment, all results were well-within acceptable limits established from various EQAs.

**Conclusion:** In summary, we have validated a matrix-matched method that allows for accurate determination of trace metals at clinically relevant concentrations on NexION2000 ICP-MS. Improved test performances and good comparability with existing Elan method will allow for better clinical assessment and continuity in monitoring of patients on the new ICPMS platform, particularly in the areas of nutritional assessment and management of Wilson's disease.

## B-083

### Development of Derivatization-free ID-LC/MS/MS Method for Simultaneous Measurement of Human Serum Monosaccharides

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**Background:** According to the CDC's National Diabetes Statistics Report published in 2020, 1.4 million new cases of diabetes were diagnosed in 2019. Besides glucose being the major biomarker for diabetes diagnosis, high fructose consumption has been associated with increased de novo lipogenesis in the liver leading to non-alcoholic fatty liver disease (NAFLD). Emerging evidence suggests that measuring fructose, galactose, and other saccharides in addition to glucose may be helpful for understanding their associations with various metabolic disorders and other diseases. Therefore, there is a need for an accurate and precise laboratory method for measuring serum monosaccharide panels. Traditional monosaccharide assays used in patient care often can only quantitate one monosaccharide at a time. We aim to develop a high-throughput analytical method for simultaneous measurement of serum monosaccharides that is suitable for large scale epidemiologic studies. **Methods:** Our assay is based on a derivatization-free ID-LC/MS/MS procedure. Certified primary reference material NIST

SRM 917c was used for assay calibration for glucose. Serum samples and calibrators were spiked with  $^{13}\text{C}$  labeled internal standards. Monosaccharides were isolated from serum matrix by acetonitrile protein precipitation and filtration in 96-well filter plate (0.2 $\mu\text{m}$ , porous PTFE). Aliquot of the acetonitrile filtrate was applied on an AB SCIEX Triple Quad™ 6500 LC/MS/MS system for analysis. Monosaccharides including glucose, galactose, fructose, mannose, and potential interferences were resolved from each other on Unison UK Amino column (3x250mm) eluted with acetonitrile and water gradient. Selective reaction monitoring in negative electrospray ionization mode was used for quantitation of monosaccharides in the samples. **Results:** The analytical measurement range covers 10 – 402 mg/dL for glucose ( $R^2 = 0.999$ ), which is sufficient to monitor serum glucose levels in normal, hypo-, hyperglycemia patient samples. Three other monosaccharides were linear between measurement range of 1.35 – 14.73 mg/dL ( $R^2 = 0.999$ ). Using serum-based reference material NIST SRM 965b (values assigned using GC-MS based reference measurement procedure), the method demonstrated excellent average measurement accuracy of 98%, with intraday and inter-day imprecision of less than 2% for serum glucose. Comparison with CDC glucose GC-MS RMP was performed using 20 single donor serum samples (glucose concentration range from 23.11 to 376.53 mg/dL), with the mean %bias of 1.70% (95% CI 0.14% to 3.27%) was observed. Imprecision and accuracy of lower abundant serum monosaccharides were lower than 6% and above 96%, respectively. The application of 96-well format filter plate and automated liquid handler system significantly improves the throughput of sample preparation. **Conclusion:** The current analytical method can be used to simultaneously measure the concentration levels of a panel of serum monosaccharides with appropriate accuracy and precision. Free of derivatization, simple sample procedure and application of automation system significantly improves the

throughput of the assay which are suitable for routine serum monosaccharides assay in clinical laboratories and for large biomonitoring studies.

## B-084

### Naphthalene-Functionalized Macrocyclic as Selective, Fluorescent Self-Quenching Sensor for Kynurenic Acid

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**Background:** Kynurenic acid is a by-product of tryptophan metabolism and a known antagonist of excitatory amino acid receptors. Abnormal serum levels are major confounding factors in neurodegenerative diseases and are therefore used to monitor the therapeutic outcome of antiepileptic drugs. We report a high affinity and selective water-soluble receptor for fluorescence detection and quantification of kynurenic acid.

**Methods:** We synthesize an *N*-naphthalene ammonium resorcinarene chloride receptor with an extended hydrophobic cavity capable of host-guest complexation with kynurenic acid through non-covalent interactions. Experimental results from 1D & 2D NMR, isothermal titration calorimetry, and electronic absorption and fluorescence spectroscopies support high-affinity binding and selectivity for kynurenic acid over tryptophan in water and human plasma. In silico DFT calculations and computational modeling are used to support experimental results.

**Results:** The measured binding constant ( $K = 1.46 \pm 0.21 \times 10^5 \text{ M}^{-1}$ ) is at least one order of magnitude larger than that observed with other resorcinarene receptors. The macrocyclic sensor is highly selective for kynurenic acid even in excess tryptophan in spiked plasma. UV absorbance and fluorescence spectroscopy were used to quantify kynurenic acid in pathological and physiological ranges, respectively. Computational studies reveal the critical roles of a series of attractive cooperative intra- and inter-molecular interactions to this binding process

**Conclusion:** Considering the rising interest in supramolecular chemistry for analytical applications of host-guest complexes, the present two-component system can be exploited as a sensory recognition element for kynurenic acid. This receptor can be further employed in electrochemical, chemical, or mass sensors using a covalent or non-covalent attachment to the sensor interface.

## B-085

### Cortisol Extended Measuring Interval Validation Study

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**Background:** Primary aldosteronism is the most frequent cause of secondary hypertension. Timely diagnosis and management can reduce hospitalizations due to cardio-

vascular events. The diagnostic work-up for primary aldosteronism involves measuring cortisol levels using adrenal vein sampling (AVS). These cortisol concentrations can be as high as 1500 ng/mL, much higher than the analytical measuring range (AMR) of chemistry analyzers. Although onboard autodilution is a common feature of these instruments, it is often insufficient for clinical management. The analytical measurement range (AMR) for cortisol is 0.5-75ng/mL on Centaur Siemens analyzer with an option of 1:2 auto dilution, as recommended by the manufacturer. With this autodilution, 150 ng/mL is the maximum cortisol concentration that can be measured without any significant precision or accuracy issues. However, this recommended 1:2 dilution was not sufficient in our institute as patients often had values >150 ng/mL. For these reasons, clinicians expressed the necessity of the measurement ability of higher cortisol levels for more effective patient management. Therefore, using CLSI EP34 guidelines, we developed a dilution scheme so that accurate results could be reported beyond the instrument's analytical measuring range. **Method:** To determine the Cortisol Extended Measuring Interval, we followed the Specimen Dilution and Spiking method, as described in the CLSI EP34 Establishing and Verifying an Extended Measuring Interval document. We used five patient samples at concentrations of 40%-100% of Upper Limit of Quantitation (ULOQ) and two high concentration levels of Cortisol calibrators. Two matrices were tested: reagent grade water (RGW) providing minimal matrix effect and MultiDiluent 3 (MD3) as recommended by the manufacturer. In addition to measuring the undiluted sample, we further diluted them with MD3 or RGW according to the following scheme: 1:2, 1:5, 1:10, 1:20, and 1:100, and ran them in triplicates on the Siemens Centaur instrument. **Results:** The results were reviewed based on the dilution recovery acceptance criterion of 100+/-10%. A random variation in recovery results showing no trend vs the dilution ratio was observed. The mean recovery percentages were between 89% and 104.83% for all dilutions tested with MD3 and between 103.32% and 117.27% for all dilutions tested with RGW. While all dilutions with MD3 had acceptable recovery results, only 1:100 dilution with RGW recovery percentage was acceptable. **Conclusion:** Our study showed that the highest acceptable dilution for Cortisol on Centaur Siemens instrument with acceptable accuracy and precision was 1:100 using Multi Diluent 3 as the diluent. This study extended the clinical reportable range from 150 ng/mL to 7500 ng/mL. **Key words:** Cortisol, Extended Measuring Interval, EMI, Analytical Measurement Interval, AMI.

## B-086

### Estimation of the Reference Interval for Growth Hormone in Newborns by a new Analytical Method Using Dried Blood Spots

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**Background:** Severe deficiency of GH (GHD) of the newborn is a rare but potentially life-threatening disease. GH can be measured during the first week of life when levels are physiologically higher (neonatal hypersomatotropism). GH evaluation using dried blood spots (DBS) may offer several advantages: small sample volume, easier transportation and storage, reduced costs, allowing centralization and method standardization. Aim of the study was to estimate the reference interval for GH in newborns by a new analytical method using dried blood spots.

**Methods:** GH reference interval was estimated in 812 healthy newborns (M:F 48:52%) attending the Neonatology Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan in the period July-October 2021. Heel-prick samples, spotted onto Guthrie cards (LTA Srl) and dried at RT, were stored at -20°C until analysis. GH was determined by a new developed analytical method. Briefly, from DBS of each sample (newborns, calibrators, high or low controls), 3 disks (diameter 5.5 mm) were punched out into a 2 mL polypropylene tube and 250  $\mu\text{L}$  of PBS 1X were added. Samples were incubated at RT on an orbital shaker for 16 hours and then centrifuged at 12500 rpm for 1 min. GH in supernatants (SN) was measured by Immulite 2000 (Siemens Healthineers). Reference limits for GH deficiency was estimated at percentiles 2.5<sup>th</sup> and 5.0<sup>th</sup> by the Harrell-Davis bootstrap method, with 90% CI calculated by the bias-corrected and accelerated bootstrap method (BCa), using 5000 bootstrap replicates.

**Results:** Linearity was verified ( $R^2 > 0.99$ ) up to a GH serum concentration of 50  $\mu\text{g/L}$ . Precision was 11.1% at 1  $\mu\text{g/L}$  (LOQ) and between 2.4% and 6.1% in the range 1.5-50  $\mu\text{g/L}$ . No appreciable differences were found between samples stored at  $-20^\circ\text{C}$  up to 2 months or directly processed (recovery > 90%). No interference due to hemolysis, present in SN after extraction, was detected (recovery > 99%). All GH measurements required 21 analytical sessions (6 months): CV% for the 21 calibration curve slopes, low (3  $\mu\text{g/L}$ ) or high (10  $\mu\text{g/L}$ ) controls were, respectively, 6.9%, 14% and 6.5%. Median (IQR) GH levels were 18.2  $\mu\text{g/L}$  (12.1-25.2  $\mu\text{g/L}$ ). Reference limits for GH deficiency, estimated at percentiles 2.5<sup>th</sup> and 5.0<sup>th</sup>, were, respectively, 5.9  $\mu\text{g/L}$  (90%CI 5.2-6.4) and 7.1  $\mu\text{g/L}$  (90%CI 6.6-7.4). GH levels were not associated to sex, height or weight standard deviation scores, birth weight, gestational age, type of delivery or mother's variables (age, smoking habit, gestational diabetes). **Conclusion:** Validation data suggest that this method can be used to measure GH in newborns using DBS. To our knowledge, this is the largest monocentric study combining DBS samples and GH measured by an automatic immunoassay analyzer. The reference limits estimated in this study are in accordance with previous published works using ELISA and may help confirming the clinical suspicion of neonatal GH.

**B-087**

**A novel ultrasensitive, high throughput and quantitative SARS CoV 2 neutralizing antibody titer measurement assay in dried blood spot samples**

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COVID-19 pandemic caused by SARS-CoV-2 is still an ongoing health crisis, leading to severe social and economic disruption in the world. With vaccines targeting SARS-CoV-2 being ubiquitously administered in the society, there is a critical and urgent need to develop a standardized and scalable method to evaluate the vaccine efficacy, particularly with the continuous emergence of SARS-CoV-2 variants. Neutralizing antibodies (NAb) against viruses are critical to protect the host from viral infection and to facilitate clearance of viruses. Several clinical studies have shown that the level of neutralizing antibodies in the blood after SARS-CoV-2 vaccine administration correlates with the protection rate against developing symptomatic infection. Serologic detections of viral antibodies have been traditionally performed using ELISA. However, this method has limited sensitivity and specificity in detecting low abundance targets and most assays measure the viral protein binding but not the neutralization capability of the antibodies. Furthermore, the low sensitivity of traditional methods restricts the samples to serum/plasma from venous blood, limiting wide-scale sample collection. Here, we present a new surrogate virus neutralization assay, SPEAR-NAB, and its application in ultrasensitive, high-throughput and quantitative measurement of anti-SARS-CoV-2 neutralizing antibody titers. More importantly, we demonstrate the utility of SPEAR-NAB in dried blood spot samples thanks to the ultrasensitive detection capability of SPEAR-NAB, enabling population-scale sample collection. We first demonstrate that SPEAR-NAB can robustly differentiate neutralizing and non-neutralizing anti-SARS-CoV-2 monoclonal antibodies. We then show that SPEAR-NAB has higher sensitivities compared with other assays including cPass<sup>TM</sup> and pseudo-virus neutralization assays in differentiating SARS-CoV-2 convalescent patients and vaccine recipients from negative samples. We validate accuracy by comparing the titers measured from serum/plasma samples using the gold standard technique, PRNT, with the titers measured from DBS samples collected from the same group of subjects using SPEAR-NAB. We show that the SPEAR-NAB result is in concordance with the PRNT result, with a coefficient of determination ( $R^2$ ) equal to 0.91. We also use the assay to measure the neutralization titers against SARS-CoV-2 variants (including Alpha, Beta, Gamma, Delta and Omicron), and show a decrease of titers against variants compared with the original strain, which is consistent with reports published by peer scientists. Using SPEAR-NAB, we also conduct a longitudinal study to track the neutralization titers after vaccination. We observe the titer change before and after vaccination and COVID infection as well as the titer decline post vaccination as a function of time. Finally, we show the adaptation of NAbSure<sup>TM</sup> into automated platforms, enabling thousands of samples to be processed in a single lab per day. More importantly, we demonstrate the high consistency of results across labs (less than 10% CV), which has been a significant challenge for traditional cell-based neutralization assays. In summary, SPEAR-NAB provides a novel, ultrasensitive, scalable and affordable method to widely assess neutralization antibody titer in the population and can assist the society to track the vaccine efficacy and to prevent future potential viral outbreak.

**B-088**

**Comparative Evaluation of Different RNA Extraction Methods for the Detection of SARS-CoV-2**

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**Background:** Obtaining a good quality of RNA sample is critical for the accurate detection of SARS-CoV-2. Multiple RNA extraction kits have been employed to counter the shortage of reagents. This study evaluates several methods for RNA extraction from nasopharyngeal (NP) swabs on the detection of SARS-CoV-2 using RT-PCR. **Methods:** A serial of synthetic SARS-CoV-2 RNA-spiked NP swabs were used to compare the limit of detection (LoD) and PCR efficiency, while 30 positive NP swabs were used to compare the sensitivity in this study. Total RNA was isolated using 3 commercial kits based on organic phase separation (OPS), silica spin column (SSC), and magnetic particle (MP), respectively. Extracted RNA was then subject to RT-PCR for the detection of virus genes (N1, N2). **Results:** As shown in Table 1, the different RNA extraction methods varied in sample volume and RNA yield. OPS and SSC-based manual extraction have significantly smaller batch size, longer extraction time, and higher cost compared to MP-based automatic extraction. While A260/A280 > 1.8 was observed in RNA isolated using all methods, a PCR efficiency higher than 100% suggests the presence of inhibitors in the RNA samples extracted using either OPS or SSC-based method but not MP-based method. The lowest LoD was observed using MP-based RNA extraction, even after being normalized to the same sample volume. Additionally, SARS-CoV-2 was detected in 28, 29, and 30 out of 30 positive samples using OPS, SSC, and MP-based methods, with sensitivity of 93.3%, 96.7%, and 100%, respectively. **Conclusion:** This study demonstrates that MP-based automatic RNA extraction in junction with RT-PCR is the most cost-efficient method for the detection of SARS-CoV-2, offering the highest sensitivity and throughput. OPS and SSC-based manual RNA extractions are less efficient but still suitable alternatives in light of a reagent shortage, offering acceptable LoD and sensitivity with a lower capital investment.

Table 1 Comparison of different virus RNA extraction methods

	OPS	SSC	MP
Input swab sample volume	50 $\mu\text{L}$	140 $\mu\text{L}$	200 $\mu\text{L}$
Output RNA volume	40 $\mu\text{L}$	40 $\mu\text{L}$	50 $\mu\text{L}$
Capital investment	Low	Low	High
Mode of operation	Manual	Manual	Automatic
Batch size	Up to 24	Up to 24	Up to 96
* Length per extraction	5 min	8 min	1 min
* Cost per extraction	\$7.21	\$12.06	\$4.34
PCR efficiency	123.2%	116.7%	90.8%
LoD	20 copies/ $\mu\text{L}$	10 copies/ $\mu\text{L}$	0.125 copies/ $\mu\text{L}$
<sup>§</sup> Normalized LoD	6.25 copies/ $\mu\text{L}$	8.75 copies/ $\mu\text{L}$	0.125 copies/ $\mu\text{L}$
Sensitivity	93.3%	96.7%	100%

\* calculated based on the maximum batch size; \* cost includes both material and labor; <sup>§</sup> normalized LoD based on 200  $\mu\text{L}$  of swab sample

**B-089**

**Serum Free Light Chain Quantification Testing: Comparison of Binding Site and Diazyme Methods**

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**Background:** Levels of free immunoglobulin light chains in serum and urine are a sensitive measure of dysregulated immunoglobulin synthesis. The development of an assay for free light chains was a major advance in testing for monoclonal gammopathies. The original assay by The Binding Site, called Freelite has been in common use in laboratory monitoring of monoclonal gammopathies and serves as the industry standard for next generation assays. Additionally, two clinical indices, myeloma-defining condition and light chain-predominant multiple myeloma rely on quantitative measurements of serum free light chains.

**Objective:** Evaluate the comparability of data generated from a new assay by Diazyme Inc. that with parallel data from the Freelite assay.

**Method:** The study included 541 specimens collected from 169 subjects at three sites, namely, Augusta University-Medical College of Georgia (AU), San Francisco General Hospital (SFGH), and University of California San Francisco Health (UCSF). Freelite testing was performed as standard laboratory evaluation at each institution with aliquots of three to nine specimens per patient of each specimen also analyzed at Diazyme headquarters' laboratory. Parallel testing by the two methods used differ-

ent sources of antibodies but similar turbidimetry platforms. The validity of the new assay was also tested by correlation with clinical parameters of patients with multiple myeloma at various stages of the disease.

**Results:** The correlation coefficient for Kappa, Lambda light chain levels and Kappa/Lambda ratio were 0.89, 0.85, and 0.98 respectively. The strength of positive correlation is supported by a P-value of <0.0001. Performance of the Diazyme and Freelite methods with respect to sensitivity and specificity is shown in Table 1. **Conclusion:** The results of Diazyme Inc. assay showed good concordance with the clinical assessment and results of the industry standard Binding Site/Freelite assay.

**Table 1.** Comparison of Diazyme and Freelite serum free light chain quantification results with respect to clinical assessment determinations.

		Clinical Assessment		
		Progression <sup>c</sup>	No Progression <sup>d</sup>	Total
Change in Diazyme FLC	Positive <sup>a</sup>	17	5	22
	Negative <sup>b</sup>	26	324	350
	Total	43	329	372
Clinical Sensitivity: 40% (17/43) (95% CI: 25.0% to 55.6%)				
Clinical Specificity: 98% (324/329) (95% CI: 96.5% to 99.5%)				
		Clinical Assessment		
		Progression <sup>c</sup>	No Progression <sup>d</sup>	Total
Change in Binding Site/ Freelite FLC	Positive <sup>a</sup>	19	9	28
	Negative <sup>b</sup>	24	320	344
	Total	43	329	372
Clinical Sensitivity: 44% (19/43) (95% CI: 29.1% to 60.1%)				
Clinical Specificity: 97% (320/329) (95% CI: 94.9% to 98.7%)				

The performance of the two methods in terms of clinical sensitivity and specificity with respect to disease progression and no progression is also similar. Comparison of the sensitivities of the two methods by the chi-square test. P-value of 0.662 indicates there is no significant difference between the two methods.

<sup>a</sup>Positive represents increase in serum free light chain levels.

<sup>b</sup>Negative a decrease in serum free light chain levels.

<sup>c</sup>Progression and <sup>d</sup>no progression are clinical response measures based on International Myeloma Working Group and National Comprehensive Cancer Network criteria.

## B-090

### A Prospective De-glycosylation Workflow for Reflex Analysis of Suspected Light Chain N-Linked Glycosylation of Paraproteins Identified Using the EXENT® System

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**Background:** N-Linked glycosylation of monoclonal light chains (LC) is a post-translational modification (PTM) of diagnostic interest in several plasma cell disorders. Frequency varies between different plasma cell disorders, with higher prevalence reported in AL amyloidosis. PTMs indicative of N-linked glycosylation have been reported in 5-9% of patients with monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma. N-linked LC glycosylation has been reported to be associated with increased risk of MGUS progression and may assist in the earlier identification of amyloidogenic LC. The immunoglobulin isotypes (GAM) assay for the EXENT Analyser (under development) can rapidly identify monoclonal proteins, including those with suspected N-linked LC glycosylation, during routine clinical analysis. These samples display LC spectra with a characteristic 'hedhog' pattern of peaks indicating the addition of different numbers of monosaccharide residues to the glycan PTM on the LC. The methodology workflow described here utilises enzymatic cleavage of the PTM to yield the un-glycosylated form of the paraprotein. This reflex approach was used on a panel of clinical samples with spectra suggestive of the co-expression of N-linked glycosylation and a non-glycosylated monoclonal LC form to explore the relationship between the two. **Methods:** Clinical screens were carried out using the matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) based EXENT assay (The Binding Site UK). Diluted serum was incubated with paramagnetic beads conjugated to antisera specific for IgG, IgA, IgM, total kappa, and total lambda; and additionally, free kappa and free lambda-conjugated beads. Following a series of washes to remove unbound serum components, samples were reduced and eluted from the beads. They were then co-spotted with matrix onto MALDI plates and dried, ready for acquisition by MALDI-TOF MS. Upon detection of a suspected N-linked glycosylated LC, samples were reflexed to on-bead de-glycosylation treatment. Samples were incubated with paramagnetic beads for the involved specificities identified in the initial screen, washed to remove non-immunoglobulin proteins, and incubated with Peptide-N-Glycosidase F (PNGase-F) to remove N-linked glycans. The samples were then washed to remove

PNGase-F. De-glycosylated immunoglobulins were reduced and eluted from the beads, co-spotted with matrix onto MALDI plates and data acquired by MALDI-TOF MS. **Results:** Samples from several clinical studies (BLOOM, MXI, ALLG, MM17 and MM21) were identified as having spectra suggestive of N-linked glycosylation using the EXENT GAM isotype assay, supplemented with free kappa and free lambda assessment. Samples with sufficient residual volume following initial testing underwent reflex de-glycosylation treatment. Effective de-glycosylation was achieved using the on-bead technique, confirming that the suspected glycosylated peak(s) were indeed glycosylated forms of the paraproteins. One of the samples presented with two groups of glycoforms and was suspected to be double glycosylation of the LC. This was confirmed when de-glycosylation resulted in a single monoclonal peak. **Conclusion:** On-bead de-glycosylation of immobilised immunoglobulins provided an effective methodology to confirm suspected LC glycosylation. The simplicity of this workflow, coupled with the use of minimal additional reagents lends itself to automation or semi-automation and the possibility of using this methodology as a reflex workflow to investigate glycosylated LC samples.

## B-091

### The Real Time Quaking Induced Conversion (RT-QuIC) Prion Assay Moves Mainstream - Development and Performance Assessment in an International Reference Laboratory

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#### Background:

There is a need to urgently determine the underlying etiology in patients with rapidly progressive dementia (RPD). Sporadic Creutzfeldt-Jakob disease (sCJD), a fatal neurodegenerative disease, is a common cause of RPD but can be difficult to distinguish clinically from other treatable causes of disease. sCJD is characterized by the accumulation of misfolded prion protein that rapidly propagates throughout the brain leading to irreversible damage. The development of a prion-specific assay, termed real time quaking induced conversion (RT-QuIC), has revolutionized the role of the laboratory in the ante-mortem diagnosis of sCJD. Newer generation RT-QuIC assays have been reported to achieve diagnostic accuracies >95%. However, clinically validated RT-QuIC prion assays are only available in a few national prion disease surveillance centers globally. Here, we report the development of an RT-QuIC assay in an international reference laboratory. We compared the clinical performance of this assay to the only clinically available RT-QuIC assay in the US at the National Prion Disease Pathology Surveillance Center (NPDPS) as well as to non-specific neurodegenerative biomarker assays commonly used in clinical practice.

#### Methods:

A second-generation RT-QuIC prion assay was developed in-house based on an assay previously reported (Rocky Mountain Laboratories, Hamilton, MT). Clinical residual CSF samples were collected from patients with suspected sCJD who had CSF testing performed at NPDPS for RT-QuIC, 14-3-3 and total tau (t-Tau) (n=141). A second cohort of samples was collected from patients who were not previously tested by RT-QuIC but had either a final diagnosis of CJD (n=17) or non-CJD neurological diseases (n=152). Patient charts were reviewed by a neurologist with expertise in RPDs. All samples were tested by the in-house RT-QuIC assay, and the diagnostic performance was compared with that of NPDPS RT-QuIC, 14-3-3 and t-Tau assays.

#### Results:

Of the 141 patients tested by the NPDPS, 44 had a final diagnosis of CJD of which 41 were positive by RT-QuIC at NPDPS (clinical sensitivity=93.2%). All samples positive at NPDPS were positive on the in-house RT-QuIC assay. Two additional samples were positive only on the in-house RT-QuIC assay; both patients had autopsy-confirmed CJD (clinical sensitivity=97.6%). In this cohort no false positive results were observed by either assay. In comparison, the NPDPS qualitative 14-3-3 assay was positive in 36 of the 44 CJD cases (clinical sensitivity=81.8%) and 22 of the 97 non-CJD cases (clinical specificity=77.3%). Using the rule-out cut-off of 500 pg/mL for t-Tau at NPDPS, clinical sensitivity and specificity were 97.6% and 46%, respectively. Of the 169 previously untested samples, 1 sample of the 152 non-CJD cases was false positive on the in-house RT-QuIC assay (clinical specificity=99.3%). Of the 17 probable/definite CJD cases, 16 were positive on the in-house RT-QuIC assay (clinical sensitivity=94.1%). The overall clinical sensitivity of the in-house RT-QuIC assay was 96.7% (95% CI, 88-99.6%) and specificity was 99.6% (95% CI, 97.8-99.9%) indicating a diagnostic accuracy of 99% (95% CI, 97.2-99.8%).

#### Conclusion:

RT-QuIC assays can be deployed in a reference laboratory and achieve robust clinical performance.

**B-092****Analytical Performance Comparing the Siemens Whole Blood Point of Care Atellica VTLi High-Sensitivity Cardiac Troponin I and the Central Laboratory Atellica High-Sensitivity Cardiac Troponin I Assay Concentrations**

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**Background:**

Cardiac troponin (cTn) is the standard biomarker for the diagnosis of acute myocardial infarction (AMI) in patients presenting with symptoms suggestive of ischemia. The IFCC Committee on Clinical Applications of Cardiac Bio-markers (C-CB) and the American Association for Clinical Chemistry (AACC) Academy have defined criteria for high sensitivity (hs) cTnI and cTnT assays which clarifies reporting of very low cTn concentrations below the 99<sup>th</sup> percentile. Development of rapid hs-cTn point-of-care (POC) assays may provide more timely clinical decision-making. The goal of the current study was to compare absolute concentrations and analytical performance of the Siemens whole blood POC Atellica VTLi hs-cTnI assay against the central laboratory Siemens Atellica hs-cTnI assay in patients presenting to an emergency department.

**Methods:**

Fresh whole blood specimens collected at 0 and 2 hours from 1089 consecutive patients enrolled in the SEIGE (NCT04772157) and SAFETY (NCT04280926) clinical trials.gov studies were analyzed for hs-cTnI using the Siemens whole blood POC Atellica VTLi assay and with lithium heparin plasma utilizing the central laboratory Siemens Atellica assay. hs-cTnI concentrations between each assay limit of detection (LoD) up to 500 ng/L were evaluated using regression analysis and concordance based on overall and sex-specific 99<sup>th</sup> percentile upper reference limits (URLs) for each assay as follows: Atellica VTLi, LoD 1.6 ng/L; 99<sup>th</sup> percentiles: overall 23 ng/L, male 27 ng/L, female 18 ng/L; Atellica, LoD 1.6 ng/L; 99<sup>th</sup> percentiles: overall 45 ng/L, male 53 ng/L, female 34 ng/L.

**Results:**

The MI rate for the study was 8.4%. 1923 paired samples at the two timepoints were analyzed. Using overall and sex-specific URLs, the concordance between the two assays at their respective 99<sup>th</sup> percentile cutoffs were: overall 92%, female 91%, male 94%. There was a 3-5% discordance between POC VTLi +/- and Atellica +/- results. Linear regression analyses showed a slope of 0.385, intercept 5.3 ng/L,  $r = 0.765$ . In comparison, the Passing-Bablok regression demonstrated a slope of 0.475, intercept 1.8 ng/L,  $r = 0.765$ .

**Conclusion:**

Our study validated excellent concordance between the Atellica VTLi whole blood POC hs-cTnI assay and central laboratory Atellica plasma hs-cTnI assay results predicated on 99<sup>th</sup> percentile URLs, despite the known lack of standardization between cTnI assays and an approximate 43-62% variance between hs-cTnI absolute concentrations. Both hs-cTnI assays are acceptable for use in clinical practice but cannot be interchanged for a patient's acute care.

**B-093****Evaluation of Point-of-Care Whole Blood Creatinine Assays and eGFR Concordance against the Abbott Alinity**

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**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). The average turnaround time for a traditional laboratory creatinine result is longer so a quick creatinine/eGFR screening test would be beneficial for patient risk management. Several whole blood (WB) creatinine assays along with eGFR capability are now available and provide a point-of-care (POC) alternative to the lab. The goal of this clinical evaluation was to compare the analytical performance of two on-market WB POC creatinine assays and the clinical concordance of eGFR to an established lab method.

**METHODS:** Remnant heparinized WB samples along with age, race and gender were obtained from the Emergency Department and Intensive Care Unit at Liège University Hospital (Liège, Belgium). The WB samples were analyzed on the GEM Premier ChemSTAT (Instrumentation Laboratory, Bedford, USA) and StatSensor (Nova Biomedical Cooperation, Waltham, USA) systems. The plasma portions were assayed on

an Alinity analyzer (Abbott Laboratories, Lake Forest, USA) as the reference method. The eGFR based on the Modification of Diet in Renal Disease (MDRD) Study equation were obtained for both WB and plasma creatinine. The clinical concordance of categorizing patients with abnormal kidney function (eGFR < 60 ml/min/1.73 m<sup>2</sup>) and overall concordance of patients (eGFR < 60 + eGFR ≥ 60) vs. plasma eGFR were assessed. 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 eGFR.

**RESULTS:** A total of 96 patient samples were analyzed. The two WB creatinine assays correlated well with plasma samples on the Alinity across the tested ranges with Passing-Bablok regression results of: ChemSTAT\_Creatinine = 1.15\*Alinity\_Creatinine - 0.079,  $r = 0.997$ ; StatSensor\_Creatinine = 1.14\*Alinity\_Creatinine + 0.09,  $r = 0.944$ . The Bland-Altman analysis showed mean biases (SD) of 0.064 (0.136) mg/dL and 0.16 (0.334) mg/dL for ChemSTAT and StatSensor against Alinity, respectively. When identifying patients with abnormal kidney function (eGFR < 60), ChemSTAT and StatSensor WB eGFR showed 100% (29/29) and 79% (23/29) concordance against the plasma eGFR, respectively. The overall clinical eGFR concordance of 94% (90/96) and 81% (78/96) was observed for ChemSTAT and StatSensor, respectively. The error grid analysis indicated 100% and 96% of WB eGFRs (Zones A and B combined) had no implication to clinical management for ChemSTAT and StatSensor, respectively.

**CONCLUSIONS:** Strong correlations were observed between the two WB POC creatinine assays and eGFR versus the lab method. Although positive regression slopes were observed for WB creatinine assays against the Alinity, both WB POC methods produced small mean biases within the measured range. Based on the good clinical concordance of WB eGFR and low mean bias, GEM Premier ChemSTAT demonstrated better performance between the two POC methods for rapid and lab quality renal function assessment.

**REFERENCE:** (1) Snaith B, *et al.* Clin Chem Lab Med 2018;56:1269-1276.

**B-094****Automating sample extraction for mass spectrometry analysis of tetrahydrocannabinol metabolite in urine**

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**Background:** In clinical laboratories, automated liquid handling platforms (ALHs) and dispensers (ALDs) are utilized to perform pipetting, liquid dispensing and mixing with greater precision and efficiency compared to manual methods. For MS-based toxicology assays, ALH and ALD systems are increasingly utilized to automate extractions before analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). We present a comparison study based on the verification of an automated sample extraction workflow for urine quantitative tetrahydrocannabinol metabolite (THCA) assay.

**Methods:**

The ALH (Tecan Fluent) is programmed to add 20 µL methanol containing deuterated THCA (THCA-d3) to 200 µL patient urine, mixed with 400 µL 5% KOH solution in glass lined 96-well plate. Hydrolysis is incubated offline at 55 °C for 30 minutes, and then quenching by adding 250 µL 17% acetic acid in methanol by ALH. The ALH then transfers the sample mixtures to a pre-equilibrated extraction plate (Strata-X-Drug B). The ALD (Tecan Resolvex A200) dispenses acetonitrile and water to equilibrate the extraction sorbent, then applies positive pressure to load the hydrolyzed urine mixture onto the sorbent. The ALD further dispenses 2% acetic acid in acetonitrile and water to wash extraction sorbent. The elution in two steps of 300 µL acetonitrile is performed on ALD using positive pressure, collected in another glass lined 96-well plate. Additional 600 µL water is dispensed to each well and the plate is vortexed for reconstitution. The extracted sample is analyzed by LC-MS/MS (Agilent 1260 Infinity - Sciex 5500). The eluent THCA is detected via negative ESI mode, with two MS/MS transitions (343.1/245.0, 343.1/191.0) for identification and quantification. The calibrators and quality controls are blank urine freshly spiked with THCA stock in methanol. The calibrators are stored in glass vials or glass culture tubes prior to sample extraction to minimize adsorption loss. **Results:** Automated sample preparation is compared to manual across five 96-well plate runs including de-identified residual patient samples, QC replicates, spiked linearity samples, and residual proficiency testing samples. The extraction methods show overall agreement in method comparison (Passing-Bablok regression: intercept -0.38, slope 0.995,  $r = 0.996$ ). Linearity replicates across assay's measurement range (n=5; 15 - 500 ng/mL) exhibit imprecision CV% between 1.2% and 10.1%. The recoveries are consistently less than spiked targets (-18% to -10%). Similar CV% are observed in QC replicates (n=18) at four different THCA concentrations between 6.7% and 8.4%, consistent with manual

extraction. The QC replicates recovery are approximately 86% relative to the manual extraction. The negative biases are corrected if the QC replicates are pipetted out of glass tubes prior to extraction, rather than plastic standard transport tubes. When residual PT samples are extracted by automation method, the results are comparable to manual extraction results. The negative biases observed in linearity and QC replicates are mostly likely due to adsorption loss of free THCA to plastic surface.

#### Conclusion:

Automated sample extractions can produce comparable or even superior performance characteristics compared to manual methods. Different tubes and pipetting materials may cause shifts in accuracy evaluation.

### B-095

#### A Dilute-and-Shoot Method for the Determination of THC-COOH and THC-COOH-Glucuronide in Serum by LC-MS/MS

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**Background:** Cannabis is one of the oldest and most commonly used drug in the world. It is widely used as a psychoactive drug for medical and recreational purposes. The main psychotropic agent of natural cannabis is delta-9-tetrahydrocannabinol (THC). 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) and THC-COOH-glucuronide are major metabolites of THC. THC-COOH and THC-COOH-glucuronide are desirable biomarkers for cannabis usage due to their longer half-lives and higher concentrations. In this study, we developed a dilute-and-shoot method for rapid analysis of THC-COOH and THC-COOH-glucuronide in human serum using LC-MS/MS.

**Methods:** Serum samples were treated by protein precipitation using acetonitrile containing 0.1% formic acid. The supernatant was centrifuged, filtered using 0.2 µm PTFE, and further diluted with water to result in a 20-fold dilution. 10 µL of the final extract was analyzed. The LC-MS/MS analysis was carried out on a Sciex QTRAP 5500 in negative MRM mode coupled with Agilent 1260 Infinity HPLC. Separation was performed using an Eclipse Plus Phenyl-Hexyl column (4.6mm×100 mm, 5µm). Gradient elution was performed with 0.1% formic acid in water and acetonitrile at an initial flow-rate of 1000µL/min and the supernatant was analyzed by LC-MS/MS using a 15.5 minute gradient.

**Results:** The LOQ for both THC-COOH and THC-COOH-glucuronide was determined to be 1 ng/mL. The method was shown to be linear from 1-2000 ng/mL for THC-COOH and 1-1000 ng/mL for THC-COOH-glucuronide ( $R^2 > 0.999$ ). The accuracy ranged from 90.8% to 118.4% for THC-COOH and 86.7% to 117.8% for THC-COOH-glucuronide. The method demonstrated no significant matrix effect, and no interferences were observed. No carryover was observed up to 2000 ng/mL for both analytes. Method validation results demonstrated good reproducibility at three levels of low, medium and high quality controls. Coefficients of variation (%CV) for intra-day and interday precision for all quality controls ranged from 2.5% to 10% for THC-COOH, and 1.6% to 8.3% for THC-COOH-glucuronide. The recovery ranged from 95.1% to 106.6% for THC-COOH and 97.8 to 106.2% for THC-COOH-glucuronide. The extract was found to be stable for up to 8 days.

**Conclusions:** A fast, simple, sensitive and robust method for the determination of THC-COOH and THC-COOH-glucuronide in human serum using LC-MS/MS was developed and validated. The method achieved a low LOQ for THC-COOH-glucuronide compared with other published methods. The application of this method has facilitated the analysis of serum samples for a large-scale biomonitoring study in Alberta.

### B-096

#### The determination of Tacrolimus, Sirolimus, and Everolimus in whole blood samples by a sub-1 minute LC-MS/MS method

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**Background:** Tacrolimus, Sirolimus, and Everolimus are immunosuppressant drugs used in transplant recipients. These kinds of drugs are successfully applied in many organ transplantations to avoid organ rejection. Because of the narrow therapeutic range of this drug class, monitoring the levels in the blood is part of immunosuppressive therapy. At low blood levels, there is a risk of organ rejection. At high blood levels, serious side effects may arise, including nephrotoxicity, cardiotoxicity, neurological effects, and elevated risk infections. The correlation between drug concentrations and clinical outcomes is an important factor for the use of therapeutic monitoring of immunosuppressive medications. Several studies have been demonstrated the benefit

of this monitoring and the liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods are considered the gold standard in therapeutic monitoring of this drug class. Analytical methods that deliver accuracy and speed of results are increasingly necessary for monitoring these drugs. **Objective:** This study aimed to develop and validate a reliable and fast LC-MS/MS method for the quantification of Tacrolimus, Sirolimus, and Everolimus in whole blood. **Methods:** 100 µL of whole blood was precipitated with a solution of zinc sulfate and acetonitrile. After homogenization and centrifugation, 2.0 uL of supernatant is injected into the LC-MS/MS system. Chromatographic separation was performed on a Waters ACQUITY UPLC equipped with Ascentis Express C18 column with 20 mm of length and using a mobile phase constituted by methanol with 0.1% of formic acid and 5 mM of ammonium formate to perform a rapid gradient separation. Detection was performed on a Waters XEVO TQ-S Micro mass spectrometer with electrospray ionization (ESI+). The total chromatographic time is 0.75 min and the three drugs are monitored simultaneously. We use Ascomycin, Sirolimus-D3, and Everolimus-D4 as internal standards to minimize the effects of the matrix in quantification. **Results:** Tacrolimus, Sirolimus, Everolimus were determined with a retention time of approximately 0.45 minutes. During selectivity experiments, there are no interfering peaks in retention time and any of the MS/MS fragments. We select two fragments of each analyte to ensure the correct identification. The method showed linearity between 1.0-51.0 ng/mL for Tacrolimus, and 2.0-51.0 ng/mL for Sirolimus and Everolimus. The precision was less than 10% for all analytes and recoveries were between 92.4-113.0%. The samples were compared with a reference method showed an excellent correlation and agreement of the results for the three drugs. One of the most advantages was obtained in productivity, analyzing more than 40 samples per hour. This is very important for urgent cases that are suspected of intoxication or low immunosuppression and need a rapid response.

**Conclusion:** We develop and validate a very fast and simple LC-MS/MS method to quantify three immunosuppressant drugs in whole blood samples. The method is very important in urgent cases and allows a short turnaround time for the clients.

### B-100

#### CSF Free Kappa Light Chain for The Diagnosis of Demyelinating Disorders: Experience from a Tertiary Care Center

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**Introduction:** Isoelectrofocusing (IEF) to detect oligoclonal bands (OCB) in cerebrospinal fluid (CSF) is the gold standard approach for evaluating intrathecal immunoglobulin synthesis in multiple sclerosis (MS) but the kappa free light chain (KFLC) is emerging as an alternative marker, and the combined/sequential use of IEF and KFLC have never been challenged. The aim of our study was to test the accuracy for KFLC in detecting MS, using OCB via IEF as gold standard. **Materials and Methods:** OCB via IEF is routinely performed at the Section of Chemical Pathology for patients with suspicion of MS or demyelinating disorders. Paired patient CSF and serum specimens are collected. The oligoclonal bands are tested by IEF on agarose gel using Interlab diagnostics CSF IEF kit and manual immunoblotting steps to transfer the proteins on transfer membranes. Simultaneous analysis is also conducted on serum samples. Sixty-four samples received during June to August, 2021 for OCBs via IEF are saved in the lab in frozen conditions, -20°C stable for analysis. 0.5 ml CSF sample will be utilized for KFLC analysis by nephelometry on Beckman Immage-800 analyzer using kits from Freelite, The Binding Site, according to the manufacturer's instructions. A cost and time analysis were also performed to identify the test performance characteristics against the OCB via IEF. A KFLC cutoff of 1 mg/L was used to assign the patients as positive. Measures of sensitivity and specificity were calculated for the 1 mg/L cutoff, and an ROC curve analysis was performed to identify an optimal cutoff using Youden's method, as well as diagnostic performance measures for this cutoff. **Results:** Considering the OCB criteria for MS diagnosis, there were no significant differences in age or gender between positive and negative patients. Positive patients showed significantly higher levels of KFLC compared to negative patients (Median and IQR 5.12 (3.15-5.12) vs. 0.31 (0.20, 0.51),  $p < 0.001$ ). The McNemar test did not find significant change in categorization using either OCB criteria or KFLC > 1 mg/L to diagnose patients. The cutoff provided an overall error rate of 10.9%, with 87.9% sensitivity and 90.3% specificity. ROC curve analysis confirmed the high diagnostic performance of KFLC levels, with an AUC of 93.6% (95% CI 87.6% - 99.6%,  $p < 0.001$ ). Using Youden's method, a slightly lower cutoff of 0.92 mg/L was considered optimal, providing the same specificity as the previous cutoff, but higher sensitivity at 90.9%. The analytical time is 3 hours and 55 minutes for OCB via IEF and 25 minutes for KFLC, a reduction of 3 hours and 30 minutes. The cost of a single OCB via IEF test at our lab is PKR 12,000 (USD 68.17) while that for KFLC is PKR 4,150 (USD 23.58). **Conclusions:** KFLC is a cheaper and time-saving alternative to

OCB via IEF and should be performed prior to the contemporary testing. Implementing this will not only reduce laboratory burden but will also be cost-effective and time saving for patients as well.

### B-101

#### Evaluation of immunological response in healthcare professionals against covid-19 in Belem PA

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**Background:** Covid-19, SARS-CoV-2 infection can cause mild symptoms as it can progress to severe acute respiratory syndrome. Detection of the virus by real-time RT-PCR (reverse transcription polymerase chain reaction) remains the laboratory test of choice for the diagnosis of symptomatic patients in the acute phase. And serologies are tests that detect the levels of antibodies produced in the body in response to a particular infection. Generally, the levels of IgM and IgG antibodies against specific proteins of the pathogen are evaluated. IgG is produced later and remains longer, being associated with protection. The time required for seroconversion has not yet been defined, nor the dynamics of production and duration/stability of antibodies over time, especially in vaccinated individuals. **Methods:** 855 healthcare professionals working on the front lines of hospitals and laboratory in Belem PA were recruited in July 2021; samples were analyzed by LIAISON SARS-CoV-2 S1/S2 IgG, chemiluminescence immunoassay (CLIA) for quantitative determination of specific anti-S1 and anti-S2 IgG antibodies against SARS-CoV-2. Provides indication of the presence of IgG antibodies. **Results:** Following reference guidelines, values below 12 UA/mL are considered Negative, values below 12 to 15 UA/mL Indeterminate and Positive values above 15 UA/mL are considered. It was possible to verify the following results: 42 negative, 77 indeterminate and 806 positive were obtained. **Conclusion:** Laboratory tests are an important tool for the evaluation of persistent or past infections, for screening potentially immunized people, as an aid in the decision when releasing patients from medical assistance and in epidemiological studies of the population, as well as evaluation of return to work for health professionals, from the seventh day of symptoms. It was possible to verify that 94% of the professionals evaluated already have immunity against SARS-CoV-2, not all of them had covid-19, but they already have immunity.

### B-104

#### The decrease in TSI during the first 6 months of treatment for Graves' disease may predict disease remission?

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**Background** Graves disease (GD) is an autoimmune disease mediated by immunoglobulins (Igs) that activate TSH receptor (rTSH). The TSI assay measures thyroid stimulating immunoglobulins (TSI), and it is able to reflect the probability of Graves' remission after treatment. As it is a relatively recent test, there is still not enough data to clarify if the percentage of TSI decrease during treatment could contribute to the assessment of disease remission. The aim of this study was to evaluate, prospectively, if the decrease in TSI during treatment of Graves' disease is capable of predicting disease remission. **Patients and Methods** Patients were treated for DG for 1 year. TSI was measured at the first visit, at six months during treatment, and at the time of ATD withdrawal. Remission was considered if the patients were clinically euthyroid, with normal TSH, freeT4 and total T3, and if TSI was negative. TSI was determined by chemiluminescent assay Siemens Diagnostics. According to a previous study by the authors TSI was negative if was less than 1.4 IU/L. **Results** We studied 79 untreated patients with GD, 75 women (94.9%), aged 41.8 ± 3.45 years. Forty-two (53.17%) went into remission after treatment for one year, and in 37 (46.83%) the disease recurred. TSI results and percentual of TSI decrease during treatment are in the table. In 40/42 patients (95.23%) with went to GD remission, the TSI dropped by more than 50% in the first 6 months, while 1/37 (2.7%) had a TSI drop above 50% in this period; In this last group, there was no decrease in TSI of more than 50% even after 12 months of treatment. **Conclusions** A decrease in TSI above 50% in the first 6 months of treatment was able to predict GD remission. **Reference** Fontes et al. Journal of Endocrine Society 2021 5 (suppl1):835

Table - TSI results and percentual of TSI decrease during treatment

	Remission	Non remission
Basal TSI	4.48 ± 3.77	10.99 ± 12.22
TSI during treatment – 6 months	2.02 ± 0.22	9.07 ± 6.52
Percentage decrease in TSI *	54.91%	17.47%
TSI during treatment – 12 months	1,39 ± 0.20	5.83 ± 4.57
Percentage decrease in TSI*	76.78%	43.95%

TSI: thyroid stimulating immunoglobulins; Thyroid n: Number of patients; Basal TSI: TSI before hyperthyroidism treatment; TSI was measured in units in IU/L. Percentage decrease in TSI\*: compared to basal levels.

### B-105

#### Thromboelastographic Profile of Covid-19 Patients with Severe Bilateral Pneumonia

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**Background:** Despite the fact that COVID-19 is usually a disease with a benign course, and mainly affects the respiratory system, a small proportion of affected patients evolve unfavorably, developing systemic complications and requiring hospital admission. Among such complications, a profound alteration in the coagulation state occupies a central role in the pathophysiology of the disease. In many cases, these complications are not detectable by standard coagulation studies, which provide information on the clots formation but do not address its stability and dissolution. Therefore, standard studies only provide partial information on this complex process. On the contrary, viscoelastic tests (VET) can evaluate the mechanical properties of clot formation, maintenance and lysis. In addition, VET also estimates the contribution of platelets and fibrinogen to their formation. It has been shown that viscoelastic systems demonstrate a state of hypercoagulability characterized by an increase in maximum clot firmness, and a decrease in clotting time (CT) and clot formation time (CFT). The aim of this study was to characterize the thromboelastographic profile of COVID-19 patients admitted to the Intensive Care Unit (ICU) with severe bilateral pneumonia.

**Methods:** We carried out a thromboelastometry study on 14 COVID-19 patients admitted to the ICU of the Virgen del Rocío University Hospital (Seville, Spain) with severe bilateral pneumonia, between October-November 2021. The exclusion criteria were chronic liver disease, pre-existing congenital hemorrhage or acquired coagulopathies, pregnancy, active oncological disease and/or chemotherapy. The thromboelastography study was performed using the ClotPro analyzer (Haemonetics Corporation, Boston, USA). Blood samples were collected in a tube containing trisodium citrate 0.129M (3.8%) on the same day of admission to the ICU. The extrinsic and intrinsic coagulation cascades were evaluated using the EX-TEST and IN-TEST tests, respectively. The contribution of fibrinogen to clot formation was evaluated using the MCF parameter of the FIB-TEST. The parameters analyzed were coagulation time (CT, s), clot formation time (CFT, s), and maximum clot firmness (MCF, mm). Results were expressed as median ± interquartile range (normal range). For statistical assessment, we used MedCalc 13.0 (MedCalc Software, Ostend, Belgium).

**Results:** Median values for CT were observed within the normal range: INTEST=161±49s (96-235s); EXTEST=71s±26s (38-85s). Median CFT values were also normal: INTEST=74±48s (42-93s); EXTEST=78±77s (52-139s). MCF values were found in the upper limit of the normal range: INTEST=63±17mm (49-66mm); EXTEST=66±16mm (53-68mm); FIBTEM; 26±10mm (12-27mm).

**Conclusion:** The results of this study do not coincide with the state of hypercoagulability described in previous studies, although the values of maximum firmness of the clot were close to the upper limit in all tests. Discrepancies with respect to other publications may be due to the small sample size.

### B-106

#### Measurement of Glucose-6-Phosphate Dehydrogenase (G6PD) In Packed Red Blood Cells for Patients With Elevated White Blood Cell Counts

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**Background:** Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common human enzymopathies. Those with G6PD deficiency may experience

acute hemolytic anemia when exposed to fava beans, infection, or certain drugs which in some cases can be life-threatening. Elevation of white blood cells (WBCs) may show falsely elevated G6PD activity in patients that are otherwise G6PD deficient. Manufacturer recommendations indicate using a buffy coat free sample to mitigate interference in these cases, however no workflow has been described for this with G6PD analysis. The aim of the current study was to evaluate a method of measuring calculated G6PD (CG6PD; G6PD U/g Hgb) in packed red blood cells (RBCs) to obtain accurate CG6PD measurements in patients with elevated WBCs. **Methods:** Whole blood specimens with varying concentrations of WBCs were obtained (4.3-60.0 K/ $\mu$ L; n=25). Additional specimens with varying CG6PD were obtained, pooled and aliquoted into three separate aliquots for each pool (0.8-20.73 U/g Hgb; n=4). All specimens were stored for a maximum of 3 days total (2-8°C). Each specimen and aliquoted pools were mixed by inversion and transferred to 12 x 75 mm clear plastic tubes (1.5-3.0mL) then centrifuged at 2000 RCF for 5 minutes. Using a 200  $\mu$ L pipettor with a fine tip (VWR Cat# 87001-698) the plasma and buffy coat layers were gently pierced and 200  $\mu$ L packed RBCs were obtained and transferred to a separate 12 x 75 mm tube after wiping clean the exterior of the pipet tip. From the extracted packed RBCs, 100  $\mu$ L of specimen was pipetted and mixed with 900  $\mu$ L of lysis buffer (Triton X-100, 0.05%) from specimens with normal WBCs (4.3-11.3 K/ $\mu$ L; n=10) and aliquoted pool specimens. These specimens were left to sit for 3-5 minutes and centrifuged for 5 minutes at 1244 RCF. G6PD testing was performed on the prepared hemolysate using the Pointe Scientific G6PD and Hemoglobin (Hgb) assays configured on a Roche cobas c501 analyzer. The remaining extracted packed RBCs were diluted using saline (0.85%; x2), and WBCs were measured using a Beckman Act Diff 2 analyzer. For applicable specimens, CG6PD or WBCs were also measured on a neat whole blood aliquot to be compared to measurements using packed RBCs. **Results:** For specimens with normal WBCs, the CG6PD measured in neat whole blood compared to packed RBCs demonstrated acceptable correlation (slope: 1.008; Bias: -6.22%). On average, CG6PD had an absolute change of  $-0.6 \pm 1.3$  U/g Hgb for specimens with normal WBCs. For specimens with elevated WBCs ( $>11.3$  K/ $\mu$ L; n=15), there was an average of 93.4% reduction in WBCs between WBCs measured in whole blood compared to packed RBCs. The precision of measuring CG6PD in packed RBCs was as follows (mean  $\pm$  SD, %CV): CG6PD (0.80  $\pm$  0.20, 25.0), (6.90  $\pm$  0.10, 1.4), (11.90  $\pm$  0.20, 1.7), (20.73  $\pm$  0.25, 1.2). **Conclusions:** Measuring CG6PD in packed RBCs demonstrated acceptable accuracy and precision and was effective at eliminating WBCs from whole blood. Further studies are warranted to better understand the effectiveness of detecting G6PD deficiency in affected patients using this method.

## B-108

### Modulating aberrant phagocytosis in Parkinson's disease (PD) using human PD macrophages and LRRK2 G2019S PD models

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**Background:** Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder worldwide and presents as a progressive movement disorder in affected individuals. Phagocytosis a key host-defense mechanism of the innate immune system have been implicated in enhancing neuronal damage and neurodegeneration in Parkinson's disease (PD) and other neurodegenerative diseases. We developed a sensitive, high-throughput and inexpensive method to screen thousands of compounds to identify and test those that are able to modulate aberrant phagocytosis in experimental PD.

**Methods:** We developed a highly sensitive assay to screen for phagocytic modulators in the National Institute of Neurological Disorder and Stroke (NINDS) custom collection 2 library. 75% of these compounds have been approved by the US FDA. To screen the library, bone marrow-derived macrophages (BMDM) were plated in 96-well plates containing single aliquots of compounds from the library at a density of 50,000/ well (200ul). Each plate contained wells were treated with or without lipopolysaccharide (LPS) (100ng/ml). Phagocytosis assay was carried out using pHrodo E.coli bio-particles and analyzed using microscopy. Of the 1040 compounds screened, 42 drugs can modulate phagocytosis in BMDM in the presence or absence of lipopolysaccharide (LPS). Of these, 12 were non-toxic to cells and able to cross the blood brain barrier (BBB). Three (3) candidates were finally selected and were further tested for phagocytosis modulation using macrophages (M1 and M2) derived from peripheral blood mononuclear cells (PBMCs) isolated from PD patients' blood samples and immune cells (microglia and BMDM) derived from LRRK2 G2019S mice.

**Results:** Our result show increased basal phagocytic activity in PD patients' M1 and M2 macrophages (n=15) as well as LRRK2 G2019S immune cells (microglia and BMDM) primed with LPS. Exposing these cells to our drug candidates led to significantly reduced phagocytosis more than Cytochalasin D (a potent phagocytosis inhibitor) via modulation of WAVE2 protein and actin assembly.

**Conclusion:** Our drug candidates were able to modulate increased phagocytosis reported in PD using macrophages and immune cells from PD patients and animal models. By screening drugs which are already FDA approved and known to enter the brain, our candidates can be evaluated in clinical trials almost directly in an off lab-label capacity.

## B-110

### A Facile Strategy for Ultracentrifugation-free Enrichment and Quantification of Exosomes

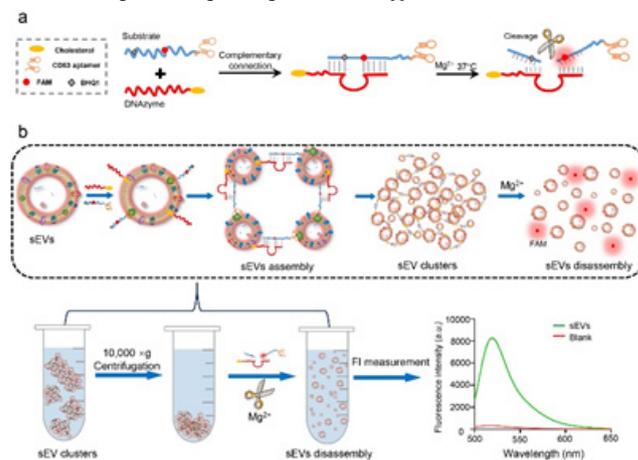
Y. Luo, X. Yu, X. Chen, R. Niu. Chongqing University, Chongqing, China

**Background:** Within the past decade, exosomes have emerged as important mediators of intercellular communications. Efficient separation and accurate identification of exosomes play essential roles in facilitating disease diagnosis and therapeutics. Most existing approaches for exosomes enrichment require tedious ultracentrifugation procedure. Thus, a convenient strategy to realize controllable enrichment and rapid quantification of exosomes remains an unmet need for disease diagnosis and fundamental research applications.

**Methods:** As DNAzyme and the substrate probes establishment, we proposed a facile DNAzyme-triggered assembly and disassembly system that converts single nano-sized exosomes into clusters that can be conveniently enriched by ordinary centrifugation, and then be broken into single exosomes in the presence of magnesium ions. After that, these free single exosomes could be adopted for the following quantification or biological applications.

**Results:** The feasibility of the DNAzyme system through various *in vitro* experiments was verified. Confocal localization implied that both DNAzyme and substrate probes could anchor on the surface of exosomes to form exosome clusters to permit the subsequent aggregation, which could be enriched by ordinary centrifugation at 10,000 g. The amount of isolated exosomes by our strategy was consistent with those from conventional ultracentrifugation. Transmission electron microscopy showed the extracted exosomes maintained complete membrane structures, and specific proteins were still retained by western blotting. Wound healing assay further demonstrated the extracted exosomes maintained their original biological functions. These data jointly proved the separated exosome could keep its physical structure and biological characteristics.

**Conclusion:** We described a DNAzyme-assembly approach for efficient separation and quantification of exosomes. The whole analytical procedure could be completed in 1.5 h without any assistance of ultracentrifugation. The proposed approach integrates the self-assembly and biological cleavage properties of DNAzyme probes for enrichment and quantification of exosomes, broadening the applications of exosomes in varied biological, bioengineering, and medical applications.



## B-111

## DNAzyme Encapsulated Nano-vesicle for Non-Destructive Detection of Exosomal miRNA

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## Background:

Exosomes and their inclusions are closely related to the occurrence and development of various diseases. However, most existing detection techniques for miRNAs embedded in exosomes still rely on membrane-rupture extraction, leading to mass loss during the process of analysis.

## Methods:

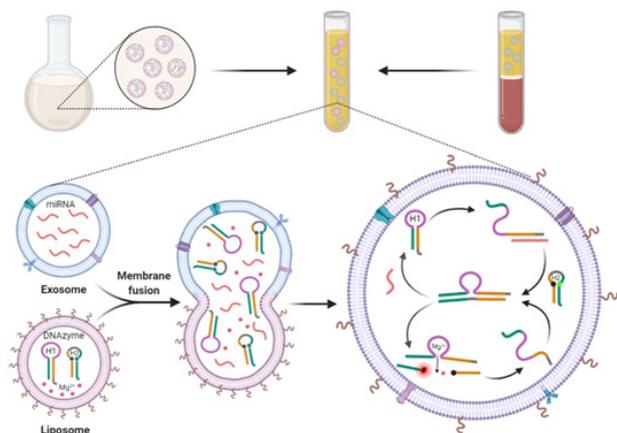
We deliberately designed a nano-vesicle that consists an engineered liposome, encapsulated DNAzyme and substrate probes, by which prompt membrane fusion with exosomes and non-destructive detection of single exosome-derived inclusions. Once the nano-vesicle fused with target exosomes, the introduced two DNAzyme molecular beacon probes (H1 and H2) could initiate a DNAzyme-recognizable structure in the presence of target miRNA. The introduced magnesium ion would trigger the cleavage of the DNAzyme structure. Consequently, target miRNA could be replaced and released to trigger the second run of DNAzyme-recognizable structure formation, by which a dual amplification could be realized in situ.

## Results:

Transmission electron microscopy (TEM) images showed the synthesized liposomes are bilayer vesicles with an average diameter of 120.8 nm. Fusion experiment demonstrated that the diameter of liposome obviously increased upon fusion with exosome within 0.5 h, which was further verified by the confocal images. We observed a target concentration-dependent fluorescence intensity increase within a linearity range from 10 pM to 10 nM. Both the results from upregulated and downregulated samples indicated the proposed nano-vesicle and RT-qPCR could yield identical results. Additionally, the proposed nanoprobe could accurately differentiate cancer patients from healthy individuals within 1 hour, further validating the feasibility of the approach for detecting exosomal miRNA from varied samples.

## Conclusion:

We describe a robust and reliable non-destructive approach for in situ detection of miRNAs in a single exosome. Excellent sensitivity empowers the proposed strategy a universal sensing platform for analyzing exosomal biomarkers without damaging the exosomal membrane, paving the way toward more accurate tumor diagnosis and reliable fundamental researches.



## B-112

## Single Blind, Prospective, Multicenter Comparison of TSH-Receptor Antibody Immunoassays

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**Objective:** In this multicenter study, sensitivity and specificity of two immunoassays were compared with two functional bioassays for the measurement of thyrotropin receptor antibodies (TSHR-Ab).

**Methods:** Two hundred two unselected, consecutive, well-documented subjects with various thyroid disorders and controls were prospectively enrolled. All antibody measurements were done in a blinded manner. The Bridge (Siemens) and Cobas (Roche) automated immunoassays were performed according to the manufacturer's instructions (cut-off <0.55 IU/L and <1.75 IU/L). Stimulating (TSAb) and blocking (TBA) TSHR-Ab were measured with cell-based reporter bioassays (Quidel), and TSAb was reported as percentage of specimen-to-reference ratio (<140 SRR %). Blocking activity was defined as percent inhibition of luciferase expression relative to induction with bovine TSH alone (cut-off >34% inhibition).

**Results:** The four TSHR-Ab assays were negative in healthy controls (n=10), patients with euthyroid nodular goiter (n=11) and non-autoimmune thyrotoxicosis (n=21). In contrast, in patients with Graves' disease (GD), irrespective of the disease duration, TSHR-Ab positivity was present in 72/114 (63%), 69 (61%), and 91 (80%) for the Bridge, Cobas immunoassays and TSAb bioassay, respectively (p<0.001). The TSAb and automated assays detected TSHR-Ab in the untreated hyperthyroid GD patients, while all TSHR-Ab negative patients had definitive treatment (surgery/radioiodine), were in remission or on antithyroid drugs. Concordant positive results in the two immunoassays and TSAb bioassay were noted only in 69/114 (61%) patients with GD. Nineteen of 114 (17%) GD samples were positive in the TSAb bioassay but negative in the Bridge assay. Patients with Graves' orbitopathy were TSHR-Ab positive in 23/25 (92%), 20 (80%) and 17 (68%) with the TSAb bioassay, Bridge and Cobas assays, respectively (p<0.001). The two immunoassays highly correlated in GD patients (r=0.8, p<0.001), with a lower correlation between the TSAb bioassay and the Bridge- (r=0.49, p<0.001) or Cobas (r=0.52, p<0.001) assays. In 27 patients with Hashimoto's thyroiditis (HT), four were TSAb positive (15%), of which three and one were Bridge and Cobas positive, respectively. In 160 patients with autoimmune thyroid disease (AITD), TBAb were present in 32 (20%), HT 11/27 (41%) and GD 21/133 (16%). Thirty (94%) and 28/32 (88%) of the TBAb positive samples were also Cobas and Bridge assay positive but TSAb bioassay negative.

**Conclusions:** Highly variable sensitivity and specificity values were observed for the detection of TSHR-Ab. In patients with AITD, both Bridge and Cobas immunoassays were positive in the presence of blocking TSHR-Ab measured in a TBAb bioassay.

## B-114

## Development of internal standard for lipoprotein subclass analysis using dual detection gel-permeation HPLC system

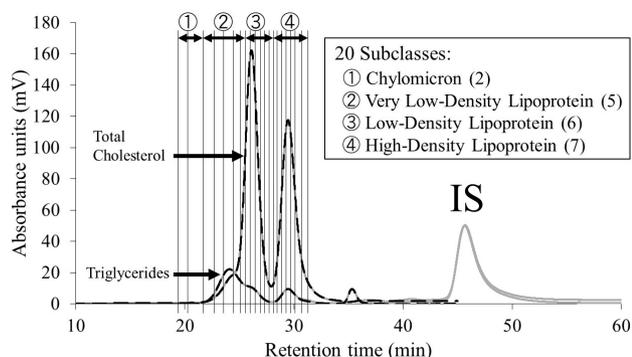
M. Ogino<sup>1</sup>, T. Kameda<sup>1</sup>, Y. Mutsuda<sup>1</sup>, H. Tanaka<sup>2</sup>, J. Takahashi<sup>2</sup>, M. Okazaki<sup>1</sup>, M. Ai<sup>1</sup>, R. Ohkawa<sup>1</sup>. <sup>1</sup>Tokyo Medical and Dental University, Bunkyo-ku, Japan, <sup>2</sup>Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan

**Background:** The LipoSEARCH® System is a breakthrough lipoprotein subclass analysis method based on gel-permeation HPLC (GP-HPLC). This system separates the major serum lipoprotein subclasses according to particle size, and splits them into two pathways to measure total cholesterol and triglycerides concentrations simultaneously and further analyses by fitting Gaussian curves to profile the 20 lipoprotein subclasses. An important assumption of this system is its simultaneous detection of two pathways to guarantee the accuracy of each analysis. Therefore, we investigated the development of an internal standard (IS) that can ensure the simultaneous detection.

**Methods:** To seek a candidate of IS, several quinone pigments with a maximum absorption wavelength of 550 nm, the primary wavelength used in this system were analyzed by GP-HPLC. After one optimal pigment was selected, the effect of pigment addition to serum on the lipoprotein analysis was evaluated. IS stability was examined using the pigment solution stored at 22-24°C or -80°C for 10 days. For evaluation of IS efficacy in detecting accidental errors in dual detection, serum in the presence/absence of IS was analyzed under the condition that were changed deliberately in flow rate of the dual pumps.

**Results:** According to the GP-HPLC profile, N-ethyl-N-(3-sulfopropyl)-3-methoxyaniline (ADPS) was selected as a IS candidate. Using the derived ADPS, we succeeded in producing overlapping pigment peaks that appeared after the analytical chromatograms in two pathways. Comparing the cholesterol and triglycerides concentrations in 20 lipoprotein subclasses with/without IS, IS addition did not affect the serum analysis, and the IS pigment was stable for 10 days in frozen storage. When two pairs of pumps were intentionally malfunctioned at a flow rate, the accidental errors was detectable as IS peak shift.

**Conclusion:** We newly developed an ideal IS for a GP-HPLC system using a derivative from ADPS to ensure the synchronization of dual detection.



## B-115

### Development and validation of novel automatable assay for cholesterol efflux capacity

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**Background:** Cholesterol efflux capacity (CEC) is one of the anti-atherosclerotic functions of high-density lipoprotein (HDL). In recent years, in addition to the HDL-cholesterol (HDL-C) concentration, which reflects the amount of HDL, evaluation of HDL quality by measuring HDL-CEC has attracted attention for predicting cardiovascular diseases (CVD) risk. However, CEC has not yet been introduced into the clinical setting because of several technical issues including use of radioactive material and differentiated cells handlings in the assay. Previously, we developed a radioisotope- and cell-free CEC assay named an immobilized liposome-bound gel beads (ILG) method to replace the conventional method. However, the separation process of the supernatant by a spin down centrifuge was not still suitable for installation on an automatic analyzer. Therefore, this study aimed to further develop a new method with easier operation.

**Methods:** We assumed that use of a magnetic bead instead of the gel bead enabled to skip a centrifugal process for the separation. First, in a similar manner of the ILG method, porous magnetic beads were treated with liposome containing fluorescently labeled cholesterol. Immobilization of the fluorescent-labeled liposome to the magnetic beads was confirmed by a fluorescence microscope. The effect of immobilization was investigated by measuring the fluorescence intensity of the supernatant during the immobilizing process. To estimate the stability of the liposome-immobilized magnetic beads (ILM), the ILM solution was kept in the dark at 4°C for 60 days. Next, using the ILM, we conducted to perform CEC assay. Serum samples were obtained from healthy volunteers (n = 15). HDL was isolated from the serum samples by ultracentrifugation, and apolipoprotein B-100 depleted serum (BDS) was prepared using 20% polyethylene glycol. Total cholesterol levels in the HDL fraction were determined using a commercial enzymatic assay kit: T-CHO (S) (Denka Co., Ltd.). Using the HDL and BDS samples as cholesterol acceptors, validation tests for ILM method were performed, and compared to ILG method.

**Results:** The fluorescence was observed as dots on the beads indicating that the liposome containing fluorescence-labeled cholesterol was entered into the beads and then fixed successfully. The efficiency of the immobilization was comparable to the ILG method, and the ILM solution was stable until 30 days. The newly produced ILM allowed to perform CEC assay without the centrifugal step. The new ILM method showed sufficient basic performance, and CEC values of various concentrations of HDL and BDS samples increased in a dose-dependent manner. When 24-dilution series of HDL samples were measured by both ILM and ILG methods, CEC values by ILM method was correlated with those by ILG method ( $r = 0.987$ ). Moreover, CEC values of BDSs from 15 human healthy serums by ILM method also showed a good correlation with those by ILG method.

**Conclusion:** In this study, we succeeded in immobilization of liposome containing fluorescence-labeled cholesterol on magnetic beads. Our novel ILM method can be available for evaluation of CEC with less complicated process implying automatization potential. For clinical application of CEC measurement, further assessment of CEC by ILM method for CVD would be required.

## B-116

### Does the Eurachem/CITAC Uncertainty of Qualitative Results Guide Comply with ISO Standards?

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#### Background:

The Eurachem/CITAC Guide Assessment of Qualitative Analysis [1] is an innovative document, very useful for laboratories. False qualitative analysis results are extremely relevant, e.g., in forensics or doping analysis and in medical examinations.

We see at least two critical issues in the EURACHEM document: lack of the precision component and ambiguity between qualitative results and intermediate steps with quantitative measures.

#### Methods:

We compared EURACHEM statements with ISO 3534-2 [2], VIM [3], ISO Guide 99 [4], ISO 17025 [5], ISO 15189 [6], ISO 20914 [7], and ISO 16393 [8] requirements. In VIM, measurement uncertainty includes a statistical analysis of values obtained under conditions such as repeatability, intermediate precision, and reproducibility. ISO 17025 requires laboratories to identify contributions to measurement uncertainty, and does not exclude qualitative results, but includes contingencies that preclude rigorous statistical evaluation. ISO 15189 and ISO 20914 require uncertainty in the intermediate measurement steps of qualitative results. ISO 16393 describes the precision of qualitative results.

#### Results:

There is no feature in EURACHEM Guide resulting from replicates of measurements, repeatability, intermediate precision, and reproducibility. All the characteristics in Table 2 of the Guide (True positive rate, and so on) derive from the comparison of results with a reference external to the laboratory method.

Examples 7.3, 7.4, 7.5 and 7.6 in EURACHEM document show qualitative results derived from quantitative measurements.

#### Conclusion:

Eurachem/CITAC Guide is an important tool to apply ISO 17025 statement that, when evaluating measurement uncertainty, all contributions which are of significance, including those arising from sampling, shall be taken into account. ISO 17025 does not distinguish between quantitative and qualitative results. ISO 15189 and ISO 20914 do it, but are both under revision. The characteristics of precision, repeatability and reproducibility of methods are useful in the laboratory for the verification and quality control of examination methods. EURACHEM Guide does not resolve the case of qualitative results derived from quantitative measurement.

**References:**[1] R Bettencourt da Silva and S L R Ellison (eds.) Eurachem/CITAC Guide: Assessment of performance and uncertainty in qualitative chemical analysis. First Edition, Eurachem (2021). ISBN 978-0-948926-39-6. Available from <https://www.eurachem.org>[2] ISO 3534-2:2006 Statistics — Vocabulary and symbols — Part 2: Applied statistics

[3] International vocabulary of metrology - Basic and general concepts and associated terms (VIM) 3rd edition 2008. <https://www.bipm.org/en/publications/guides>

[4] ISO/IEC Guide 99:2007. International vocabulary of metrology — Basic and general concepts and associated terms (VIM)

[5] ISO/IEC 17025:2017. General requirements for the competence of testing and calibration laboratories

[6] ISO/DIS 15189:2021. Medical laboratories — Requirements for quality and competence

[7] ISO/TS 20914:2019. Medical laboratories — Practical guidance for the estimation of measurement uncertainty

[8] ISO/TS 16393:2019. Molecular biomarker analysis — Determination of the performance characteristics of qualitative measurement methods and validation of methods

## B-118

### Development of A Highly Sensitive Influenza Test Kit for Fully Automated Analyzer LUMIPULSE G1200 Based on Chemiluminescent Enzyme Immunoassay.

M. Imaizumi, H. Sagi, T. Ohshima, Y. Kitamura, A. Kaneko, S. Yagi, K. Aoyagi. FUJIREBIO INC., Tokyo, Japan

**Background:**The limited sensitivity of point-of-care testing (POCT) for influenza (Flu) often requires clinician to diagnose comprehensively considering with clinical

symptoms and local epidemic situation, especially, in the early stage of infection. The similarity of clinical and epidemiological features between Flu and COVID-19 that caused by SARS-CoV-2 would be serious concern during Flu-season under COVID-19 endemic. Rapid and high-throughput test system, as IVD for COVID-19 on fully automated immune-assay system, have been helping health professionals to reduce the burden during COVID-19 endemic waves. We hypothesized that a high-throughput diagnostics kit for Flu also will help them during Flu season under COVID-19 epidemic. We developed the highly sensitive Flu antigen test “Lumipulse® G Flu-A&B” for the detection Flu antigen using the same sample as SARS-CoV-2 test, “Lumipulse® G SARS-CoV-2 Ag”, on the same fully automated chemiluminescent enzyme immunoassay (CLEIA) system (LUMIPULSE® G1200). In this study, we examined the fundamental performance of Lumipulse® G Flu-A&B by comparing with two POCT IVD for Flu approved in Japan.

**Methods:** Lumipulse® G Flu-A&B (Lp-Flu) can detect Flu-A and Flu-B nucleoproteins without distinction, and the result can be obtained 30 minutes from sampling. Lp-Flu is a two-step sandwich CLEIA with an automated sample treatment process to extract the nucleoprotein from the virus during the 1st immunoreaction. Therefore, Lp-Flu can assay several kinds of samples directly, not only the specimen extracted from nasopharyngeal swab (NPS) or nasal swab (NS), but viruses stored in VTM. NPS-derived specimens (NPSS) were purchased from ProMedDx, LLC. Two POCT kits, ESPLINE Influenza A&B-N (EL-Flu, FUJIREBIO Inc.) and Quick Navi-Flu2 (QN-Flu, Denka Co., Ltd) were used for comparison with LP-flu.

**Results:** Using positive NPSS for Flu-A and Flu-B serially 2-fold diluted with sample extraction solution for NPS, we compared the sensitivity of LP-flu with two POCT kits: LP-Flu sensitivity to Flu-A was 8-fold and 32-fold higher, and that to Flu-B was 16-fold and 32-fold higher than EL-Flu and QN-Flu, respectively. Among 55 Flu-A or Flu-B positive NPSS, the number of LP-Flu-positive to POCT-negative discrepancies were 5 and 18 with EL-Flu and QN-Flu, respectively. These discrepancies were positive for RT-PCR for influenza virus. Negative percent agreement was 100 % with both POCT using Flu negative NPSS (n=50). Negative NPSS spiked with each 9 strains of Flu-A and 5 of Flu-B inactivated virus gave positive with LP-Flu. LP-Flu gave less than 0.015% values for all negative NPSS spiked with each recombinant nucleocapsid protein of seven coronaviruses including SARS-CoV-2, and this result indicate that LP-Flu could distinguish Flu-A/B from SARS-CoV-2.

**Conclusion:** The higher sensitivity of LP-Flu to Flu-A/B would allow to diagnose the flu-infected patients at earlier stage post-infection than two POCT. IVDs for SARS-CoV-2 and Flu on the same automated immune-analyzer using same samples would provide user-convenience in discriminate COVID-19 from Flu in the flu-seasons under COVID-19 endemic.

## B-119

### SARS-CoV-2 IgG analyte in dried blood spot (DBS) samples stability for a commercial fluoroimmunoassay test under cold storage and ultra-freezing conditions - a long-term analysis

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**Background:** The methodology of collecting clinical samples on filter paper is well described, from the dosage of biochemical analytes to serological tests, several assays have already been standardized, bringing the ease of collecting blood without performing venipuncture, in addition to the ease of material transport. Infectious, viral, and even parasitic diseases have already had their serological diagnosis standardized using this methodology. One of its advantages is the stability of the sample for long term periods. In this project, we aim to evaluate the stability of collected material on filter paper for the anti-SARS-CoV-2 IgG detection assay, since the manufacturer establishes in the package insert the period of only 7 days after collection in cold storage from 2 to 8°C.

**Methods:** Samples were obtained by finger prick from 200 volunteers of both sexes between 18 and 80 years old for analysis in the time-resolved fluoroimmunoassay, using the qualitative kit GSP®/DELFLIA® Anti-SARS-CoV-2 IgG kit (PERKINELMER®) for detection of IgG antibodies on filter paper. 100 samples were stored under refrigeration (2 to 8°C) in the presence of a desiccant, another 100 samples were stored in an ultra-freezer at -80°C also in the presence of a desiccant.

**Results:** The results obtained immediately after sample obtaining were compared with the data analyzed after 6, 9, and 12 months under cold storage (2 to 8°C) and after 9 months under ultra-refrigeration (-80°C). Our results demonstrate good analyte stability after 6 or 9 months under cold storage, with 97% agreement and 0,94 Kappa index after 6 months, and 93% agreement and 0,86 Kappa index after 9 months. When analyzing the samples after 12 months, we observed a decrease in agreement to 84%

and Kappa index at 0,68, indicating loss of stability of this analyte under this storage condition. The samples kept in ultra-freezer were analyzed after 9 months, maintaining a 95% agreement with the previous analysis and 0,90 Kappa index.

**Conclusion:** These results indicate that the SARS-CoV-2 IgG analyte remains stable for storage and dosage after long periods, as expected for this type of sample collection methodology. Our analysis will still occur during 2 years at ultra-freezing conditions to evaluate the stability of this analyte at long-term storage conditions.

## B-120

### Improved method for quantification of apoA-I/apoA-II heterodimer in high-density lipoprotein

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**Background:** Ischaemic heart disease is the top cause of death in the world. The disease is caused by atherosclerosis. Therefore, the development of testing methods for atherosclerosis is an important research issue. High-density lipoprotein (HDL) is known to play an anti-atherosclerotic role, and HDL-cholesterol level provides important information in clinical laboratory tests. On the other hand, it is known that apolipoprotein A-I (apoA-I), the primary functional and structural protein of HDL, is changed to a free radical form by myeloperoxidase (MPO) secreted by macrophages in atherosclerotic lesions and binds to apolipoprotein A-II (apoA-II) to form apoA-I/apoA-II heterodimer. We previously reported that the concentration of apoA-I/apoA-II heterodimer in the serum of patients with acute myocardial infarction was higher than that in the control group. We had also reported that when the complex was produced in HDL, some anti-atherosclerotic functions of HDL were attenuated. Thus, the apoA-I/apoA-II heterodimer may be a useful biomarker for atherosclerotic disease. However, the previous measurement method required a complicated pre-treatment using an anti-apoA-I antibody affinity gel. In this study, we improved the method to a simple measurement system that does not require pretreatment. Moreover, we quantified the heterodimer in serum of healthy subjects and MPO-treated HDL to confirm that the assay worked.

**Methods:** Serum and plasma samples were collected from healthy volunteers. HDL (1.063 < d < 1.210 g/mL) was obtained from the plasma by ultracentrifugation. The quantification of apoA-I/apoA-II heterodimer was performed by sandwich ELISA with anti-apoA-II antibody for capture and biotin-labeled anti-apoA-I antibody for detection antibody. Then, horseradish peroxidase-labeled streptavidin was reacted for detection by QuantaBlu™ Fluorogenic Peroxidase Substrates. The fluorescence intensity was measured at 340(ex)/460(em) nm. The validation study was conducted for linearity, detection range, and precision. The level of apoA-I/apoA-II heterodimer was measured in healthy serum samples (n = 12) and MPO-treated HDL sample. HDL isolated from pooled healthy human serum (2 mg protein/ml in PBS) was incubated with 0.1 mM H<sub>2</sub>O<sub>2</sub>, 0.2 mM diethylenetriamine pentaacetic acid, 0.4 mM L-tyrosine, and 20 nM MPO for 2 hours at 37°C. In previous assays, serum samples were pre-treated with anti-apoA-I antibody coupling CNBr-activated Sepharose 4B to remove excess amount of free apoA-II, and signals were detected using 3,3',5,5'-tetramethylbenzidine (TMB) substrates.

**Results:** The detection signal was enhanced by changing the TMB substrate to fluorescent substrate. As a result, the signal-to-noise ratio was improved from 0.36 to 0.45. In the improved method, linearity was observed at dilution rates of 10,000 to 80,000 times of serum. When serum was measured in the above range, the amount of apoA-I/A-II heterodimer ranged from 26.8 to 149.8 (arbitrary unit) between individuals. MPO-treated HDL presented significantly 16.2 times higher fluorescence intensity than untreated HDL samples, and the linear relationship was also observed. The coefficient of variations was less than 4.53% in this measurement.

**Conclusion:** In this study, we have achieved the omission of pre-treatment by using fluorescent substrate. This improved simple method is suitable for clinical test and can be applied as an indicator for risk assessment and treatment of cardiovascular diseases.

## B-121

### Biomarker for acute kidney injury: performance assessment of a point of care platform for Proenkephalin penKid testing

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**Background:** Acute kidney injury (AKI) is frequent in critically ill patients, and biomarkers can facilitate its early diagnosis. Proenkephalin (penKid) is a stable opioid

peptide cleaved from the same precursor peptide (preproenkephalin A) of endogenous opioids as enkephalins. penKid has been identified as a functional biomarker for kidney function and its plasma concentration are inversely correlated to the glomerular filtration rate (GFR). Our study objective was to evaluate the reliability of a new point-of-care testing (POCT) system for measuring penkid with a short turnaround time of analysis (TAT). **Methods:** Imprecision of the IB10 sphingotest® penKid® assay was assessed by repetitive measurement of human EDTA plasma pools. Method comparison was performed in reference to Immunoluminometric assay (ILMA) by measuring 50 EDTA plasma samples. Correlation and agreement between methods were tested using Bland-Altman plot and Passing Bablok linear regression. Usability of the POCT instrument was assessed by healthcare workers through a dedicated questionnaire. **Results:** The inter-assay CVs of the IB10 sphingotest® penKid® test were 19.7% for a concentration of 113 pmol/L and 9.3% for a concentration of 307 pmol/L penKid. The IB10 sphingotest® penKid® significantly correlated to the ILMA ( $r=0.97$ ). The Passing-Bablok linear regression showed a slope of 0.905 and an intercept of 7.49. The Bland-Altman plot showed a mean bias of 4.93% between the two methods. The POCT usability questionnaire was overall satisfying and the TAT analysis with the POCT was below 20 minutes.

**Conclusions:** Our results showed a good analytical performance of the IB10 sphingotest® penKid® combined with an excellent correlation to the reference method. Our study also confirmed that the usability of the instrument was satisfactory alongside a short TAT, two important elements to contribute to a fast clinical decision.

## B-124

### Investigation of very low-density lipoprotein-triglyceride hydrolysis assessment

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**Background:** Cardiovascular disease (CVD) is one of the leading causes of death in the world. In addition to hypercholesterolemia, hypertriglyceridemia is also the major risk factor of CVD, and triglycerides (TG) levels in blood are measured routinely. However, temporal TG concentration is not enough to predict CVD because it tends to vary during the day. Blood TG is classified into exogenous and endogenous TG: the former affected by meal is carried by chylomicron (CM), and the latter produced in the liver is carried by very low-density lipoprotein (VLDL). As for TG metabolism, TG is hydrolyzed to glycerol and free fatty acid by lipoprotein lipase (LPL). LPL is attached to the endothelial cells, and regulated by apolipoprotein C-II (apoC-II) and apoC-III which are present on the surface of CM, VLDL and high-density lipoprotein (HDL). Since the amounts of these apolipoproteins in each lipoprotein are different individually, as a result, resistance of TG hydrolysis by LPL is assumed to vary from person to person even if they have the same TG concentration. Therefore, assessment of the degree of TG hydrolysis individually can be useful for prediction of CVD in detail. However, assay for TG hydrolysis has not been developed yet. In this study, we aimed for establishing the method to measure VLDL-TG hydrolysis, which reflected TG metabolism capability of individual. Moreover, we examined the effect of HDL and lipoprotein depleted plasma (LDP) on the VLDL-TG hydrolysis.

**Methods and Results:** Fasting plasma samples were collected from healthy volunteers. VLDL, HDL and LDP were obtained from the plasma by ultracentrifugation. The plasma or mixtures (VLDL plus HDL or LDP) adjusted to 60 mg/dL TG at final concentration were mixed with LPL and incubated at 37°C for 24 h. During the incubation, a part of the mixtures was transferred to another tube at each time point (0.5, 2, 6, and 24 h), and then, TG level of each sample was quantified at the same time using TG-EX “SEIKEN” (enzymatic method, Denka Company Limited). As a first step, to establish a condition of the assay, the reactivity of plasma to various amounts of LPL was monitored. Consequently, we decided the optimal amount of LPL 12 U/mL which decreased plasma TG concentration by half. Under this condition, when TG hydrolysis was compared between plasma and VLDL derived from same blood, VLDL-TG hydrolysis was quite faster than that of plasma. Thus, since VLDL-TG hydrolysis occurred promptly, after reevaluating the optimal level of LPL 1.2 U/mL for assessment of VLDL-TG hydrolysis, the effect of HDL and LDP on VLDL-TG hydrolysis was investigated. As a result, LDP did not affect VLDL-TG hydrolysis, while HDL suppressed VLDL-TG hydrolysis.

**Conclusion:** We established the assay for evaluating plasma TG and VLDL-TG hydrolysis, and revealed that our assay is assessable for the effect of LDP and HDL on the VLDL-TG hydrolysis. Using this assay, further studies on the investigation of related factors such as apoC-II and apoC-III levels in HDL on VLDL-TG hydrolysis and the assessment of patients with hypertriglyceridemia can be expected.

## B-125

### Side-by-side Comparison of two automated computer-assisted antinuclear antibody indirect immunofluorescence assay systems

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**Background:** Fully automated computer-assisted antinuclear antibody (ANA) indirect immunofluorescence assay (IFA) systems have great potential to reduce assay variability and interpretation subjectivity and facilitate standardization in ANA pattern identification. Commercially available platforms vary in HEP-2 cell lines, screening titers, dilution schemes, and pattern reorganization algorithms. We performed a side-by-side comparison of two platforms for their performance in determining ANA positivity, pattern, and titer.

**Methods:** Thirty residual patient and ten healthy donor samples were assayed using two platforms: QUANTA-Lyser 3000 in combination with NOVA View (Inova Diagnostics, CA) and Sprinter XL IFA 2400 processor with EUROPattern (Euroimmun US Inc, NJ). Results were compared to our current method (manual testing using reagents from Inova Diagnostics at 1:40 dilution with fluorescence microscopy for reading slides). All samples were also tested for a panel of 10 extractable nuclear antigens (ENA) using Bioplex 2000 (Biorad) to address discrepancies. Assays were performed per the manufacturer's instruction.

**Results:** The EUROPattern ANA assay (1: 40 dilution) showed 100% positive and 46% negative (overall 85%) agreement with our manual method. 5 out of 11 negative samples were classified as positive by EUROPattern. The NOVA View assay (1: 80 dilution) initially misclassified 67% negative samples (very low fluorescence intensity and no pattern) as positive. After technologists' review, the NOVA View assay showed 90% positive and 100% negative (overall 93%) agreement with the current method. 3 out of 29 positive samples by the current method were classified as negative by NOVA View. All discrepant samples were low titer ( $\leq 160$ ) with either speckled or homogenous patterns. ENA panel results of all discrepant samples were negative and patient chart review showed no history of autoimmune disease. Therefore, these discrepant samples were likely to be true negative. For pattern analysis, the EUROPattern suggested one or more patterns for all positive samples. Thirteen percent (5 of 38) of the suggested patterns were incorrect. An additional 24% (9 out of 38) reported both speckled and homogenous patterns required technologists to differentiate. The EUROPattern results after technologists' review showed 96% (25 out of 26) agreement with the current method in pattern identification. The NOVA View failed to suggest a pattern for 53% of samples. After technologists finalized the results, the NOVA View showed 92% (24 out of 26) agreement in pattern identification with our current method. A centromere pattern (1:2560) and a nucleolar pattern (1:160) were missed by the NOVA View. Titers for positive results were determined via serial dilutions by the EUROPattern. Agreement with the current manual method was 45% within  $\pm 1$  titer and 69% within  $\pm 2$  titers. Titers by NOVA View were generated using its single well titer function. The results showed 75% within  $\pm 1$  titer and 83% within  $\pm 2$  titers agreement with our current method.

**Conclusion:** Both platforms can reduce manual processing and improve ANA testing. The EUROPattern demonstrated strong ANA pattern identification but optimizing screen dilution might be needed to avoid false-positive results. The NOVA View could generate acceptable titers without serial dilutions but ANA pattern recognition needs to be improved.

## B-126

### Fluorescent microsphere intensity reference assigned with ERF values for quantitative clinical flow cytometry

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#### Background:

Multicolor flow cytometry assays are used routinely in biomedical laboratories to differentiate and count cells in blood and other body fluids. To obtain quantitative and comparable measurements, fluorescent microspheres with MESF (molecules of equivalent soluble fluorophore) values were used as intensity references. However, existing protocols employing MESF microspheres have not yet to produce consistent results that are independent of the flow cytometer used. A main reason for this

inconsistency is that the methods employed to assign MESF values can vary from one vendor to another. Additionally, the number of reference fluorophores for MESF value assignment is limited.

**Methods:**

To overcome these challenges, a new intensity measurement unit, equivalent number of reference fluorophores (ERF), has been proposed and implemented by the National Institute of Standards and Technology (NIST) through a measurement service. Notably, MESF is a special case of ERF where the labeling fluorophore on the microspheres and the reference fluorophore in solution are the same. To ensure quality and traceability of the intensity references, all fluorophores used for ERF value assignments are NIST certified standard reference or research grade reference materials. High-quality reference fluorophores are essential because most commercially available fluorescent dyes, currently used to assign MESF values, are sold with an approximate purity and no measured uncertainty.

**Results:**

Through collaborative research with NIST, a set of multi-color fluorescent microparticles (Invitrogen™ AccuCheck™ ERF Reference Particles) were developed. These particles have NIST-traceable ERF values assigned to 26 commonly used flow cytometer fluorescence detection channels. Using these particles for quantitative flow cytometry, will assure consistency and traceability of the sample intensity value, thus, enabling intra- and inter-laboratory data comparison.

**Conclusion:** Proper use of AccuCheck ERF Reference Particles will enable the standardization of the fluorescence intensity scale and performance characteristics of flow cytometers. In combination with a biological standard, these particles can also be used to determine the expression level of surface and intracellular protein biomarkers in an assessable unit such as antibodies bound per cell (ABC).

## Data Analytics and Informatics

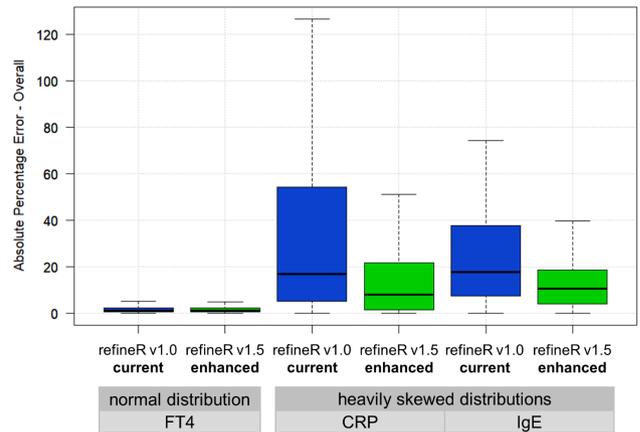
### B-127

#### Enhancing refineR - Improving Performance of Reference Interval Estimation for Skewed Distributions

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**Background:** Precise reference intervals (RIs) are essential for the interpretation of laboratory test results in medicine. The current gold standard is to determine the central 95% range of test results from apparently healthy subjects (direct method). However, this method is practically, logistically and ethically challenging. Recently, we published a novel indirect method using real-world data (RWD) to estimate RIs (refineR). In the published simulation study, refineR outperformed another indirect method (kosmic) and the direct method with N=120. For RWD, the algorithm achieved results comparable to the direct method. However, performance of refineR for heavily skewed distributions has not been studied so far. **Methods:** We provide an update to refineR that accounts for challenges associated with skewed distributions. First, we adapted the definition of the region of test results that characterizes the non-pathological distribution. Second, we introduced a more fine-grained search region for the power parameter lambda. Third, we adapted the cost function by introducing a factor to account for measurement imprecision in RWD. The updated version was applied to simulated test cases published for refineR v1.0 and to simulated data of two heavily skewed distributions (C-reactive protein, CRP and Immunoglobulin E, IgE) and one normal distribution (Free Thyroxine, FT4), all with varying location and fraction of pathological samples. **Results:** The updated refineR algorithm substantially improves performance of RI estimation for skewed distributions of CRP and IgE, while maintaining the performance for the normal distribution of FT4, shown by the median percentage error of 17%/18%/1.3% (refineR v1.0) compared to 8%/11%/1.3% (enhanced refineR v1.5), respectively. Performance for the previously published simulation studies was comparable to refineR v1.0 (overall median percentage error of 1.66% (enhanced refineR v1.5) compared to 1.53% (refineR v1.0)). **Conclusion:** We present an update to the refineR algorithm, which improves the estimation of RIs from RWD for skewed distributions.

Performance Evaluation of Current and Enhanced refineR Algorithm



Smaller absolute percentage error represents better performance.

### B-128

#### Automation of isolate Whole Genome Sequencing (WGS) to empower detection and surveillance of Healthcare-associated Infections (HAI)

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**Background:** Healthcare-associated Infections (HAI) affect approximately 1 in 25 patients causing nearly 100,000 deaths annually. CDC reports that among hospitalized patients, approximately 1.7 million people acquire an HAI in the United States, costing over \$30 billion dollars annually. Catheter-associated urinary tract infections (CAUTI), ventilator associated pneumonia (VAP), surgical site infections (SSI), and central line associated bloodstream infections (CLABSI) linked to hospital care are identified as the most common routes of HAI, however many more go undetected from various unidentified sources. Identifying sources of transmission is the first step towards improving infection prevention control (IPC). To that end, whole genome sequencing (WGS) of bacterial isolates that cause HAI, e.g. methicillin-resistant Staphylococcus aureus (MRSA), carbapenem-resistant Enterobacteriaceae, Clostridium difficile etc., coupled with the mining of Electronic Health Records (EHRs) may identify transmission sources that can result in prevention of larger outbreaks. In order to make this technology accessible, a next-generation sequencing system should be affordable, easy-to-use, rapid and fully integrated into existing routine clinical workflows.

**Methods:** A fully automated turn-key isolate WGS sequencing platform, developed by Clear Labs, was utilized for this study. The Clear Dx™ system performed sample lysis, DNA extraction, library preparation, sequencing, bioinformatic analysis and generated a sample sequence similarity report. To evaluate the accuracy of the Clear Dx system to correctly identify and flag a cluster of related sequences; a panel of bacterial isolates associated and not associated with a hospital outbreak was sequenced and compared to results generated from a traditional manual setup.

**Results:** No significant differences in single nucleotide polymorphisms (SNP) or indels were found when sequencing was conducted on the panel of real world HAI outbreak samples using the Clear Dx platform versus a traditional manual sequencing method. HAI outbreak strains were detected through the Clear Dx bioinformatic data processing pipeline and similar sequences were flagged for further investigation. Results generated from the automated Clear Dx system versus the traditional manual method are statistically indistinguishable. Automating the extraction, library preparation, sequencing and bioinformatic analysis did not impact the quality or accuracy of the sequencing or analysis of sequence data.

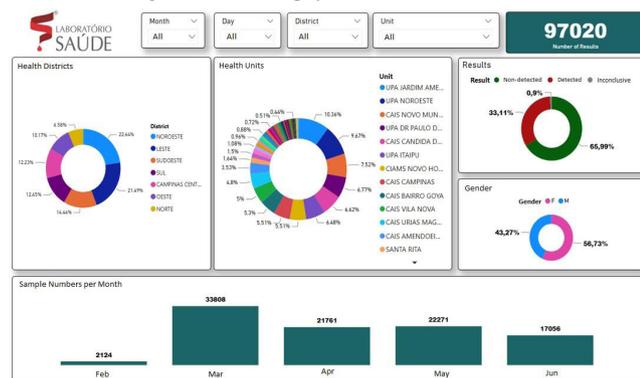
**Conclusion:** Reducing HAI will save lives, reduce financial penalties implemented by the Center for Medicare Services (CMS) and lower the total cost burden on the healthcare system. Once similar sequences are identified, IPC teams or clinicians can follow up to assess whether there are common source(s) or modes of transmission (e.g. procedures, provider, location etc.) to help minimize HAI in a hospital. Traditional manual sequencing is cumbersome, time consuming and requires technical expertise. An affordable, fully automated turn-key isolate WGS system that provides sample-to-answer in nearly 24 hours and can be easily integrated into routine clinical testing could enhance the way that a hospital performs IPC surveillance and outbreak monitoring and tracking.

## B-129

### Turnaround Time (TAT) and Presentation of Results in Real Time (Power BI) for the Municipality of Goiania, Brazil in the Fight Against the SARS-CoV-2 Pandemic

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**Background:** The emergency of different SARS-CoV-2 variants, the increased viral transmissibility and immune evasion to vaccination and past infection has maintained the viral spread around the globe and reinforced the need to monitor and to evaluate data for continuous epidemiological surveillance by the health authorities. Consequently, clinical laboratories have had the necessity to set up high-throughput computer-integrated diagnostic platforms in order to generate and analyse data much faster than before. Since the beginning of the SARS-CoV-2 pandemic, clinical laboratories have been facing problems like shortage of supplies and this has resulted in delayed laboratory reports, jeopardizing data management for prompt medical and public health decisions. This study aimed to evaluate the turnaround time (TAT) given by our laboratory facility when carrying out a project with the local City Secretary of Health to perform RT-qPCR viral diagnosis in nasopharyngeal patient samples obtained from different geographic public health districts in the city of Goiania, Goias State, Central Brazil, from February to June of 2021. **Methods:** 97,026 patient laboratory reports were evaluated through a Power BI tool which allowed real-time data analysis and provided the TAT for patient samples from all different city public health facilities. The “views” with the necessary columns were created within the database (Sqlyanywhere 8), later logged and synchronized (ODBC synchronization) within Power Bi for constant data update. A dashboard was generated and optimized according to the needs of the City Secretary of Health as the pandemic evolved. **Results:** An average TAT of 13 hours, 4 minutes and 40 seconds was obtained with an average SARS-CoV-2 positivity rate of 33.11% (32,126/97,020). The developed dashboard (Figure 1) allowed general and detailed assessments of each health district and unit of the City Secretary of Health with respect to sample hour of ordering and reporting, gender and number of patient samples in a given period. **Conclusion:** In this study, the prompt response to the public health system was shown through SARS-CoV-2 lab TAT of less than 14 hours and this represented a major contribution to epidemiological surveillance and rapid decision making by local health authorities.



## B-130

### Assessment of a Combined Biomarker-EMR Data Machine Learning Model for Sepsis-3

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#### Background:

Early administration of broad-spectrum antibiotics for patients at high risk of sepsis leads to reduced morbidity and mortality. However, timely broad-spectrum administration for these patients is often challenging due to the lack of information readily available to rapidly and accurately identify patients at high risk of sepsis. Current machine learning models (MLM) and diagnostic tests for sepsis suffer from poor clinical performance or are unable to provide a result within a reasonable time-limit. In

this work, the diagnostic and prognostic capabilities of a novel MLM that predicts the 24-hour-septic risk within 3.5 hours from the first blood culture order (BCO), is explored.

**Methods:** Adult patients suspected of sepsis in an emergency department or hospital, as defined by a BCO, with white blood cell count and basic metabolic panel measurements were recruited from three U.S. hospitals (N=2379). IL-6, PCT, and CRP measurements were measured from discards of samples drawn +/-3 hours from the first BCO using a magnetic bead assay. 23 other clinical features (results of a Complete Blood Count, a Complete Metabolic panel, vitals, and demographic information) were extracted from the Electronic Medical Records. A random forest model was trained (N=1433) to provide a sepsis risk score and a prognostic risk category (low, medium, or high, or very high). Diagnostic performance for the MLM's ability to predict a Sepsis-3 event within 24 hours of the BCO was quantified and compared to PCT and CRP in a test cohort (N = 946). The utility of the prognostic risk categories was explored by examining the differences in Length of Stay (LOS), 5-day-mortality, ICU transfer within 24 hours, time to vasopressor first and time to first ventilator event in the Test Cohort.

**Results:** The MLM exhibited an Area Under the Receiver Operating Curve (AUROC) of 0.856 compared to an AUROC of 0.709 and 0.686, 0.844 for PCT, IL-6, and EMR data only machine learning algorithm respectively. The MLM showed separability among the prognostic risk groups for LOS ( $p < 0.001$ ), 5-day-mortality ( $p < 0.001$ ), ICU transfer within 24 hours ( $p < 0.0001$ ), time to vasopressor first administration ( $p = 0.0001$ ), and time to first ventilator event ( $p = 0.0001$ ).

**Conclusion:** In this work, an MLM which integrates 3 sepsis biomarkers (IL-6, PCT, and CRP) with 23 other routinely measured clinical parameters was shown to have excellent diagnostic performance and prognostic utility. Such a tool could help physicians quickly and accurately determine which patients should be prioritized for rapid administration of broad-spectrum antibiotics in hospital and emergency department environments.

## B-131

### Demonstrating Siemens and Roche near-equivalence of big data-derived RCVs of common clinical chemistry analytes

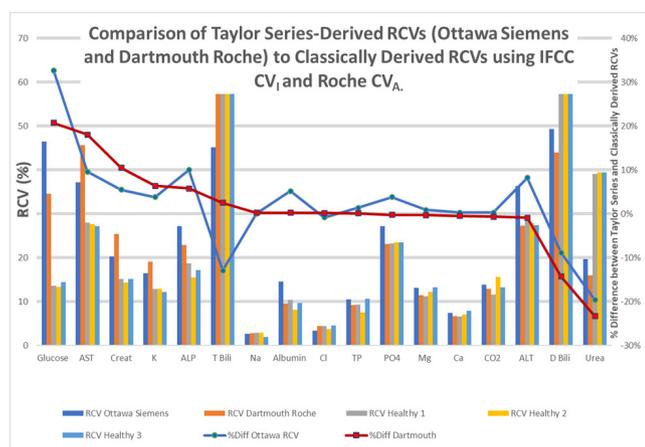
G. Cembrowski<sup>1</sup>, M. Cervinski<sup>2</sup>, Y. Qiu<sup>1</sup>, J. Qiu<sup>1</sup>, C. McCudden<sup>3</sup>. <sup>1</sup>University of Alberta, Edmonton, AB, Canada, <sup>2</sup>Geisel School of Medicine at Dartmouth, Hanover, NH, <sup>3</sup>University of Ottawa, Ottawa, ON, Canada

**Background:** Serial differences between inpatient consecutive measurements can be transformed into Taylor series of variation vs time with the intersection at time=0 ( $y_0$ ) equal to the total variation (analytical + biological + preanalytical). With small preanalytical variation,  $y_0$ , expressed as a percentage of the mean, is equal to the multiplicand of the Reference Change Value (RCV) calculation:  $(CV_A^2 + CV_I^2)^{1/2}$  (PMID 35137000). We compare the between day RCV of patient data for 17 analytes measured by the Siemens Vista to recently published RCVs of a US academic hospital running Roche analyzers and 3 virtual hospital Roche laboratories.

**Methods:** We obtained 5 years (2014-2019) of Ottawa Hospital Siemens Vista general chemistry data. The serial patient values of 17 analytes were transformed into 95% 2-sided RCV. RCVs were calculated for three virtual Roche hospital laboratories incorporating 3 Roche Modular analyzers' quality control summaries and  $CV_I$  derived from IFCC biological variation studies that employed healthy subjects.

**Results:** For sodium, chloride,  $CO_2$ , calcium, magnesium, and total protein, the RCVs are equivalent. For ALT, albumin and phosphate, Siemens RCVs are 5 to 10% higher than the virtual and real hospital laboratories. Similar to the the Roche hospital laboratory, increased, clinically explainable Siemens variation was found for glucose, AST, creatinine, K and ALP and lower Siemens variation was found for urea and direct and total bilirubin.

**Conclusion:** As RCV is calculated from  $CV_I$  and  $CV_A$  and as the clinical laboratory industry reduces  $CV_A$  compared to  $CV_I$ , then RCV becomes a function of  $CV_I$  which is relatively constant. It is then not surprising that so many analytes share similar RCVs as the clinical laboratory industry is producing analyzers in which  $CV_A \ll CV_I$ .



**B-132**

**Assessment of LDL C Calculation Using the Newly Adopted NIH LDL C Equation in Pediatric Population**

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**Background:** The Friedewald equation for calculating low-density lipoprotein cholesterol (LDL-C) has been widely adopted for clinical use since its introduction in 1972. One of the well-recognized shortcomings of this equation is its overgeneralization of the physiological ratio between masses of triglyceride (TG) and LDL-C, leading to erroneous estimations of LDL-C at low levels and in patients with abnormally high TG levels. Over the years, many attempts have been made to address this issue with the development of more sophisticated equations. In 2020, Sampson and colleagues developed a new equation using lipid samples from patients tested at the National Institutes of Health (NIH) Clinical Center. This new NIH equation was proposed to allow for a more accurate estimation of LDL-C at low levels compared to the Friedewald equation. In this study, a retrospective assessment of LDL-C calculations was done using the new NIH equation to investigate its benefits over the Friedewald equation in pediatric patients. **Methods:** Patient data was extracted from the lab information system, EPIC Beaker, which included results for total cholesterol, HDL cholesterol, non-HDL cholesterol, and TG between calendar year 2019 and 2021. A total of 14,356 results from 8719 unique patients aged <1 month to 21 years, median age 14 years, were analyzed in RMarkdown accessed through RStudio. Calculations of LDL cholesterol levels for each result set were conducted independently using the Friedewald and NIH equations. **Results:** Concordance in LDL-C estimation was 95% (13636 out of 14356) using the Friedewald versus NIH equations in this cohort patient data, 15% of all “Low” results determined by the Friedewald equation have been re-classified as “Normal” using the NIH equation, and 2% of “Normal” were re-classified as “High”. A small fraction of the dataset, 2.27% (326 out of 14355), had elevated TG between 400 and 800 mg/dL, and 0.07% (106 out of 14355) had TG >800 mg/dL. Of the samples with TG between 400 and 800 mg/dL, 44% (145 out of 326) resulted in a Friedewald LDL-C estimation of less than 70 mg/dL, which had been classified as “Low” based on the population reference range. Using the newly adopted NIH equation for LDL-C, 41% (59 out of 145) of said fraction were re-classified from “low” to “Normal”. **Conclusion:** When compared with the Friedewald equation, the newly adopted NIH equation allowed for a uniformly higher estimation of LDL-C levels in pediatric patients with TG levels between 400 and 800 mg/dL. This is consistent with other published reports that it has a higher degree of accuracy and is less likely to overestimate patient risk from false classification. We expect this to better facilitate medication management for patients’ receiving statin therapy post heart transplantation.

**B-133**

**Implementation of Alert System for Early Diagnosis of Acute Kidney Injury: Paving the Way for National Public Health Advisory in India**

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This study is the first attempt to create and implement an alert system to facilitate early diagnosis and timely intervention for management of Acute Kidney Injury (AKI) in India. An algorithm was created to retrospectively track rise in serum creatinine (SCr) as per the KDIGO guidelines. Participants >18 years of age with a baseline SCr <4 mg/dl were included. Clinical history, including 53 features associated with AKI, were recorded and statistical analyses were performed. With excellent sensitivity (99.53%), specificity (98.60%), and diagnostic accuracy (Youden’s index 0.98), true positive alerts were generated for 214 of 4439 patients. 75.2% patients were critically-ill, with primary diagnosis of cardiac, pulmonary and nephrological events and co-morbidities such as hypertension, diabetes mellitus and CKD. Only 40.2% patients had a documented clinical diagnosis of AKI. The overall in-hospital mortality rate was 21%. Sub-group analysis revealed worse outcomes in stage 3 AKI patients. De-novo AKI patients had a higher risk of death compared to AKI on CKD patients. We classified the 55 cases where AKI episodes occurred in patients with pre-existing CKD as AKI on CKD patients. The other were classified as de novo-AKI patients (table 1).

**Table 1.** Comparison of AKI on CKD vs AKI patients

	AKI on CKD		AKI	
	No. of patients	%Incidence	No. of patients	%Incidence
<b>Total</b>	55	25.70%	159	72.43%
<b>Stage 1 AKI</b>	28	50.91%	111	69.81%
<b>Stage 2 AKI</b>	4	7.27%	18	11.32%
<b>Stage 3 AKI</b>	23	41.82%	30	18.87%
<b>Nephrology consultation</b>	47	85.45%	69	43.40%
<b>AKI Diagnosed</b>	32	58.18%	54	33.96%
<b>ICU Status</b>	44	80.00%	117	73.58%
<b>COVID</b>	11	20.00%	20	12.58%
<b>Dialysis</b>	16	29.09%	16	10.06%
<b>Mortality</b>	7	12.73%	38	23.90%

This study revealed that in the current scenario, a substantial number of AKI cases are being missed and thus, patient outcomes are poor. This study has also shed light on the prevailing epidemiological trends of AKI and shall provide a benchmark for future studies in India to implement an alert system for accurate and early diagnosis of AKI in India.

**B-134**

**The Use of Outlier Relative Marker MoM Ratios (RMMRs) for the Identification of Analytical and Demographic errors in Maternal Serum Screening Risk Calculations**

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**BACKGROUND:** Quality assurance in maternal serum screening (MSS) for trisomies (T18, T21) and neural tube defects (NTD) are of particular importance to ensure accuracy of results. MSS utilizes four markers: alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE3), and dimeric inhibin A (DIA). Concentrations of these analytes, converted into multiples of the median (MoMs) utilizing medical provider-submitted demographic parameters (including gestational age; GA), are the basis for calculation of fetal abnormality risk. Examination of Relative Marker MoM Ratios (RMMRs) (for example: uE3 MoM/AFP MoM) represents a potential novel quality assurance method in addition to standard QC practices for the detection of analytical or demographic errors in MSS.

**OBJECTIVE:** To examine distributions of the six different possible RMMRs (uE3/AFP, uE3/INHA, uE3/hCG, AFP/INHA, AFP/hCG, INHA/hCG) in a large sample set and investigate the significance of observed RMMR outliers. **METHODS:** Analyte concentrations were measured in a cohort of 1522 samples on a Beckman Access DxI 800 analyzer. MoMs were calculated based on provider-submitted patient demographics. The base ten log of each of the six RMMRs were plotted versus relative reported

T21 and T18 risk scores. Log RMMRs greater than an absolute value of |1.0| were denoted as outlier RMMRs. Submitted patient demographics were examined for transcription errors, and marker analyte concentration measurements were repeated for all RMMR outliers. RESULTS: Eleven samples (0.7%) exhibited at least one outlier RMMR. In general, increased trisomy risk was not associated with RMMR outlier. Analytically, all immunoassay components repeated from these outliers within 20% of the original values with the following exceptions. One sample had an abnormally high INHA/hCG RMMR that was traced to a substantially altered initial uE3 MoM (+320%) determination due to bovine alkaline phosphatase assay interference. Three other samples (one with a low uE3/hCG, and two with low uE3/INHA RMMRs), were associated with sporadic artifactually low (20-30%) initial uE3 concentrations.

Demographic examination yielded potential explanations for the other seven outlier RMMR samples. One sample was associated with unexpectedly high hCG which was suspicious for an erroneously early GA (abnormally low UE3/hCG, AFP/INHA and AFP/hCG RMMRs). This patient's GA for this sample was substantially revised to a later date by the client. Four other samples exhibited unexpectedly low hCG MoMs (yielding two high UE3/hCG ratios and two high INHA/hCG RMMRs), which were consistent with possible misdated (early) GA, although further provider confirmation of GA was unavailable. Finally, two samples had abnormal RMMRs associated with abnormally high AFP measurements (low UE3/AFP and high AFP/hCG), which were deemed likely to be associated with the presence of a neural tube defect (associated risk score of >1/2).

CONCLUSIONS: Investigation of outlier RMMRs identified a combination of demographic and analytical errors which were not detected by standard quality control/assurance methods. While these RMMRs are not suitable as the sole method of assessing the accuracy of MSS, the identification of patient

specimens exhibiting abnormal RMMRs could potentially be incorporated as an alert parameter for further investigation of analytical or demographic information errors during the testing and reporting process.

### B-135

#### Reliability Monitoring. A practical example with Elecsys® Troponin T Assay Linearity and Calibration Performed with two cobas® e-601 for Four Years.

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**Background:** Reliability is the aspect of quality reproducibility over time that impacts the customer the most. The reproducibility of Elecsys® Troponin T Gen 5 STAT (cTnT) calibration and linearity, as assessed with two cobas® e-601 analyzers for four years, was evaluated with a weighted polynomial regression model testing for reproducibility of linearity and equality of regression lines. **Methods:** Two cobas e-601 analyzers (Roche Diagnostics). Elecsys Troponin T Gen 5 STAT (Roche Diagnostics) reagent. Calibration with Cal Set® (Roche Diagnostics) was performed for each new reagent lot, then every five days and when required by regulatory criteria (CLIA/CAP). Calibration and linearity were assessed every six months with Cal Check® (Roche Diagnostics) performing five independent assays, per each level of calibration material, throughout twenty-four hours. The data were transferred to Minitab® (Version 17, Minitab Inc.) statistical software and analyzed for linearity and equality of regression lines with polynomial regression analysis techniques, residuals diagnostics, their graphical representations and the locally weighted scatterplot smoother (lowess). **Results:** Multiple comparisons and Levene's statistical tests for equality of variance and their graphical representation with 95% Bonferroni's confidence intervals, showed increasing variance for increasing values of the calibrator material ( $P < 0.001$ ). Consequently a weighted polynomial regression model was employed. The pure error test for linearity showed probabilistically significant lack of fit ( $P < 0.001$ ). However, this was due to very small mean sum of square pure error (=1ng/L) which was not clinically significant. The test for equality of regression lines did not show probabilistically significant differences between either instruments ( $P=0.9$ ) or years ( $P=0.8$ ). The plot of the standardized residuals by the references values showed seven potential outliers with values between -3 and -4.5 standard deviations for the regression line as determined on December 2021. The lowess showed that all regression lines were quasi-linear and those for December 2021 had a negative slope visually different from the others. Additionally, the graph of the relative difference showed that while values >6000 ng/L for December 2021 had a negative bias exceeding -10%, all the other values had a relative difference between +5% and -5%. These results clearly showed that the regression line for December 2021 was different from all the others, in spite of the probabilistic test for equality of regression lines militating against inequality. This was caused by increased variance for values >6000 ng/L. **Conclusion:** This practical example of reliability monitoring showed that the cTnT Gen 5 method calibration and linearity were reproducible for four consecutive years prior showing a

negative bias for values >6000 ng/L at the end of the fourth year. Recalibration of the method corrected this negative bias. Since the negative bias exceeding the total error criterion (Target value  $\pm 10\%$ ) would have affected patient values >6000 ng/L, this would not have affected the diagnosis of early myocardial infarction. Additionally, this practical example illustrated the limitations of the interpretation of probabilistic statistical F test for equality of regression lines and linearity. Residuals diagnostics assessing the method variability in light of the acceptable total variance offered a more accurate evaluation.

### B-136

#### A Clinical Decision System Using Deep Learning for Accurate and Timely Sepsis Diagnosis Based on Electronic Health Records of Patients in the ICU

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**Background:** Prediction of sepsis using machine-learning approaches has gained traction over the past few years. However, lack of translation of these algorithms into clinical routine remains a major pain point. Existing methods of early detection of sepsis are either flawed by a design that prevents them from being successfully integrated into routine clinical care or do not achieve the level of performance required for accurate detection of sepsis, leading to high frequency of false-positive alerts. This well-known issue of clinician's "alert fatigue" leads to decreased responsiveness and identification, ultimately resulting in delayed clinical intervention. Hence, there is a fundamental, unmet need for a clinical decision system capable of accurate and timely sepsis diagnosis, running at the point of need.

**Methods:** In this work, a deep-learning framework based on long short-term memory (LSTM) networks was developed to predict early onset of sepsis in real time for patients admitted to the ICU. The models are trained and validated with data from the PhysioNet Challenge, which consists of 40,336 patient data files from two healthcare systems: Beth Israel Deaconess Medical Center and Emory University Hospital. The algorithm tracks, in the short term, frequently measured vital signs, sparsely available lab parameters, demographic features, and certain derived features for making the predictions. A real-time warning system, which monitors the trajectory of the predictions, is developed on top of the deep-learning framework to minimize false alarms.

**Results:** On a balanced held-out test dataset, the model achieves an accuracy of 92.01% and precision of 96.96%, with sensitivity and specificity values of 87.03% and 96.99%, respectively. The model predicts the onset of sepsis at a median of 6 hours (interquartile range 6 to 8 hours) ahead of time to alert clinicians for early intervention. Most importantly, the model achieves a false-alarm per true-alarm rate of less than 3.5%, which is significantly less than other sepsis alert systems.

**Conclusion:** The proposed algorithm might serve as a clinical decision support system to assist clinicians in accurate and timely diagnosis of sepsis. With an exceptionally high specificity and low false-alarm rate, this algorithm also helps in mitigating the well-known problem of clinician's alert fatigue that arises from currently implemented sepsis alert systems. Consequently, the algorithm addresses the challenges of successful integration of machine-learning algorithms into routine clinical care.

### B-137

#### A Novel Laboratory-Based IT-Driven Clinical Pathway to Improve Detection of Familial Hypercholesterolemia: the Intelligent Lipid (iLipid) Concept

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**Background:** Familial Hypercholesterolaemia (FH) is an inherited condition carrying a high risk of cardiovascular morbidity and mortality. Early diagnosis enables effective treatment, reducing cardiovascular risk to the same life expectancy as in the general population. FH affects 1 in 250 people, yet the vast majority remain undiagnosed. A method to improve identification of FH patients, is, therefore, desirable. We describe a novel laboratory-based IT-driven solution to identify patients with possible FH, incorporating automatic referrals to our regional West Midlands FH Service (WMFHS) for genetic testing, whilst keeping patients and primary care physicians (PCP) informed of the decision processes.

**Aim:** To develop a laboratory information system (LIMS) based algorithm that identifies lipid results indicative of FH, prompting an intelligent lipid (iLipid) profile request, and incorporates an iLipid profile which: 1) Rules out secondary causes of

hyperlipidemia. 2) Calculates a FH risk score (Welsh score). 3) Automatically refers patients at high risk of FH to the WMFHS. 4) Automatically informs patient and PCP of decision processes.

**Methods:** An iLipid profile has been developed in the pathology e-requesting system (Sunquest ICE). The requestor inputs information from drop-down boxes on patient and family history of cardiovascular disease, clinical features of FH, and statin treatment, which is transmitted into the LIMS (Winpath Enterprise, Clinisys). If total cholesterol is  $\geq 7.5$  mmol/L and triglycerides are  $< 5.0$  mmol/L, an algorithm in the LIMS reflexes HbA1c, TSH, albumin and UE. After exclusion of secondary hyperlipidemia, a Welsh score is calculated using age and treatment corrected LDL cholesterol, triglyceride results, and inputted clinical information.

**Results:** If secondary causes of hyperlipidemia are present, characterized by one of the following: HbA1c  $> 58$  mmol/mol, TSH  $\geq 10$  mIU/L, albumin  $\leq 30$  g/L, or chronic kidney disease stage 4 or 5, a comment is appended advising the requestor on appropriate management. Otherwise, a Welsh score is calculated. A Welsh score  $\geq 5$  categorizes the patient at high risk of having FH, generating an automatic referral to WMFHS. The patient and PCP are informed of the referral by automated text message, and appended comment on the laboratory result, respectively. The WMFHS then undertake FH genetic testing. Those with FH are referred to local lipid clinic and the WMFHS undertake cascade genetic screening. Those without FH are discharged back to the PCP. A Welsh score  $< 5$  categorizes the patient at low risk of having FH, generating an automatic text message informing the patient to contact their PCP. An interpretative comment is appended on the test result advising the PCP on appropriate management.

**Conclusion:** This is a proof of concept IT based solution for improving detection of FH and streamlining the patient pathway, allowing for earlier intervention and treatment of patients. The iLipid profile is now being made available for PCP to request. Uptake of the iLipid profile, and its impact on the diagnosis of FH, will be audited over a 12-month period. If successful, this concept of direct laboratory referral to secondary and tertiary care can be applied across multiple patient pathways.

### B-139

#### Understanding the Rheumatoid Arthritis Patient Journey Through the Lens of Real World Data

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##### Background:

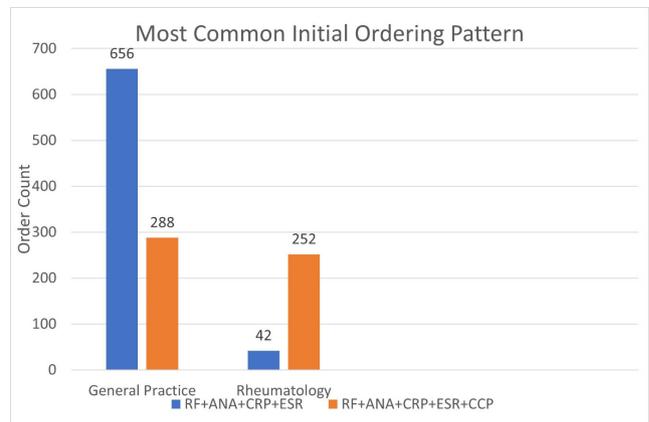
The most recent 2010 ACR/ EULAR Rheumatoid Arthritis (RA) classification criteria included the addition of Anti-Cyclic Citrullinated Peptide (CCP) to aid in more accurately identifying a key subset of early RA patients who may potentially benefit from early therapeutic intervention. We reviewed 8 years' worth of RA specific real world laboratory data to determine the existence of potential diagnostic care gaps.

##### Methods:

Data extraction spanning 2014 - 2021 was pulled from a US based laboratory, using Rheumatoid Factor (RF) and Anti-Cyclic Citrullinated Peptide (CCP) positivity as the primary entry criteria. Additional information pulled for this data set included sex, age, ordering physician specialty, ICD-10 codes, test order date and test codes (ESR, CRP, ANA screen, RF isotypes).

**Results:** The full data set includes 12,455 unique patients who tested positive for RF and/or CCP, with a total of 78,762 individual lab test orders. 37.83% (4,712) of these patients were classified with RA based on an associated RA ICD code. Average time from first RA associated lab order to RA diagnosis was 855.77 days (2.34 years). The initial test order combination most requested through general practice clinicians included RF, ANA screen, CRP and ESR (Figure 1). Among rheumatology specialists, the most common initial order combination was RF, ANA screen, CRP, ESR and CCP (Figure 1).

**Conclusion:** Our data set highlights an RA diagnostic care gap between clinician type (general practice and rheumatology). In particular, general care clinicians seem to be omitting a critical laboratory test, CCP, within the initial screening of suspected RA patients. Further analysis will need to be conducted to determine the downstream implications resulting from this care gap. This data brings to light the need for continued education around appropriate RA diagnostic testing within the primary care setting.



### B-140

#### An Open Source Software Tool Supports Next Generation Sequencing Performance, Quality Control, and Data Sharing

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**Background.** Laboratories are expanding genomic sequencing capacity to modernize and improve infectious disease services and personalized medicine. While sequencing data adds valuable information to aid in metabolic disorders, outbreak detection, transmission chain tracing, and understanding large-scale dynamics of pathogen evolution, it also poses new challenges for laboratories. For example, performing sequencing workflows requires new laboratory processes and new biostatistical expertise. Presently, a lack of standardization in sample processing into sequencing libraries, implementation of bioinformatics pipelines, and result reporting makes it difficult to reliably reproduce and report results. This presents an urgent need for a unified informatics ecosystem to support standardized pathogen sequencing workflows and analyses, and to facilitate efficient data sharing for public health. **Methods.** Several key features of an effective informatics laboratory solution for next-generation sequencing were documented as functional requirements and built into a new suite of open-source software tools. Functionality includes increased accessibility and auditability of sequencing. A user-friendly interface to implement click-through protocols for conducting sample workflow, execution of bioinformatics pipelines, viewing of results, and data sharing all in one platform. This decreases the advanced technical computing expertise typically required to set up and perform advanced bioinformatics analyses of sequencing data. The tool also includes a partner communication portal that facilitates the secure sharing of sample metadata, input files, and results to appropriate partners. The platform was built as a set of containerized open-source modules enabling local- or cloud-based deployments that can be rapidly scaled for an efficient response to emerging outbreaks. The platform laboratories have the software and infrastructure required to establish consistent end-to-end sample processing from specimen preparation through in-silico bioinformatics analysis and data reporting to facilitate near real-time clinical testing and surveillance activities. **Results.** This poster will communicate the innovative LIMS Lite platform that CDC and J Michael Consulting have built. LIMS Lite is an open-source unified informatics ecosystem designed to facilitate the execution of standardized laboratory workflows and bioinformatics analyses, to ease reporting of genomic attributes, and to simplify efficient data sharing among public health agencies. Currently being built to support multiple pathogens including Influenza, SARS-CoV-2, and Anthrax, LIMS Lite aims to offer unified sequencing methodologies used across public and clinical laboratories as starting points for local innovation and validation. Uniting laboratory analysis and reporting will improve process validation, reproducibility, and traceability of sequencing data, advancing our ability to monitor and understand disease etiology, infectious outbreaks, and links to pharmacotherapy response. **Conclusion:** The LIMS Lite tools, documentation, and infrastructure were produced on contract with the CDC. As such they are open-source and free to be used and improved upon by clinical laboratories. It is the hope of the LIMS Lite Team that by presenting this poster at AACC there will be some laboratories that find the platform useful.

## B-141

## Finding Clinical Utility in COVID-19 Serology Testing - A Retrospective Analysis

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**Background:** Serologic assessment of immune status in patients who have had exposure to the SARS-CoV-2 virus has come under a high degree of scrutiny and is met with uncertainty over its clinical utility. Employing COVID-19 serologic methods that identify immunoglobulin production against the viral nucleocapsid enables clinicians the ability to differentiate between immune response to a natural infection and the immune response to a vaccination, which elicits a response against the receptor binding domain.

**Objectives:** Evaluate our medical center's patient data of those testing positive for SARS-CoV-2 IgG nucleocapsid to determine incidence rates among those who have been previously tested for COVID-19 using PCR and assess positivity rates among those who have been fully vaccinated in an attempt to establish clinical utility of COVID-19 serology testing.

**Methods:** We performed a retrospective analysis and chart review of 5000 patients who were tested for SARS-CoV-2 IgG for response to the nucleocapsid antibody using the Abbott Architect method (Abbott Park, Illinois) between June 2020 and December 2021. For all patients who tested as positive with a manufacturer's specified signal to cutoff (S/C) ratio of greater than 1.40 S/C, we assessed previous PCR test data and vaccination status, and evaluated S/C versus time from the PCR diagnosis.

**Results:** The overall positivity rate of those tested for SARS-CoV-2 IgG for the nucleocapsid was 26.7%. Of those testing as serologically positive, 13.6% were symptomatic but had no prior COVID-19 PCR test documented in their medical records. 15.4% of those testing as serologically positive had a negative PCR test documented within the previous 30 days. Among those testing as serologically positive after January 2020, 92% were not fully vaccinated. The S/C response rates were shown to be highly variable, with a median of 6.3 S/C peaking within 30 days from the PCR diagnosis date with median S/C values progressively decreasing over the period of a year to 2.0 S/C approaching the limit of positivity.

**Conclusions:** The clinical utility of this serologic test has been demonstrated in our patient population. Despite diagnostic efforts to identify COVID-19 using sensitive real-time PCR methods, 15.4% of our symptomatic patients who tested negative by PCR were subsequently found to have a positive immune response to the nucleocapsid antigen and could thus be targeted to be followed for COVID-19-related complications. Among patients who have tested as seropositive, 13.6% of symptomatic patients had no prior PCR test but were able to have viral exposure and immune response confirmed. Further, despite documented full compliance with vaccination regimens, we identified that 8% of our vaccinated patients tested positive for antibodies against the nucleocapsid. Serologic assays that detect SARS-CoV-2 IgG against the spike antigen of the receptor binding domain would not be able to differentiate such immune response, and this need was demonstrated during the Omicron variant phase of this pandemic.

## B-142

## Big data Reference Change Value (RCV) analysis of Calgary and Singapore serial patient cTnT demonstrates population and diurnal variation dependencies

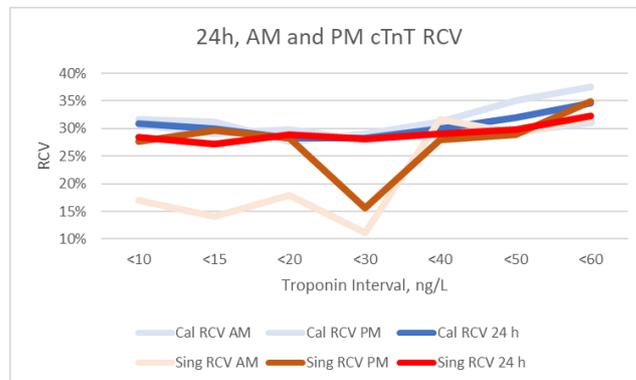
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**Background:** Reference change value (RCV), the smallest significant change in a laboratory test, is usually derived from analytical and biological variation ( $CV_A$ ) and biological variation ( $CV_I$ ). We use an RCV calculation that incorporates serial differences between consecutive intrapatient measurements with a Taylor series transformation (PMID 35137000) to characterize the RCV of Roche troponin t (cTnT) in Singapore and Calgary, Alberta.

**Methods:** Singapore Changi Hospital ED patients [July 2018-July 2020] and Calgary patients [December 2017-December 2021] had cTnT measured with the Roche Elecsys assay on multiple, local Cobas e801's. We determined 24-hour RCV at 7 TnT intervals. As the Roche cTnT demonstrates diurnal variation (in type 2 diabetes patients), we determined and contrasted the AM (midnight to noon) and PM (noon to midnight) RCV. 95% RCV were calculated from  $RCV = 1.96 \times 2^{1/2} \times 100\% / \text{mean TnT}$ .

**Results:** 520,000 and 1,031,000 paired cTnT were available from Singapore and Calgary patients (average age 66.4y and 68.5y, respectively). Calgary patients have higher 24-hour RCVs with a 24h RCV of 32% for low cTnT, and gradually increasing to 35%. Singapore 24h RCVs are 26% for low cTnT increasing to 30% and 32%. For Singapore, the PM and AM RCV demonstrate different patterns. For Calgarians, PM and AM RCV resemble the 24h RCVs.

**Conclusion:** For lower troponins, the lower Singapore AM RCVs are statistically different from Calgary's ( $p < .02$ ) and are consistent with lower Singapore cTnT levels, probably related to the low prevalence of obesity in Singapore (7% in the elderly). European rules for identifying patients with significant myocardial ischemia may lack sensitivity, especially in low troponin patients. TnT diurnal variation in Singapore with low range troponins may mask or heighten the awareness of ischemic troponin increases.



## B-143

## Implementation of a Business Intelligence Dashboard for monitoring COVID-19 in a large-scale laboratory

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**Background:** Since the arise of the COVID-19 pandemic at the end of 2019, clinical laboratories have had a major impact on their operation. Most large laboratories are used to perform massive numbers of common tests, such as biochemistry, hematology, urinalysis and so on. Due to several restrictive measures, like lockdowns, the number of routine tests has dropped drastically. Moreover, the gold standard method for diagnosing COVID-19 has been established to be a molecular test (RT-PCR), which is a complex test that requires highly specialized personnel to perform it. Most laboratories had to re-structure their operations to adapt to this new format and continue to deliver reliable results in time for a suitable treatment. Once the first wave softened, laboratory scenario started to normalize. However, we are facing several subsequent waves of infection to which laboratories must rapidly adapt. In this study, we share our experience in developing and using a business intelligence (BI) tool to monitor all COVID-19 tests to respond to the waves as fast as possible. **Method:** Since early 2020, a multidiscipline team of medical, laboratory and information technology professionals worked together to develop a BI dashboard to monitor COVID-19 tests in the laboratory. The panel was developed using QlikSense®. It includes a quantitative panel with the number of tests requested and released and a positivity panel. All dashboards can be drilled down per type of test, methodology, material, location, client, time, and so on. They are available in a private server of the laboratory. **Results:** Since 2020, 1.8 million COVID tests have been performed in our laboratory. Most of them (over 1.5 million) are RT-PCR tests, followed by rapid tests for antigen detection (180 thousand). During the contamination peaks, over 8 thousand tests were released per day. Four peaks have been detected: the initial and longer one in early 2020, two minor ones in Dec-20/Jan-21 and Mar-21, and another major one in Jan-22. Those peaks can be easily detected by the BI dashboards and therefore allow the laboratory to manage the modification necessary for continue delivering the results with quality standards and properly time. Those modifications include recruit and re-train personnel from other departments of the laboratory, adequate physical facilities and processes, request additional amount of reagents, acquire and validate new instruments. Our laboratory offered continuously all COVID-19 tests available through all peaks, maintaining rigorous deadlines and high-quality level. **Conclusion:** BI tools are an important feature to support complex decision making. They are already consolidated in many business areas. In healthcare they are commonly used for medical purposes.

Here we present a successful use for process management which has allowed us to get prepared for the evolution of the COVID-19 pandemic and continuously deliver important information for patients and the healthcare system.

## B-144

### Implementing auto-verification algorithms in a core clinical chemistry section of a private reference lab in India

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**Background** Auto-verification is a process of building digital solution based rules to verify clinical laboratory test results using laboratory defined criteria for the efficient release and reporting of patient results without manual intervention

SRL Diagnostics, Mumbai has been an ISO 15189 & CAP accredited laboratory since 2002, processing over 5,000 samples and 12,500 tests per day, with a goal to continuously improve turnaround time and efficiency for both routine and STAT samples

The objective of this poster is to share our approach of implementing auto-verification using cobas infinity middleware which connected eight high throughput analysers across disciplines with our existing LIS system to create a central command center and to bring efficiency and bringing consistency in post analytical patient reporting.

**Methods** All processes, workflows and verification rules (35) were constructed in middleware in adherence to the CLSI Auto10-A Guideline and CAP checklist. We chose a 2 phase approach starting from Oct 2021 in deploying and testing the auto-verification.

Creating the Framework Phase 1 included setting up of the rule based intelligent auto-verification by •Defining Analytical measuring ranges, reference ranges, and critical ranges for 92 assays •Redefining Instrument Interface for all results including calculated parameters •Configuring pre-analytical and analytical systems flags Expanding the Framework Phase 2 included defining an automated process by •Diligent QC based auto-verification using Westgard rules •Better insight on out of range results to inform and standardize next steps such as rerun, reflex, dilution or repeat analysis •Multi-disciplinary and comprehensive reporting for each sample using clinically correlating algorithms

Each of the phases were tested for user acceptance using clear defined outcome criteria's such as, •Total auto-verification percentage over manual verification •Verification for accuracy and documentation of actual test results that were auto-verified by matching results on respective analyzers, middleware, LIS and final patient report generated for a week. •Simplification of non-valued steps in post analytical workflow leading to improvement of TAT.

#### Results

a. The auto-verification process today allows automated release of 49% of all results across the core clinical chemistry section. b. Today we are able to meet our service level agreement commitment of 4 hours TAT in 95 % of all routine and 2 hours for all STAT results. c. We have reduced paper based instructions and log sheets by 65%. d. Today we only require 4 staff members instead of 6 for final result release allowing us to redeploy them to other critical work.

#### Discussion & Conclusion

During acceptance testing, comparison between manual validation and auto-verification showed 100 % compliance which also included stopping of all out of criteria results. We have streamlined and automated result validation decision making by eliminating 10 process steps. The ability to auto-verify a high percentage of results increases productivity and allows staff to focus on the results that require manual review and investigation.

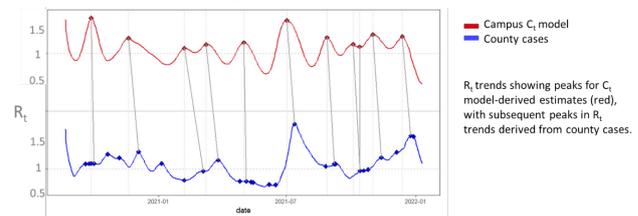
## B-145

### Cross-sectional Viral Load Distributions for Early Detection of COVID-19 Transmission Peaks on Campus and in the Community

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**Background:** During the COVID-19 pandemic, SARS-CoV-2 PCR testing data is widely used for disease monitoring. However, the binary, positive/negative result, approach provides a limited picture of the pandemic trajectory. To overcome this bias, a mathematical approach utilizing the cycle thresholds ( $C_t$ ) values obtained from PCR tests to estimate the epidemic trajectory from cross sections of all screened samples

has recently been developed by Hay et. al. The goal of this study was to assess the effectiveness of  $C_t$ -derived epidemic trajectories for sentinel surveillance eg early detection of transmission peaks on campus and in the community using testing results from Northeastern University (NEU). **Methods:** Cross-sectional  $C_t$  values from RT-PCR data collected weekly at NEU between August 2020 and January 2022 were used to generate the effective reproductive rate ( $R_t$ ) and estimate campus epidemic trajectories.  $R_t$  estimates from the  $C_t$  model were compared to those generated from NEU cases and Suffolk county cases. These epidemic dynamics were also tracked through three waves of SARS-CoV-2 variants. **Results:**  $C_t$  model-derived  $R_t$  estimates showed a maximal lagged correlation of 0.38 and 0.59 compared to University and Suffolk county case-based  $R_t$  estimates, respectively. The correlation analysis also showed that the incidence generated from the  $C_t$  model preceded the incidence on campus and county cases by 14 days. High  $R_t$  values from the  $C_t$  model were reported early after the beginning of each new variant wave, indicating higher transmission rates which preceded peaks in campus and county case-based  $R_t$  estimates, with a median lag of 11 days. **Conclusion:**  $C_t$  values from PCR-based screening programs can provide valuable data to estimate epidemic growth and detect transmission peaks earlier in the community. Earlier prediction of peak incidences can inform policies and public health countermeasures to mitigate spread.



## B-146

### Reassessment and revision of anion gap reference interval at our institution: a study prompted by results from two recent JALM papers

A. S. Hrizat, D. F. Stickle. *Jefferson University Hospital, Philadelphia, PA*

**Background:** Two recent JALM papers (Ayala-Lopez & Harb, 2020; Wu et al., 2021) describe reassessment and alteration of the reference interval for calculated anion gap (AG) at their institutions, leading to a broadening in one case and a contraction in the other. Our institution's history was similar to those in these reports, that despite analytical platform changes over the years in which reference intervals for AG components were verified, a corresponding reassessment of the reference interval for the calculated AG had not been made for some time. Prompted by these papers, we reassessed the reference interval for AG at our institution. **Methods:** Anion gap ( $AG = Na - (Cl + CO_2)$ ) is reported for a number of laboratory panels, such as the Basic Metabolic Panel. AG components (Na, Cl,  $CO_2$ ) are measured by Roche Cobas assays. To assess AG reference interval, same-day admission (SDA) patients were used as a surrogate for a reference population, anticipating no significant preselection among SDA for abnormalities in AG (following procedures of Wu et al.). Using our hospital information system database (Epic/QLIK), we retrieved all AG reported for SDA over a 6-month interval (Jan-Jun 2021). Data analysis to determine AG reference interval (central 95% interval) was conducted using programming in R. **Results:** Primary data comprised 9389 measurements. 4 outlier measurements ( $AG \geq 20$  mmol/L) were excluded (0.04% of total). Using our current AG reference interval (4-16 mmol/L), only 0.4% of AG from this presumed-normal population were flagged as being outside of the reference interval. This circumstance indicated that our existing reference interval was patently incorrect. The data were close to being normally distributed ( $9.7$  (average)  $\pm 2.1$  (1 SD) mmol/L), with a parametric reference interval (6-14 mmol/L) being identical to the non-parametric reference interval (6-14 mmol/L). **Conclusions:** Reassessment of the reference interval for AG at our institution caused a significant contraction of the reference interval, from 4-16 mmol/L to 6-14 mmol/L. Previous reports have suggested that inter-institutional differences in AG reference intervals may be platform-specific.

## B-147

**Time-of-day (TOD) variation in interpatient average calcium (Ca) results: potential application to patient-based quality control (PBQC)**M. Ghafoor, D. F. Stickle. *Jefferson University Hospital, Philadelphia, PA*

**Background:** In routine quality control reviews, we had previously noted a stable TOD pattern in average interpatient Ca results having statistically significant differences across 1-h intervals. Our objective in this study was to examine whether the scale of TOD variation would warrant use of TOD-dependent Ca targets in PBQC. **Methods:** Primary data were Ca results from all-comers (outpatient (OP) and inpatient (IP)) over a 3 month interval (Jul-Sep 2021). For PBQC evaluation, data were bracketed (B) to weekdays (M-F, to exclude weekend variation in the daily IP/OP mix), and to results within the Ca reference interval (RI, 8.5-10.3 mg/dL). B data were merged to a single contiguous data set ordered by date and time. Running means were evaluated for contiguous results of length 20 (20-mers). The pertinent analysis was to determine the extent to which the standard deviation (SD) in running means (the primary parameter in PBQC used to flag deviation of running means from average) was comparable in scale to the TOD-dependent variation in means themselves. Calculations were performed using R programming. **Results:** B data were 39,629 Ca measurements (IP=75.3%). Overall Ca was  $9.29 \pm 0.47$  mg/dL (mean $\pm$ 1SD), compared to  $9.40 \pm 0.45$  mg/dL for RI. Ca distributions for IP ( $9.21 \pm 0.46$  mg/dL) and OP ( $9.55 \pm 0.37$  mg/dL) were distinct ( $p < 0.001$ ). Using 20-mers, the all-data running mean was  $9.29 \pm 0.19$  mg/dL. When parsed in 1h intervals, however, 20-mer running means for Ca ranged from 9.1-9.5 mg/dL. This TOD pattern included two time blocs of contiguous results above (0800-2300 h; 53.3% of results; IP=75.3%) and below (2300-0800 h; 46.7% of results; IP=99.9%) the all-data mean. The SD within 1h means was reasonably constant ( $0.12 \pm 0.01$  mg/dL). Compared to the all-data mean for 20-mers as a target, however, average 1h 20-mer means varied from target by as much  $\pm 0.2$  mg/dL, or  $\pm 105\%$  of the all-data SD. Accordingly, without accounting for TOD-dependence of means, there was an inherent TOD-dependent pattern of inaccuracy in the PBQC target value, the magnitude of which was of a scale that was essentially equal to that of the all-data SD used as a parameter in standard PBQC. A simple strategy can overcome this error. Characterization of the TOD-dependent means using a Fourier series allows for determination of predicted means for any arbitrary time of arrival for results of a 20-mer mean. Using this strategy reduced target comparison error for average 1h intervals of 20-mer means by a factor of 7, to  $\pm 0.028$  mg/dL [excluding one point (0000-0100 h, error=0.06 mg/dL)], with no TOD-dependence. **Conclusions:** TOD-dependent variation in running means for Ca was due in part to TOD variation in the IP/OP mix. For 20-mers, variation was of a scale that was essentially equal to  $\pm 1$ SD of the all-data SD for running means. Without accounting for this variation, there was an inherent, predictable, TOD-dependent inaccuracy of the target value for PBQC. Characterization of TOD-dependence of running means by Fourier series analysis to produce TOD-dependent targets significantly reduced this error. In principle, the strategy of using TOD-dependent targets should reduce the probability of false-positive flags in PBQC.

## B-148

**The Evaluation of Artificial Intelligence Assisted Flow Data Analysis for Diagnosing Immunologic Disorders and Monitoring Immune Status**T. S. Yeager<sup>1</sup>, S. Wang<sup>1</sup>, A. Wang<sup>2</sup>, G. Fan<sup>1</sup>. <sup>1</sup>OHSU, Portland, OR, <sup>2</sup>DeepCyto, West Lynn, OR

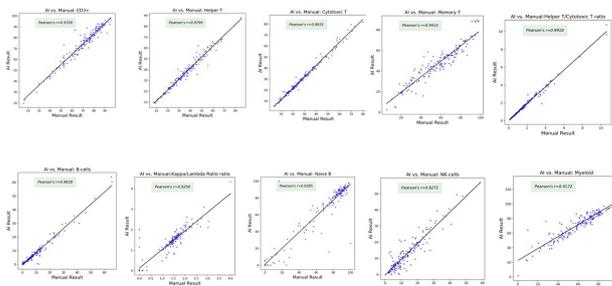
**Background:** Flow-cytometry analysis is widely used for diagnosing immunologic disorders. However, flow-data analysis is time consuming. We use 10-color flow-cytometry with a panel of 29 antibodies to evaluate immune status including immune deficiency, autoimmune disorders, post-chemotherapy, and post-transplant immune reconstitution. The flow-data analysis is performed by technologists using Kaluza (Beckman-Coulter); then reviewed by pathologists for final reporting. This study is to evaluate the accuracy and efficiency of an AI flow-data analysis system developed by DeepCyto using artificial intelligence compared to manual processing.

**Methods:** The 10-color flow-panel of 29 antibodies is designed to evaluate T-cell subsets, B cell subsets, NK-cells, neutrophils, monocytes, and dendritic cells. The Deepflow AI-assisted flow-data analysis software was developed based on DeepCyto's proprietary High Dimensional Phenotype Coupling (HDPC) algorithm. A total of 158 cases were analyzed by both AI and manual gating using Kaluza software. The correlation of each cell population by AI and manual was analyzed by Pearson correlation coefficient (r-value).

**Results:** Of 158 samples, 29 immune-cell parameters were analyzed for each sample. Correlation plots were generated for each parameter to evaluate the agreement

among the AI and manual analysis. Correlation of major parameters demonstrated r values  $> 0.90$  as shown in Figure-1, including total lymphocyte, total T-cells, helper-T, cytotoxic-T, helper-T/cytotoxic-T ratio, total B-cells, Naïve B-cells, CD5+B1 B-cell, Kappa/Lambda ratio, NK-cells, and myeloid-cells. Some lymphocytic subsets revealed r values at the range of 0.80-0.89, including Treg,  $\alpha\beta$ TCR,  $\gamma\delta$ TCR, Naïve T-cells, HLA-DR+ activated T-cells, and monocytes.

**Conclusion:** In conclusion, our study results indicate that AI assisted analysis can be used to accurately enumerate immune subsets, including adaptive and innate immune cells. Each manual analysis needs 10-15 mins of tech time while AI analysis only needs 1 minute. The AI assisted auto analysis will be able to deliver an accurate and time saving immunophenotyping solution for clinical laboratories.



## B-149

**Respiratory virus interactive panel: coexistence in Brazil**F. S. Malta, D. A. Zauli. *Pardini Group, Vespasiano, Brazil*

**Background:** Since December 2019, the world has been facing one of the most important pandemics in the recent world. COVID-19 has spread all over the world and has caused enormous threats to health and life with great socio-economic impact worldwide. According to data from the World Health Organization (WHO), there are currently more than 400,000,000 confirmed cases and almost 6,000,000 deaths caused by Severe Acute Respiratory Syndrome associated with SARS-CoV-2 (Covid-19) (<https://covid19.who.int/> viewed on 2022-02-24). Therefore, other seasonal respiratory viruses such as Influenza or syncytial virus (RSV) are expected to co-circulate with SARS-CoV-2 at some time demanding public health measures with long-term effects to simultaneously deal with both respiratory infections and their complications. In this context, the Pan American Health Organization encourages countries to continue epidemiological surveillance of Influenza and other viruses to contribute to public health policies implementation and strengthen health systems. **Objective:** The purpose of this study is to show the importance of epidemiological surveillance of other endemic diseases during the pandemic COVID-19 due to the possibility of an extra burden on the health system. **Methods:** Data were obtained from detection tests for SARS-CoV-2, Influenza and RSV carried out in the Grupo Pardini (Clinical laboratory with national coverage). The R programming language was used for data analysis and the Leaflet and Shiny libraries to plot the interactive map. A time-series graph shows the positive rate of each infectious agent evaluated in this study in Brazil since 2021-12-26. The interactive dashboard allows you to select the individual or joint view of infectious agents. The presence of the infectious agent is indicated according to the rate of positives in the municipality using a circle filled with color according to the legend on the panel. Each infectious agent is presented in a layer of the map and, therefore, the superposition of circles of different colors shows the coexistence of the viruses in the same municipality. **Results:** Map data is updated weekly and presented on the link <https://www.grupopardini.com.br/FLURONA/>. To date, 356,236 SARS-CoV-2 tests, 7,168 Influenza tests, and 5,417 RSV tests have been recorded. Since the end of 2021, it has been possible to observe a large increase in the rate of positives for SARS-CoV-2, which was compatible with the peak arising from the Ómicron variant, at the same time we had a drop in the rate of positives for Influenza A and RSV in Brazil. At the most critical moment of the coexistence of these three agents, we had a positive rate (Brazil) of 15%, 55%, and 31% of SARS-CoV-2, Influenza A, and RSV, respectively. **Conclusion:** The map records the temporal history of the coexistence of the SARS-CoV-2, Influenza A, and RSV viruses in Brazil since 12-26-2021 and serves as an epidemiological surveillance tool to monitor the presence of these viruses regionally to support a decision in the area of public health.

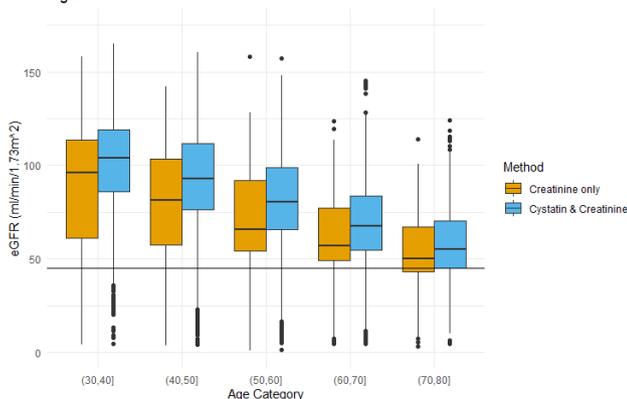
**B-150**

**Detection of Renal Insufficiency with and without Cystatin-C in a Population of Life Insurance Applicants**

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**Background:** Recent guidelines from the National Kidney Foundation regarding screening for renal insufficiency suggest using new, race-blind, cystatin C formulae to determine estimated glomerular filtration rate (eGFR) in at-risk individuals. **Methods:** In order to determine how this strategy might affect the distribution of eGFR in a generally healthy population, 43,174 life insurance applicants (mean age: 53.5, 52% male) who submitted blood for testing as part of a life insurance underwriting examination had their eGFR calculated using two different formulae. The first was the 2009 CKD-Epi formula which uses creatinine only and has independent variables of age, sex and race. Since race is not collected as a variable in life insurance underwriting, it was imputed as non-black for all samples. The second formula was the 2021 CKD-Epi formula using creatinine and cystatin C along with age and sex. The resulting distributions of eGFR were compared by age category, and the mean differences calculated. **Results:** Across all age ranges, the mean eGFR when using the cystatin C formula was higher than the mean eGFR determined by the older, creatinine-only formula (Figure 1). The mean differences for those ages 30-39, 40-49, 50-59, 60-69 and 70-79 were 11.6, 13.0, 10.4, 7.2 and 3.2 mL/min/1.73m<sup>2</sup>, respectively. The prevalence of stage 3b or worse renal insufficiency (eGFR < 45 mL/min/1.73m<sup>2</sup>) fell from 9.5% to 6.3% with the newer formula. **Conclusions:** In a generally healthy, self-selected population, the 2021 CKD-Epi formula using cystatin C produced generally higher levels of eGFR and a lower proportions of individuals identified with significant renal insufficiency.

Figure 1



**B-151**

**Parathyroid hormone reference interval adjusted for age and gender using two immunometric assays**

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**Background:** Parathyroid hormone (PTH) measurement is relevant for evaluation of osteometabolic diseases. Clinical and Laboratory Standards Institute (CLSI) recommends that reference intervals (RI) could be established through indirect data, retroactively identifying acceptable reference populations. Literature data suggest that PTH is higher in elderly, but there is a lack of data defining if RI should be established by age. **Objectives:** To define RI for PTH in the Brazilian population, assess if different RI should be defined by age, and by gender in two different assays. **Methods:** The database of a private laboratory was analyzed with the selection of subjects aged ≥20 years (y) who had PTH measured over a period of 4 years, in several technical operational centers distributed across the country. PTH was measured by electrochemiluminescence in the Cobas Roche assay (method 1) or by chemiluminescence in the Advia Centaur Siemens assay (method 2). Exclusion criteria were (and/or): hospitalized patients, estimated glomerular filtration (EGF) <60 mL/min, albumin-corrected total calcium <8.6 or >10.0 mg/dL, ionic calcium <1.00 or >1.30 nMol/L, phosphorus <2.5

or >4.5 mg/dL, vitamin D <30 ng/mL, and body mass index (BMI) < 18.5 or > 24.9. The central 95% PTH levels were considered for RI determination. The level of statistical significance was p <0.05. **Results:** Were selected 3645 subjects, 2935 (80,52%) female. PTH RI was statistically different according to age and gender in method 1 (table). Due to the low number of individuals, the RI of the elderly in method 2 could not be evaluated; the results for young subjects did not differ by gender (table). In the subjects aged 20 to 64 years RI differed between the two methods. **Conclusion:** The PTH RI should be segmented by age and gender according to the method used.

Table – Data of subjects and PTH reference intervals by age and gender using two immunometric assays

Legend	Age (years)	Number of patients	Gender	Mean age	PTH reference interval	p value between gender	p value between < 65 or ≥ 65 years
Method 1	20 – 64	2380	Female	43.6	12.5 – 58.6	< 0.01	< 0.01
		581	Male	41.0	12.5 – 52.6		
	≥ 65	555	Female	71.5	17.7 – 75.0		
		129	Male	72.7	16.8 – 63.8		
Method 2	20 – 64	599	Female	41.5	11.7 – 69.3	0.20	-
		239	Male	41.5			
	≥ 65	63	Female	-			
		19	Male	-			

PTH - Parathyroid hormone

**B-152**

**Free or Total Testosterone? Assessment of Androgens Status in Reproductive Age Females.**

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**Background:** Androgen concentrations are measured in females to investigate conditions associated with hyper-/hypo-androgenism. The most commonly performed tests include total testosterone (Te), sex hormone binding globulin (SHBG) and free Te (FTe). Concentrations of Te or FTe outside of the age-specific reference intervals are considered key diagnostic features of biochemical hypo-/hyper-androgenism. We compared distributions of Te and FTe concentrations in association with various health conditions observed in reproductive age females (RAF) and assessed diagnostic potential of the tests.

**Methods:** We reviewed data on Te and FTe concentrations measured in consecutive routine serum samples from RAF (n=11,764), for which International Classification of Diseases (ICD-10) codes were provided with the test request. We evaluated subset of specimens (age range 18-40 years; mean/median 29/29), where the ICD-10 codes corresponded to healthy females (medical examination without abnormal findings, n=413), and females with clinical conditions known to be associated with androgen excess or deficiency (n=3,419). Te was measured using LC-MS/MS method, reference interval (RI), 9-55 ng/dL; SHBG was measured using immunoassay (Roche Cobas e602); concentrations of FTe (RI 0.8-7.4 pg/mL) were calculated (Vermeulen, JCEM 1999) using measured concentrations of Te, SHBG, and albumin; the assays' imprecision were <10%.

**Results:** Mean values of measured Te and calculated FTe concentrations, concentrations distribution, percent of samples outside of the RI, and comparison of the distribution between the pathologic and healthy groups are summarized in Table below.

**Conclusion:** In all pathologic groups, the percent of samples with concentrations outside of the RI was greater for FTe than for Te. We observed statistically significant differences in distributions of Te concentrations in specimens from females of pathologic groups as compared to healthy females, for all conditions with exception of IAA and H; and FTe with exception of IAA. Our data suggest that in RAF, FTe correlates better with androgen status than Te.

Tc and FTc concentrations in samples by ICD-10 code									
Group/ ICD-10 code	N	Tc mean, ng/dL	Tc central 95%, ng/dL	Tc p-value (com-parison to Healthy group)	Tc percent of samples above (below) RI	FTc mean, pg/mL	FTc central 95%, pg/mL	FTc p-value (com-parison to Healthy group)	FTc percent of samples above (below) RI
Healthy/ Z00.00 and Z01.419	413	34	10-89	NA	9.4% (1.5%)	4.9	0.8-14.5	NA	12.8% (1.7%)
Non-scarring hair loss/ L65.9	397	27	9-61	0.0001	4.0% (2.3%)	3.3	0.7-9.4	<0.0001	4.8% (3.0%)
Decreased libido/ R68.82	152	27	9-74	0.0037	3.3% (2.0%)	2.8	0.6-8.3	<0.0001	3.9% (3.3%)
Female infertility associated with anovulation (IAA)/ N97.0	101	34	12-113	0.86	11.9% (0%)	5.8	1.4-21.4	0.087	19.8% (0.0%)
Hirsutism (H)/ L68.0	724	36	11-93	0.26	11.5% (0.7%)	5.3	1.2-14.7	0.0002	18.2% (0.6%)
Secondary amenorrhea/ N91.1	485	37	9-90	0.037	17.5% (1.9%)	6.2	1.0-15.2	<0.0001	33.2% (0.8%)
Amenorrhea, unspecified/ N91.2	448	38	9-96	0.036	16.1% (1.8%)	6.3	1.1-16.8	<0.0001	31.7% (0.9%)
Polycystic ovarian syndrome / E28.2	1036	41	12-103	<0.0001	18.6% (0.8%)	6.2	1.0-17.8	<0.0001	29.0% (1.2%)

**B-153**

**Comparison of Vitamin D Classification between Abbott Architect or BioPlex 2200 and Liquid Chromatography Tandem Mass Spectrometry.**

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**Background:** Automated immunoassays are widely utilized in the assessment of vitamin D sufficiency. However, despite efforts to standardize, variabilities between vitamin D immunoassays persist. The objective of this study was to evaluate the agreement between two commonly used vitamin D immunoassays and liquid chromatography tandem mass spectrometry (LC/MS-MS) in the classification of vitamin D sufficiency.

**Methods:** Serum specimens (n=100) collected for routine vitamin D assessment by an LC/MS-MS at a reference laboratory were identified. Split aliquots sufficient for vitamin D (Total) analysis by Abbott Architect (Abbott Diagnostics, Lake Forest, IL) and BioPlex 2200 (Bio-Rad Laboratories, Des Plaines, IL) immunoassays were obtained and stored until analysis. Vitamin D sufficiency was defined according to the cutoffs (deficient ≤ 20 ng/mL, insufficient 21-29 ng/mL, sufficient ≥ 30 ng/mL) provided by the Endocrine Society clinical practice guideline. For simplicity, results below or above linearity (n=2) were imputed to the nearest whole number.

**Results:** Median Abbott Architect, BioPlex 2200, and LC/MS-MS vitamin D results were 23.0 ng/mL (IQR, 15-38.0; range, 5.0-119.0), 29.0 ng/mL (IQR, 20.0-40.3; range, 6.0-126.0), and 27.0 ng/mL (IQR, 17.3-37.8; range, 3.5-104), respectively. The respective Pearson correlations between LC/MS-MS and Abbott Architect or BioPlex 2200 were 0.96 (95%CI, 0.94-0.97; P < .0001) and 0.92 (95%CI, 0.88-0.95; P < .0001). The correlation between Abbott Architect and BioPlex 2200 was 0.92 (95%CI, 0.88-0.95; P < .0001). The overall vitamin D classification agreement between LC/MS-MS and Abbott Architect was 83% (Kappa, 74%; 95%CI, 63%-85%). The agreement between LC/MS-MS and BioPlex 2200 was 81% (Kappa, 70%; 95%CI, 59%-82%). Compared to LC/MS-MS, Abbott Architect vs BioPlex 2200 Vitamin D deficient, insufficient, and sufficient classification agreements were 81% (30/37) vs 82% (23/28), 68% (17/25) vs 70% (16/23), and 95% (36/38) vs 86% (42/49), respectively.

**Conclusion:** Overall, Abbott Architect and BioPlex 2200 vitamin D assays showed comparable performance. Both assays demonstrated better agreement at lower and higher concentrations.

**B-154**

**Deriving TSH and thyroid hormone reference intervals by big analysis**

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**Background:** We sought to harmonize reference intervals (RIs) for thyroid function tests and optimize the TSH reflex algorithm across Alberta laboratories using a “big data” approach.

**Methods:** One year of thyroid hormone results tested on the major chemistry platforms used in the province (Roche cobas and Siemens Atellica) were extracted from the laboratory information system (LIS). Data was filtered to remove tests ordered by specialists (e.g. endocrinologists) as well as tests ordered on inpatients. Individuals suspected of thyroid disease (anti-TPO and TRAb), autoimmune disease (ANA), and pregnancy (hCG) were excluded. RIs were derived using a combination of statistical Bhattacharya analysis, CALIPER for pediatrics, and consultation with endocrinology.

**Results:** RIs, partitions, and sample sizes are provided in Table 1. The most significant change to RIs occurred with TSH with a higher upper limit of 6.50 mIU/L for adults compared to the current limit of 4.00 mIU/L). 13% of TSH results were found to be 4.00-6.50 mIU/L and were flagged as abnormal by current RI, suggesting an upper limit of 4.00 mIU/L was inappropriate. Analysis of paired TSH and FT4 results showed that 99% of TSH results within 4.00-10.00 had normal FT4, suggesting minimal added value to reflexing FT4 in patients with suspected hypothyroidism. In the hyperthyroidism range, 20% of TSH <0.2 had low FT4 results. This suggested that automated FT4 reflex has most value at low TSH. Automated testing of FT4 when TSH is high was removed from algorithm, which in most cases does not add clinical value. We predict that the changes in RI and TSH reflex algorithm will save the lab \$ 10,000-60,000/year.

**Conclusion:** Current TSH RIs used in Alberta flag too many patients as abnormal despite falling within the central 95%. Big data analysis allows us to indirectly derive RIs that more closely reflect our local population.

Hormone	Age partition	Derived RI	Sample (N)
TSH	0 to 30 days	1.23 to 25.0 mIU/L	77
TSH	1 to < 12 months	1.0 to 6.80 mIU/L	718
TSH	≥ 1 year	0.2 to 6.50 mIU/L	940648
ft4	0 to 14 days	13.5 to 50 pmol/L	49
ft4	15 to 29 days	8.7 to 32.5 pmol/L	256
ft4	≥ 1 month	10.0 to 25.0 pmol/L	182548
ft3	0 to 30 days	4.2 to 13 pmol/L	38
ft3	1 to < 12 months	5.1 to 8.6 pmol/L	66
ft3	1 to 13 years	4.4 to 8.1 pmol/L	2562
ft3	14 to 17 years	3.5 to 7.4 pmol/L	1850
ft3	≥ 18 year	3.0 to 6.5 pmol/L	87991

**B-155**

**Easiness of Uncertainty Estimation and use for Quantitative and Qualitative Results According to ISO**

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**Background:**

The revision of ISO 15189 retains the requirement for uncertainty estimation, referring to ISO/TS 20914 for implementation. [1] With document Q16 [2], SIPMeL collects the main ISO, literature, and CLSI guidance on the topic. Typical examples of application of the Q16 Recommendations are presented here to demonstrate their ease of use.

**Methods:**

The performance characteristics of an assay with quantitative results (ALT EC 2.6.1.2) are derived from refs [3] and [4]. A test with “qualitative” results, Abbott BinaxNOW-19 COVID-19 Ag card [5] [6], is then described.

The SIPMeL Q16 recommendations provide for quantitative results, and for quantitative phases of examinations with nominal results, the calculation of type deviation ( $\sigma$ ) from the long-term IQC data and the repeatability limit from type deviation ( $\sigma$ ) in the series. For nominal qualitative results, and for the weakly positive result of a method that provides ordinal qualitative values, the CQI for a material near the detection limit or  $C_{95}$  provides a frequency value of positives (POD%), while the repeatability check uses the equality criterion between two repeated results.

#### Results:

The uncertainty component of the calibration, the prerogative of the manufacturers, is set to zero. The precision component results for ALT  $\sigma$  2.94 U/L (CV 8.3%), extended uncertainty (U) 5.88 U/L (16.6%), while for COVID-19 Ag the precision of the chosen level is fixed at POD 95%. Results of duplicate testing are presented.

#### Conclusion:

In the medical laboratory, pressured by the amount of tests to be performed and turnaround times to be met, procedures for quality must be easily executable, simple, intuitively understandable, robust, and uncontroversial. This is what ISO 20914 and a long list of ISO and CLSI documents, collected in SIPMeL Q16, are for. Knowledge of uncertainty provides the laboratory with awareness of the reliability of results, information that is shared with users or third parties, for example, in accreditation inspections.

"Precision" requires repetitions of the measurement on the same material at a set level. For qualitative results, it is easy to determine how often observations differ from each other, rather than measuring by how much observations differ from the mean.

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## B-156

### Reference interval for fasting insulin and HOMA-IR indices in healthy adults of Rio de Janeiro.

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**Background:** Insulin resistance (IR) is considered the cause of metabolic syndrome (MS) and is associated with increased risk for several high prevalent pathologies such as type 2 diabetes mellitus, cardiovascular disease, and non-alcoholic liver disease. Insulin and especially homeostatic model assessment of insulin resistance (HOMA-IR) index are relatively simple and reliable noninvasive markers of IR. This study aimed to determine the reference interval (RI) of fasting insulin (using Roche-Cobas platform) and HOMA-IR indices in adults living in the state of Rio de Janeiro - Brazil and to verify if there was any difference in RIs between sexes.

**Methods:** Fasting serum insulin levels of 204388 subjects submitted to blood sampling from January to December of 2019 in the state of Rio de Janeiro were obtained retrospectively through access to a big database of a Brazilian laboratory. Insulin was determined by the electrochemiluminescence immunoassay (ECLIA) method, using Roche Diagnostics kits and the Roche/Hitachi Cobas e-411 analyzer (GmbH, Mannheim, Germany). After applying exclusion criteria, 31780 subjects [26628 (84%) women]  $\geq 20$  years were included (average  $42 \pm 14.5$  and range 20 to 99 years).

**Results:** Overall, 95% RIs for fasting insulin levels were, respectively, 2.46-12.97, 2.34-12.58, and 2.44-12.89  $\mu\text{U/mL}$  in women, men, and the total population. HOMA-IR index RIs were, respectively, 0.38-2.88, 0.34-2.85, and 0.37-2.88 in women, men, and the total population. Although the difference in insulin levels and HOMA-IR index between men and women were statistically significant, it does not justify using sex-specific RIs.

**Conclusion:** Since the evaluation of our data showed no clinical significance for sex-specific RIs for fasting insulin and HOMA-IR index, we conclude that separate RIs for men and women are not necessary. Reference intervals of fasting insulin and HOMA-

IR index found in the total population can therefore be applied for both sexes. Thus, we suggest the following RIs for our population: fasting Insulin: 2.44-12.89  $\mu\text{U/mL}$  and HOMA-IR index: 0.37-2.88.

## B-157

### Development and Verification of Age-Specific Reference Intervals for Serum Methylmalonic Acid Using Population based data Analysis

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**Background:** Methylmalonic acid (MMA) is an intermediate metabolite in succinic acid biosynthesis from propionic acid in the catabolic pathways of isoleucine, valine, threonine, methionine, thymine, uracil, cholesterol, and odd-chain fatty acids. Methylmalonyl-CoA mutase (MCM) catalyzes the conversion of methylmalonic acid to its structural isomer succinic acid using adenosyl-cobalamin as a cofactor. Isolated methylmalonic aciduria is an inborn error of metabolism caused by mutations in the MCM gene, or defects in the transport or biosynthesis of adenosyl-cobalamin. Serum MMA is used to diagnose and monitor patients with methylmalonic aciduria and is a better test to assess functional B12 deficiency than B12 levels alone. In our laboratory, we have observed a pattern of higher serum MMA levels in newborns (<1 year) and geriatric patients (60 years and above) compared to the rest of the population. Although there have been previous studies looking at age-specific MMA reference intervals, they have been limited due to relatively small sample sizes and have not covered the entire age range in detail to account for subtle trends in age-related changes in MMA. Ours is the most comprehensive analysis, particularly with respect to the pediatric sample set. This study aimed to establish age-specific MMA reference intervals utilizing historical data from Quest Diagnostics.

**Methods:** Age-related reference intervals were determined using historical data from serum MMA analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS) and a multi-modal decomposition (MMD) method, developed by Quest. MMD is an iterative method capable of decomposing the one-dimensional normal mixture model into multiple components of normal distribution using the expectation-maximization (EM) algorithm. The reference intervals were verified using Quest's employee health screening data and by testing deidentified specimens from patients who were previously tested for plasma amino acids or serum apolipoprotein A-1 and who had normal B12 and total homocysteine levels.

**Results:** Analysis of historical data from the last several years on more than 1,500,000 specimens predicted 6 age-specific reference intervals, including 0-30 days: 87-580 nmol/L; 1 month - 0.99 Year: 62-377 nmol/L; 1 year - 1.99 years: 55-347 nmol/L; 2 years - 59.9 years: 55-335 nmol/L, 60 years - 79.9 years: 69 -390 nmol/L,  $\geq 80$  years: 85 - 423 nmol/L. Employee health screening results and retested patient specimens fell within these age-specific ranges.

**Conclusion:** These age-specific MMA reference intervals underscore the usefulness of historical data and the population-based data analysis approach in establishing or refining normal reference intervals. When there are age-related differences in normal biomarker levels, more specific reference intervals can aid in the diagnosis and management of disorders where elevated biomarker levels are observed, such as methylmalonic aciduria.

## Hematology/Coagulation

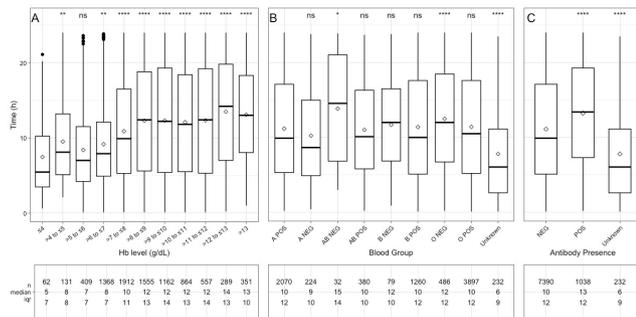
## B-159

### Time interval from hemoglobin result to red blood cell transfusion initiation as a metric for patient impact during a prolonged blood shortage

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**Background:** Supply of red blood cells (RBCs) is currently constrained by COVID-19 related impacts to blood collection. Nationally on average, RBC demand exceeds available supply, but the impact of the shortage on patient care outcomes has been difficult to measure. Established metrics, e.g., hospital use of backup suppliers and self-reported shortages, may not capture the total impact. We propose that the time interval from hemoglobin (Hb) result to RBC transfusion initiation may be a useful metric, in near real-time, to evaluate the impact of RBC shortages. **Methods:** Data extracts covering January 2020 to January 2022 including Hb results 24 hours prior to an RBC transfusion, from both inpatients and outpatients, accompanying RBC unit

information and bed side transfusion data were merged. Time from Hb result to initiation of RBC transfusion was calculated and later stratified by Hb, blood group, and antibody screen results. Generalized linear model and Wilcoxon Rank-Sum test were used for statistical assessment (R 4.0.5). Results: The time from Hb result to initiation of RBC transfusion was dependent on the Hb result ( $\beta=0.582$ ,  $p<0.0001$ ). Intervals ranged from a mean of 7.5 hours (SD: 5.5) for patients with Hb $\leq 4$  g/dL to 13.1 hours (SD: 6.3) for patients with Hb $>13$  g/dL. Positive antibody screen and AB neg and O neg blood groups were associated with modest delays to transfusion (all  $p<0.05$ ). Conclusion: Among pediatric patients with a Hb result who were ordered a RBC transfusion, Hb concentration, blood group, and antibody screen results influenced the time to transfusion. In addition to providing a near real-time assessment of patient impact, this proposed metric may help determine inventory requirements for blood banks and to set quality metrics for hospitals. Figure: Time to start an RBC transfusion in relation to Hb result(A), blood group(B) and antibody presence(C). ns:  $p>0.05$ ; \*:  $p\leq 0.05$ ; \*\*:  $p\leq 0.01$ ; \*\*\*:  $p\leq 0.001$ ; \*\*\*\*:  $p\leq 0.0001$



**B-160**

**Pediatric reference interval establishment for 79 hematology parameters on the Mindray BC6800 Plus System in the CALIPER cohort of healthy children and adolescents**

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**Background:** Hematological parameters vary significantly throughout growth and development due to physiological processes such as fetal-to-adult erythropoiesis and puberty. Pediatric age- and sex-specific reference intervals (RIs) are thus needed for appropriate clinical decision-making. Critical gaps continue to exist in pediatric hematology reference intervals for modern laboratory platforms, increasing the risk of diagnostic error.

**Methods:** 687 children and adolescents from birth to 18 years of age were enrolled in the study upon informed consent. Participants were identified from apparently healthy and metabolically stable outpatient clinics at The Hospital for Sick Children (Toronto, CA). Children and adolescents were also recruited from the community as part of Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER). Exclusion criteria included: pregnancy, history of chronic illness, acute illness within one week of collection, and regular prescribed medication use. Whole blood was collected in KEDTA vacutainers and analyzed for 79 hematology parameters, including erythrocytes, leukocytes, platelets, and research use-only parameters, on the Mindray BC6800 Plus System within 8 hours of collection. Reference intervals were established as per Clinical and Laboratory Standards Institute EP28-A3c Guidelines.

**Results:** Dynamic reference value distributions were observed for several hematology parameters. Age partitioning was required for 58 parameters, demonstrating marked concentration changes in early infancy and puberty. Sex partitioning was required for 11 erythrocyte parameters, demonstrating significant elevations in males relative to females from 14-19 years. Select parameters demonstrated no age- or sex-dependency (e.g. basophil count, high fluorescent count). Few parameters were undetectable in the pediatric population, including nucleated red blood cell count and immature granulocyte and eosinophil count.

**Conclusion:** The current study established reference standards for 79 hematology parameters on the BC6800Plus system in a healthy cohort of Canadian children and adolescents. To our knowledge, this is the first study to report health-associated pediatric concentration profiles for novel research parameters, including neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio. These data emphasize the complex biological patterns of hematology parameters in childhood necessitating age- and sex-specific reference intervals for evidence-based clinical interpretation.

**B-161**

**Post-Partum HELLP Triggers Complement-Mediated Thrombotic Microangiopathy**

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**Background:** The cause of acute kidney injury in the postpartum period can be particularly challenging to diagnose, especially when it is necessary to differentiate among preeclampsia; eclampsia; hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome; and thrombotic microangiopathies (TMAs). Various conditions and disorders can present with kidney failure, microangiopathic hemolytic anemia, and thrombocytopenia. This teaching case study highlights the interactions and impact of a DCLS, pathology resident, an interprofessional health care team and different diagnostic management teams.

**Case Presentation:** Day 1: A 24-year-old G2P1001 woman was admitted to our facility for induction of labor at 37 weeks gestation due to variable deceleration at term. She reported a 2-day history of lower abdominal and pelvic pain with dizziness and numbness. At admission, she denied vaginal bleeding or fluid leakage and had normotensive pressures. She had a previous diagnosis of gestational hypertension in her previous pregnancy. Pitocin was started on admission day.

Day 2: The patient underwent an uncomplicated, spontaneous vaginal delivery. On postpartum day 1, shortly after delivery, the patient had episodes of somnolence together with increased vaginal bleeding. Uterotonics were administered together with 1 gram of tranexamic acid. A bedside ultrasound showed no evidence of retained placental products. A cervical laceration was identified, and the patient was taken to the operating room for evaluation. Posterior cervical laceration was identified and repaired.

In the operating room intermittent periods of uterine atony with bleeding were observed. A massive transfusion protocol was initiated, and a second dose of tranexamic acid was administered. A hypotonic uterus was discovered during laparotomy. Bilateral uterine arteries were ligated, with no immediate result. A supra cervical hysterectomy was performed. Bedside viscoelastic testing showed profound coagulopathy, confirmed with fibrinogen levels of 50 mg/dL. The patient developed severe thrombocytopenia, hemolytic anemia, elevated liver enzymes, and acute kidney injury. Her estimated blood loss at the time was 2 liters. The degree of the coagulopathy was out of proportion to the bleeding observed raising the possibility of disseminated intravascular coagulation from an atypical amniotic fluid embolism or placental abruption. A total of 10 RBC, 3 FFP, 4 platelets, and 6 cryoprecipitate units were transfused. Transfusion medicine recommendations included continual hematology and coagulation studies. The patient developed acute kidney injury.

Day 3: Laboratory studies revealed serum creatinine of 2.42 mg/dL, hemoglobin 5.6 g/dL, lactate dehydrogenase (LDH)  $>6,450$  U/L, serum aspartate aminotransferase 114 IU/L, total bilirubin 2.1 mg/dL, platelet count  $64,000/mm^3$ , and undetectable haptoglobin levels. Peripheral smear revealed marked schistocytosis. The patient's condition and laboratory values continued to worsen.

Day 4: After four cycles of therapeutic plasma exchange, her laboratory values started to improve.

**Methods:** A differential consult and two DMT consults were requested for hemostatic evaluation and transfusion medicine. ADAMTS13 levels were sent prior to plasma exchange. After consult review, a kidney biopsy was performed and C3 and C4 levels were also ordered.

**Conclusion:** Pregnancy can unmask an underlying predisposition to TMA. This case illustrates the necessity of interdisciplinary diagnostic stewardship for the prompt and accurate diagnosis of complement-mediated TMA.

**B-162**

**Quality Utilization in Laboratory Testing: Quality Improvement in Disseminated Intravascular Coagulation**

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**Background:** Disseminated intravascular coagulation is a clinico-pathological disorder requiring the integration of both clinical and laboratory information for accurate diagnosis. This study aimed to evaluate the ordering behaviors of providers that have patients presenting for laboratory evaluation of DIC before and after DCLS-led PRIA, the review rate of DIC interpretations, and assess the impact of DIC interpretations on clinician ordering practices.

**Methods:** A retrospective, quasi-experimental study was performed and included data from DIC panel requests analyzed from Brooke Army Medical Center's hematology laboratory. The study aimed to evaluate PRIA interventions using non-equivalent, but similar groups of patients where one group received DCLS-led PRIA-based interpretation and the other received pathologist-interpretation (pre-PRIA intervention).

**Results:** 559 DIC were reviewed between March 2020 and December 2020. No statistical difference ( $p = 0.418$ ) in the mean number of days it took clinicians to review DIC interpretations between the pre-DCLS led PRIA intervention and the post-DCLS led PRIA intervention groups. A statistical difference ( $p = 0.003$ ) was noted in the number of replicate within DIC panel coagulation tests ordered at the same time a DIC panel was ordered, where number of replicate within DIC panel tests increased in the post-DCLS led PRIA intervention.

**Conclusion:** Findings showed that clinicians who received DCLS led PRIA interpretations ordered replicate within DIC panel coagulation tests more often. To improve laboratory DIC testing, the researcher suggests critical evaluation of how interpretations are delivered to health care providers and re-evaluation of panel data for interpretation.

## B-163

### Coagulation factor VIII enhances degradation of von Willebrand factor *in vivo*

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**Background:** Von Willebrand factor (VWF) and coagulation factor VIII (FVIII) circulate as a noncovalent complex, but each has a critical and unique function in maintaining normal hemostasis. It has been well established that binding of FVIII to VWF prolongs the half-life of FVIII and modulates its immunogenicity. However, the biological effect of this binding to VWF homeostasis or proteolysis is not fully understood. Hence, we sought to determine whether binding of FVIII to VWF would alter plasma VWF multimer distribution and its antigen levels using a murine model in the absence or presence of ADAMTS13.

**Methods:** Recombinant ADAMTS13 (rADAMTS13) and B-domain deleted FVIII (rFVIII) were prepared from stably transfected HEK293 and BHK cells, respectively, and purified to homogeneity through ion exchange and/or affinity chromatography. rFVIII with or without rADAMTS13 was injected via tail vein into *wild-type* (WT), *Adams13<sup>+/+</sup>*, and *Adams13<sup>-/-</sup>* mice (CAST/EiJ strain). Blood samples were collected 7 days prior to injection (time=0) and 24 hours following FVIII ( $\pm$  rADAMTS13). Plasma VWF multimer and antigen levels were determined by agarose gel electrophoresis and ELISA, respectively.

**Results:** Our results showed that infusion of rFVIII at the dose of 40  $\mu$ g/kg (2- to 3-fold of normal) and 160  $\mu$ g/kg into WT and *Adams13<sup>+/+</sup>* mice resulted in a dramatic reduction in plasma VWF multimer sizes. Additionally, plasma levels of VWF antigen in WT mice decreased by 17% in the low-dose group and 24% in the high-dose group, respectively, 24 h following rFVIII infusion. Plasma levels of VWF antigen in *Adams13<sup>+/+</sup>* mice decreased by ~34%, regardless of rFVIII dosages. However, rFVIII infusion into *Adams13<sup>-/-</sup>* mice even at a high dose didn't result in any change in either plasma VWF multimer distribution or antigen levels, indicating that alteration of VWF homeostasis was mediated through ADAMTS13. Again, a simultaneous infusion of both rADAMTS13 and rFVIII into *Adams13<sup>-/-</sup>* mice resulted in similar changes in plasma VWF multimers and antigen levels even when plasma ADAMTS13 activity was only restored to 5-20% of normal. All mice tolerated well to rFVIII infusion and there was no major thrombotic event during weeks of follow-up.

**Conclusion:** Our results demonstrate that rFVIII might be useful as an adjunctive treatment for thrombotic thrombocytopenic purpura or any other arterial thrombotic condition associated with increased VWF and reduced ADAMTS13 activity. This study may be a paradigm shift towards our understanding of the pathophysiological role of FVIII.

## B-164

### Oxidative stress parameters are related with the progression of Myelodysplastic Syndrome patients and 5-Azacytidine treatment

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**Background:** Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematological disorders. A number of publications showed a heterogeneity of factors involved in the inadequate hematopoiesis, dysplastic hematopoietic cells, and bone marrow stromal defects. Actually, the evidence of the involvement of free radicals in a variety of hematological neoplasms, including MDS, is increasing. This previous oxidative condition observed in MDS patients led us to investigate the existence of a dysregulation of the endogenous antioxidant defense system. Also, we evaluated whether current treatment with 5-azacytidine (5-AZA) could affect oxidative stress parameters.

**Methods:** The study included 24 myelodysplastic syndrome (MDS) patients (12 males, 12 females; mean age of 70 years) of the Hospital Universitario Clínico San Cecilio, Granada. MDS patients were diagnosed on the basis of the World Health Organization (WHO-2016) classification. Peripheral blood samples were used to determine the activity of the endogenous antioxidant defense system (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx; and reductase, GRd, activities) and markers of oxidative damage (lipid peroxidation, LPO, and advanced oxidation protein products, AOPP). The statistical software GraphPad Prism version 6.0 for Windows was used for all analyses.

**Results:** MDS patients showed less redox status based on a decrease in the GSSG/GSH ratio and in the LPO levels, as well as an increase in the CAT activity compared with healthy subjects, with no changes in the rest of antioxidants activities studied (SOD, GPx and GRd). However, when analyzing the evolution from early to advanced stages of the disease, we observed a tendency of the oxidative stress to increase, reflected by the slight increase in the GSSG-GSH<sup>-1</sup> ratio in advanced MDS stages; with an increased of LPO and AOPP levels. On the other hand, when we analyzed the effect of 5-AZA treatment on redox status, we found that 5-AZA increases oxidative stress in blood samples from MDS patients. We observed an increased of the erythrocyte GSSG-GSH<sup>-1</sup> ratio in the 5-AZA group, mainly dependent of the levels of GSSG; with a markedly reduced activity of CAT and an increased in the LPO values.

**Conclusion:** Alteration of redox status are an important factor involved in the development and progression of MDS. On the other hand, 5-AZA treatment generates oxidative stress in MDS patients. In this line, use of antioxidants with 5-AZA could improve the response of the patients to the treatment.

## B-167

### Precision of HemoSonics Quantra® QStat® Cartridge to Determine Coagulation Function and Fibrinolytic Status of Samples Assessed at 3 Locations within a Medical Center

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**Background:** The Quantra Hemostasis Analyzer and QStat Cartridge are components of a POC system for rapid viscoelastic measurement of key coagulation parameters including clot lysis. The system uses ultrasound to detect changes in clot stiffness in four separate cartridge channels. The QStat Cartridge measures clot time (CT), clot stiffness (CS), contribution of fibrinogen (FCS) and platelets (PCS) to clot stiffness, and clot stability to lysis (CSL).

**Methods:** Precision of one lot of QStat Cartridges was evaluated at 3 locations within one medical center using two Quantra analyzers run by one operator at each location. On each day of testing, 12 tubes of citrated blood were collected from one of 3 donors, with Donor 1 samples tested as collected, while Donor 2 and 3 samples had tissue plasminogen activator (tPA) added to achieve specific levels of fibrinolysis. 2 tubes were tested on each Quantra analyzer. Each donor was tested on 5 days, for 60 total results (180 total for all donors). Locations were sites where the QStat System might be operated: a clinical research laboratory; a POC area; and a clinical laboratory servicing operating rooms, intensive care units and an emergency department. Operators represented a range of experience including a nurse, a POC technician, and a certified medical laboratory scientist.

**Results:** Total imprecision was < 7.0% CV for Donor 1 and <10.0% CV for Donor 2 for all parameters. For Donor 3 total imprecision was < 11.0% CV for all parameters

except CSL, which had standard deviation <10.0%. Repeatability accounted for more than a third of total imprecision as determined by variance component analysis. Analyzer, site, and day represented smaller sources of imprecision.

**Conclusion:** The QStat Cartridge provides precise measurements of 5 coagulation parameters including clot lysis when used in a range of clinical setting by operators with varying experience.

Parameter	N	Mean	Total Imprecision SD (%CV)	Variance Component Analysis (% of contribution to total imprecision)					Total
				Repeatability (Replicate)	Between-Day	Between-Analyzer	Between-Site	Within-Site	
<b>All Locations- Donor 1 (unspiked)</b>									
CT (sec)	60	148.70	7.06 (4.75)	61	23	0*	12	4	100
CSL (%)	60	98.38	1.37 (1.39)	95	3	0*	2	0*	100
CS (hPa)	60	21.33	1.42 (6.66)	45	12	20	23	0*	100
PCS (hPa)	60	19.48	1.42 (7.30)	43	7	20	29	0*	100
FCS (hPa)	60	1.85	0.17 (9.14)	36	42	0*	22	0*	100
<b>All Locations- Donor 2 (tPA spiked to CSL threshold)</b>									
CT (sec)	60	129.62	9.22 (2.89)	84	16	0*	0*	0*	100
CSL (%)	60	85.07	5.47 (6.44)	43	27	0*	26	4	100
CS (hPa)	60	13.95	0.92 (6.61)	56	7	15	12	10	100
PCS (hPa)	60	12.75	0.94 (7.35)	53	6	15	14	12	100
FCS (hPa)	60	1.20	0.11 (9.15)	45	0*	0*	54	0*	100
<b>All Locations- Donor 3 (tPA spiked to below CSL threshold)</b>									
CT (sec)	60	132.80	7.86 (5.92)	78	3	0*	0*	20	100
CSL (%)	60	21.97	9.35 (42.56)	89	8	0*	0*	3	100
CS (hPa)	60	24.38	1.69 (6.92)	65	1	17	17	0*	100
PCS (hPa)	60	22.25	1.71 (7.69)	61	2	18	19	0*	100
FCS (hPa)	60	2.13	0.22 (10.21)	94	0*	1	5	0*	100

\*Study was developed with reference to CLSI EP05-A3, Third edition. Variance component analyses were done using the anovaVCA function from VCA library in R Version 4.0.2. Negative variance component estimates were reported as zero, and the zero value was used to calculate the total variance. The variance model included random terms for between-sites, between-analyzer, within sites, between-days, and error (between replicates) variance.

## B-168

### Validation of Blood Gases and Co-Oximetry Parameters Using the ABL™ 80 Flex Co-Ox Analyzer

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#### Background

The objective of this study is to validate the blood gases and co-oximetry parameters for Monkey, Mice, Rat, Rabbit and Dog species using the ABL™ 80 flex co-ox analyzer.

#### Methods

A total of four blood gases (pH, pCO<sub>2</sub>, pO<sub>2</sub> and cCa<sup>2+</sup>) and five co-oximetry (ctHb, sO<sub>2</sub>, FO<sub>2</sub>Hb, FCOHb and FMetHb) parameters were validated in animal species Monkey, Mice, Rat, Rabbit and Dog. Validation testing including intra-assay, inter-assay, accuracy, linearity and reference interval were performed. Blood samples for evaluation were collected in dry sprayed lithium heparinized syringe or tube. For intra-assay, blood samples from species and four level of control (Qualicheck5+ Level 1, Qualicheck5+ Level 2, Qualicheck5+ Level 3, and Qualicheck5+ Level 4) were analyzed for 10 consecutive times (one replicate) within a single run for each parameter. For inter-assay precision, four level of control were assayed on at least six different runs (one replicate per run) over a minimum of three days. Accuracy was calculated us-

ing the data obtained from inter-assay. Linearity was performed using the four level of quality control. Finally, reference interval for each parameter was calculated by analyzing at least ten different samples for each species.

#### Results

Results show that the blood gases and co-oximetry parameters met the acceptance criteria for intra-assay, inter assay, accuracy and linearity for all four species. For intra-assay, all parameters had coefficient of variation (% CV) less than or equal to 25% for all species. For inter-assay and accuracy, all parameters had coefficient of variation (% CV) less than or equal to 25% for all species. All parameters for all four species were linear because % nominal was in between 75-125 %, slope was within 0.75-1.25 and coefficient of determination (r<sup>2</sup>) was less than or equal to 0.9. Additionally, reference interval for all parameters were measured.

#### Conclusion

All blood gases and co-oximetry parameters met the acceptance criteria for each qualification testing for all five species: Monkey, Mice, Rat, Rabbit and Dog. Since, all these parameters were validated for all five species, ABL-80 Flex Co-Ox analyzer can be used for preclinical studies.

## B-169

### An Algorithm to Predict COVID-19 Positivity Using Hematology Data from the ADVIA 2120i Hematology System

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**Background:** Detection of COVID-19 can sometimes be challenging due to shortages of direct testing methodologies such as RT-PCR or rapid antigen tests. In such scenarios, tests on other commonly available instrument platforms such as hematology analyzers could be deployed to help. Hematology testing is generally done before any suspicion of disease, and the data generated represents host-response. Analysis of multiparameter hematology datasets can easily be accomplished using computational techniques such as machine learning (ML), allowing for an understanding of specific host-response hematology signatures and an opportunity to signal the likely presence of COVID-19 infection. We have developed a multiparametric ML algorithm to flag COVID-19 in patients using data from the Siemens Healthineers ADVIA® 2120i Hematology System. Since all parameters for a hematology-based algorithm are produced by the same instrument, it obviates the need to gather additional test data and thereby provides a robust alternate method to detect likely COVID-19 infection. **Methods:** Positive and negative anonymized patient datasets were obtained from a 2020 pre-vaccinated single-center cohort in Mumbai, India, with a total of 309 COVID-19 positives and 245 COVID-19 negatives as confirmed by RT-PCR. Parameters were pruned based on significance tests between positives and negatives and other clinical and instrument-specific considerations. After we identified a set of 48 parameters, we used ML to train classification models with 5-fold cross-validation to distinguish positives and negatives. We used 80% of the data for training the models and the remaining 20% as the test set.

**Results:** We developed multiple ML models. The best predictive model was an ensemble of decision tree-based methods that had a training accuracy of 75.6% and a testing accuracy of 74%, with an AUROC of 0.798 with 48 parameters. Parameters such as eosinophils and lymphocytes that have already been identified in the literature were observed to be important. We also found some RBC-related parameters to be important in distinguishing positives and negatives. Hospitalized patients were predicted positive more accurately (92% correctly classified) than non-hospitalized patients (70% correctly classified) likely because of more severe symptoms in the hospitalized individuals. **Conclusion:** We have demonstrated the opportunity to use data from routine hematology analyzers to build an algorithm for flagging COVID-19. Such an algorithm could be used for different pandemic and endemic applications. We envision use of this algorithm to flag patients for RT-PCR testing during the pandemic or even for positive calling in locations with low RT-PCR penetration. The algorithm can be used at high sensitivity to reduce total RT-PCR testing volume by eliminating RT-PCR testing in negative individuals. As an endemic use case, the algorithm can be used to call unsuspected positives at high specificity and flag them for RT-PCR testing.

**B-170****Significance of 5-19% Circulating Plasma Cells**

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**Background:**

Plasma cell leukemia (PCL) is a neoplasm characterized by malignant plasma cells circulating in peripheral blood. It can occur as a de novo disease or as a progression of multiple myeloma. Both primary and secondary forms have a dismal prognosis, so early detection is essential to initiating timely therapy. In 2021 the International Myeloma Working Group has reduced the % of plasma cells in peripheral blood required for the diagnosis of PCL from  $\geq 20\%$  to  $\geq 5\%$ . We performed a retrospective study to see how many results of PCs 5-19% and if PC% in this range were seen in patients without plasma cell neoplasms.

**Methods:**

Peripheral blood differential counts from 11/01/2019-11/01/2021 with plasma cells reported as part of the differential were reviewed. The electronic medical record was searched to determine the patients' underlying medical conditions and clinical status.

**Results:**

387 differentials were reported with plasma cells during these two years, with PC% ranging from 0.5%-96%. 93 patients had more than one instance of plasma cells reported. There were 173 results with plasma cells 5-19%, which were from 38 individual patients. There were 128 results with plasma cells  $\geq 20\%$  from 23 patients. Among patients with a maximum PC% between 5% and 19%, 18 (48.6%) had multiple myeloma and would now be considered secondary PCL. 19 (51.4%) patients had no history of a plasma cell neoplasm, and typically had other malignancies or infections (one patient had no history available). 16 (43.2%) patients in this category had expired while 21 (56.8%) were alive. Among the patients with PC%  $\geq 20\%$ , ten has primary PCL, 11 had secondary PCL, and 2 had no history of a plasma cell neoplasm (on with Steven Johnson syndrome and one with diffuse large B-cell lymphoma. 18 (78.3%) of these patients had expired while 5 (21.7%) were alive.

**Conclusion:**

PC% of 5-19% is seen rarely, but can be seen in reactive conditions as well as in patients with plasma cell neoplasm.

**B-171****Evaluation of Hemoglobin Variants on the Abbott Alinity c HbA1c Assay**J. H. Nichols<sup>1</sup>, M. Berman<sup>2</sup>, A. Carrillo<sup>2</sup>, S. Manning<sup>1</sup>. <sup>1</sup>Vanderbilt University Medical Center, Nashville, TN, <sup>2</sup>Abbott Laboratories, Abbott Park, IL

**Background:** The interaction of the more than 1000 hemoglobin variants on the HbA1c assay performance has not been well studied. In this study, we evaluated the impact of hemoglobin variants on the performance of the Abbott Alinity c and Biorad Variant II Turbo HPLC HbA1c Assays.

**Methods:** The analytical performance of the Abbott Alinity c HbA1c (enzymatic) assay was compared to the Bio-Rad Variant II Turbo HPLC method using leftover whole blood EDTA samples with and without the presence of a hemoglobin variant. Assay precision was determined from an analysis of controls. Bias was estimated from analysis of an NGSP panel set of 40 samples.

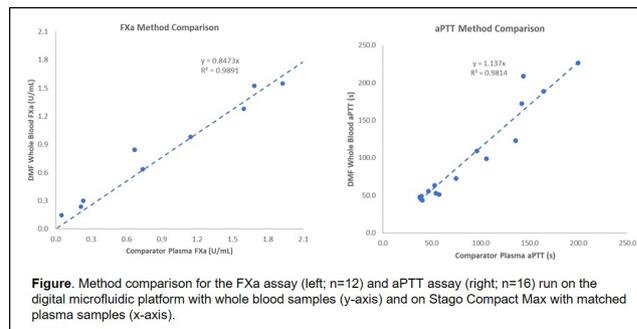
**Results:** Precision was excellent for both methods ( $<3\%$ ). Bias for both the Alinity and Bio-Rad methods met NGSP criteria of  $\pm 5\%$  to target value for  $>90\%$  of the 40 sample set in the range of 5 - 10% HbA1c results. Correlation between the Alinity and Bio-Rad methods was good for patient samples without a hemoglobinopathy ( $y = 1.028 - 0.38$ ,  $SEE = 0.16$ ,  $N=36$ , mean bias  $-0.22$ ). A total of 700 hemoglobin variant samples were evaluated on the two methods. 640/700 of the hemoglobin variants gave results on both methods: hemoglobin (Hb) S trait ( $N=452$ ), C trait ( $N=131$ ), D trait ( $N=23$ ), E trait ( $N=26$ ) and a mixture of other abnormal hemoglobinopathies ( $N=8$ ) including Beta thalassemia, high HbF, transfused HbSc, HbSD, and HbSS, or unknown variant. There was good overall agreement for the 640 hemoglobin variants between the methods with a mean difference of  $-0.10$  to  $+0.06$  depending on the variant, but more variability ( $SEE 0.25 - 0.39$ ). Sixty samples could not be compared because of either a low HbA<sub>0</sub>  $<40\%$  or elevated hemoglobin variant  $>40\%$  by HPLC. The Alinity c HbA1c would have reported 35/60 results where the HPLC method flagged the sample to suppress the HbA1c result.

**Conclusion:** To our knowledge, this study was the largest investigation of the effect of hemoglobinopathies on the Abbott Alinity c HbA1c assay. The Alinity c HbA1c assay

demonstrated excellent precision and bias. Analytical performance varied depending on the specific hemoglobin variant trait when compared to the Bio-Rad Variant II Turbo HPLC method.

**B-172****Multifunctional Platform for Viscoelastic aPTT and Fluorometric FXa Whole Blood Testing in Near-Patient Settings for Pediatric Patients on Heparin Therapy**M. Basmajian<sup>1</sup>, A. Ullal<sup>1</sup>, L. Nichols<sup>1</sup>, M. Moser<sup>1</sup>, C. Graham<sup>1</sup>, S. Adhikari<sup>1</sup>, R. Sista<sup>1</sup>, S. Emami<sup>2</sup>, S. Emami<sup>2</sup>, V. Pamula<sup>1</sup>. <sup>1</sup>Baebies, Inc., Durham, NC, <sup>2</sup>Boston Children's Hospital, Boston, MA

**Background:** Current heparin monitoring tests for Factor Xa activity (FXa) and activated partial thromboplastin time (aPTT) require frequent and large volumes of blood and can lead to iatrogenic anemia in neonatal and pediatric patients. We describe two novel assay methods using digital microfluidics (DMF) to perform simultaneous tests for FXa and aPTT directly from low whole blood volumes ( $<50 \mu\text{L}$ ) for rapid, near-patient monitoring of heparin in children. **Methods:** DMF enables electronic control of microliter droplets. All required reagents are dried within the cartridge. For FXa assay, whole blood is separated into plasma on the cartridge and the rate of fluorescence is inversely proportional to the amount of heparin. For aPTT assay, an activator initiates clotting in a shuttling whole blood droplet, changing its viscoelastic properties, which is then measured electronically as a change in droplet velocity and clotting time. Preliminary precision was obtained on the DMF platform with FXa commercial controls ( $n=13$  for FXa at 0.23 U/mL) and with commercial whole blood samples spiked with unfractionated heparin ( $n=12$  for aPTT at 54s). A preliminary method comparison study was performed using whole blood samples sourced from a commercial vendor on DMF platform and with matched plasma samples on Stago Compact Max. **Results:** For precision, we observed an assay coefficient of variation (CV) of 4.68% for FXa and 1.87% for aPTT. Both assays on the DMF platform correlate well to comparator assays, with an  $R^2$  value of 0.99 for FXa ( $n=12$ ) and 0.98 for aPTT ( $n=16$ ). **Conclusions:** Two novel multifunctional assays for FXa and aPTT performed on a DMF cartridge, using microliter reaction volumes, are suitable for frequent testing. Preliminary method comparison from whole blood correlates well with comparator methods, indicating that heparin monitoring is feasible on a point-of-care device; the clinical utility of this technology needs further investigation.

**B-174****Discrepancy of immature granulocyte (IG) counts in transplant patients by automated white blood cell (WBC) differential analysis of Sysmex XN-10 analyzer**

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**Background:** Sysmex (Hyogo, Japan) XN-10 automated hematology analyzer performs WBC auto differential analysis based on the principle of flow cytometry. Its white blood cell differential (WDF) channel can differentiate 10 cell types using side fluorescence and side scatter. Increased immature granulocytes (IG) such as promyelocytes or myelocytes can indicate presence of severe infection/inflammation, or hematological malignancy such as leukemia. In our clinical laboratory, we observed several samples with high IG count by the Sysmex XN-10 auto differential mode and found discordant results when they were further analyzed by a morphologic review.

**Methods:** Whole blood (EDTA-lavender top) samples were tested on the Sysmex XN-10 analyzer to perform complete blood count and WBC auto differential analysis. Sysmex WAM (work area manager) middleware software communicates with our LIS that was programmed with multiple set of rule cut-offs for various cell types,

that are applied to quality check of results before they are sent to LIS. The Sysmex SP-10 module is utilized for automated slide preparation and staining by Wright stain. Samples with Immature granulocytes (IG) >5% are flagged and the results are held in the WAM until verified. Flagged results are confirmed by a morphologic review with CellaVision DI-60 microscopy method and further verified by a manual slide review when discrepant. **Results:** We identified 24 samples that showed discrepancy within a week with abnormal IG > 5%. The IG % ranged from 5.3-14.7%. Morphologic review revealed no IG present in 19 (out of 24) samples and the rest had either 1 or 2 % of IG. Some of the morphologic features noted in those samples include neutrophils with hypo segmentation, hyper segmentation, Döhle bodies and toxic granulation. Reviewing the electronic medical records (EMR) of these patients revealed that a majority (20 out of 24; 83%) of patients have undergone various organ transplants such as heart, lung, kidney, liver and are on immunosuppressant therapy. The other four patients were under chemotherapy for various malignancies such as lymphoma and carcinoma. **Conclusion:** The noted discrepancy in IG% from auto differential and morphological analysis could be related to the immunosuppressant therapy. Immunosuppressant drugs such as tacrolimus (FK506), cyclosporine or prednisone are known to affect hematopoiesis. The identification of increased IG content by automated method in those under immunosuppressant therapy could be due to alteration in the cellular nucleic acid content that affects flow cytometry analysis. A large number of IG discrepant samples can affect laboratory efficiency such as increased turnaround time, more manual labor and adjusting the WAM middleware rules for Sysmex XN-10 system auto differential module can be considered to improve the efficiency.

## B-175

### Creating a Drive-Through Emergent Need INR Testing Site

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#### Background:

To decrease both patient and healthcare provider exposure risk during the COVID-19 pandemic, we developed and rapidly implemented a drive-through INR monitoring program in 2020 for patients requiring coagulation testing to monitor Warfarin.

#### Methods:

Our institution set up a drive-through INR testing tent at one of our satellite clinics using the CoaguChek Pro (Roche Diagnostics, Indianapolis, IN). The tent was designed to accommodate 2 cars at a time and utilized mobile carts to store testing supplies and mobile testing tables with variable height settings. The tent was set up in April 2020 and was in operation for five months, ending in August 2020, so temperature and humidity were a concern. Roche recommendations indicate the CoaguChek Pro should be used at temperatures between 15-32°C. To mitigate potential low temperature issues, ambient heat was generated and pumped into the tent. Insulated coolers with heating or cooling packs were also used. Devices were routinely rotated between the outside testing tent and the indoor downloaders to ensure downloading of results in a timely manner. This model required testing personnel and support staff to rotate devices and screen patients for COVID-19 symptoms prior to testing. After results downloaded from the devices, a team of nurses would call or virtually meet with the patient for their Warfarin management. Outcome measures included error rate for point of care (POC) INR testing before and after the drive through model was adopted; and informal feedback from patients and staff on the effectiveness of the model.

#### Results:

In the five months prior to the tent model when POC INR testing occurred inside a primary care clinic, there was one error in 6,345 tests equating to a 0.02% error rate. Over five months of testing in the tent model, there were nine errors out of 5,312 tests (0.17% error rate). The nine errors were ordering errors that occurred on the first day of testing. Excluding those errors, there was no difference in the error rate for POC INR testing in the tent model compared to testing in the primary care clinic. Informal feedback from patients indicated a high level of patient satisfaction with the tent model. Patients highlighted: familiarity with appointment location, ability to remain in warm vehicle during cold weather, faster TAT of testing process, and general ease of access for testing. Informal feedback from testing staff (nurses) indicated some dissatisfaction with temperature (too cold or too hot), but overall satisfaction with the model meeting patient needs.

#### Conclusion:

Based on patient and staff experiences, utilizing a tent model during public health emergencies is a viable risk mitigation strategy. When evaluating the potential use of a drive-through tent model in your facility, consider the following: (1) Are you still meeting CLIA regulations? (2) Do you have enough staff? (3) Are environmental factors like temperature, precipitation, sun exposure, etc. a concern for equipment,

personnel, and patients? If yes, what mitigation factors can be employed? If you have worked through these considerations, you will have a strong alternative to traditional INR testing.

## B-176

### First pass efficiency of Alinity hq compared to Sysmex XN-10

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**Background** The first pass efficiency rate is an indicator that can demonstrate the quality of initial specimen sample analysis of a hematology analyzer. A high first pass efficiency rate ensures timely processing of clinical results without the need to reanalyze the sample, quick clinical decision making, saving resources, decreasing cost, and ultimately benefiting the care of patients. Alinity hq is equipped with Advanced Multi-Angle Polarized Scattered Separation (MAPSS) technology to provide accurate results the first time. The aim of this multi-site study was to compare the first pass efficiency rates of the Abbott Alinity hq hematology analyzer and the Sysmex XN-10 hematology analyzer. **Methods** First pass efficiency was evaluated between Alinity hq (investigational method) and Sysmex XN-10 (reference method) from a total of 4,001 whole blood specimen replicates collected from six clinical sites. First pass efficiency was calculated by dividing the number of results that did not require further testing, either reruns or reflex tests, by the number of total results generated during the method comparison study. **Results** The overall first pass efficiencies were 82.08% (1,726/2,208) and 67.81% (942/1,793) for Alinity hq and Sysmex XN-10, respectively. Five of the six sites demonstrated higher rates for Alinity hq than from Sysmex XN-10, with the sixth site displaying similar first pass efficiency rates. See Table 1 below for first pass efficiencies rates for Alinity hq vs. Sysmex XN-10 at the six clinical sites.

Table 1. First Pass Efficiency for Alinity hq vs. Sysmex XN-10

Site	Alinity hq			Sysmex XN-10		
	No. of Specimen Sequences with Invalidations	No. of Valid Specimen Sequences	First Pass Rate (%)	No. of Specimen Sequences with Invalidations	No. of Valid Specimen Sequences	First Pass Rate (%)
1	90	455	83.49	162	378	70.00
2	54	290	84.30	135	215	61.43
3	90	200	68.97	188	129	40.69
4	103	421	80.34	132	354	72.84
5	81	328	80.20	74	305	80.47
6	45	271	85.76	160	412	72.03
	19	243	92.75			
<b>Total</b>	<b>482</b>	<b>2208</b>	<b>82.08</b>	<b>851</b>	<b>1793</b>	<b>67.81</b>

**Conclusion** Overall, the Alinity hq hematology analyzer's first pass efficiency performance exceeded that of Sysmex XN-10. Alinity hq Advanced MAPSS technology allows for accurate results more frequently during first sample analysis than Sysmex XN-10. As demonstrated by this study, Alinity hq further exemplifies its state-of-the-art technology with excellent first pass efficiency results.

## B-177

### Morphological flagging of Alinity hq compared with Siemens Advia 2120 in a tertiary care pediatric hospital

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**Background** Alinity hq is a hematology analyzer which utilizes a combination of photometry, optical counting and fluorescence analysis to provide a 6-part WBC differential including immature granulocytes (IG) and nucleated red blood cell concentration (NRBC) with every CBC sample and displays up to twenty-nine measurands. The presence of abnormal cells generates a morphological flag by Alinity hq warranting a need for secondary inspection via manual count or slide review. The flags that the analyzer generates are Blast, Variant Lymphocytes, Left Shift, PLT Clumps and RBC Fragments similar to Siemens Advia 2120 hematology analyzer. The aim of this

study was to compare the morphological flagging rates of Alinity hq with Siemens Advia 2120 hematology analyzers in a tertiary care pediatric hospital. **Methods** Four hundred and twenty-nine remnant patient samples were tested from routine laboratory work and diagnosed pediatric oncology and hemoglobinopathy cases. The samples were analyzed using Alinity hq and comparative analyzer; Siemens Advia 2120. The morphological flags raised by Alinity hq for Blast, Variant Lymphocytes, Left Shift, PLT Clumps and RBC Fragments. [FF1] were recorded for each sample and compared to Siemens Advia 2120 analyzer's flags. **Results** Alinity hq flagging showed excellent agreement with Siemens Advia 2120. The agreement between the two analyzers portrayed Blast (cutoff at 5.0 for Advia 2120) 92.5%, Left shift 82.3%, RBC Fragments 96.5%, Platelet Clumps 98.6% and Variant Lymphocyte 68.8%. **Conclusion** Alinity hq flagging showed excellent concordance with Siemens Advia 2120. Slight variation is likely due to variation of technology and parameters used in flagging analysis especially with Variant Lymphocytes. However, flagging results between Alinity hq and Siemens Advia 2120 still demonstrated high level of agreement amongst the pediatric oncology and hemoglobinopathy patient population. As the flag results demonstrate, Platelet Clumps and RBC fragments show the highest level of equivalence, all above 96.5%.

## B-178

### Adherence to Clinical Practice Guideline for Platelet Factor 4-Heparin (PF4) Complex IgG Antibody Testing in Patients Suspected of Heparin Induced Thrombocytopenia

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**Background:** Thrombocytopenia caused by antibodies against platelet factor 4-heparin (PF4) complex is called heparin-induced thrombocytopenia (HIT). Current clinical guideline (Cuker et al. Blood Adv. 2018 Nov 27;2(22):3360-3392) recommends calculating a 4T score, a pretest probability parameter, before requesting PF4 IgG (HIT) antibody testing, and advise against ordering antibody testing on patients with a low pretest probability for HIT (4T score of 0-3), except in rare exceptions. The primary objective of this study was to assess if the 4T score was appropriately utilized as a first step in the diagnosis of suspected HIT before ordering the antibody test.

**Methods:** A retrospective analysis was performed on all patient cases from the year 2021, where a PF4 IgG antibody-screening assay (Solid Phase ELISA, IMMUCOR, Waukesha, WI) was ordered for suspected HIT. Medical records were reviewed to determine if a 4T score was calculated before requesting HIT antibody testing, and whether the score was used to determine the utility of HIT antibody testing. Medical records were also reviewed for patients who had positive or equivocal HIT antibody results to extract serotonin release assay (SRA, LabCorp) results, a confirmatory functional assay.

**Results:** In total, 259 patients were identified as having HIT antibody tests performed. A 4T score was documented in the medical chart in 71% (184/259) of these cases, of which 29% (54/184) had a low score, 59% (108/184) had an intermediate score, and 12% (22/184) had a high score. Only 8.5% (22/259) and 1.5% (4/259) patients had a positive or equivocal HIT antibody result, respectively. Of these patients with either positive or equivocal results, 7% (4/54) had low, 9% (10/108) had intermediate, 23% (5/22) had high 4T scores, and 9% (7/75) did not have a 4T score documented. SRA was ordered and performed in 62% (16/26) of cases with positive or equivocal results. SRA was either not requested or discontinued for unknown reasons in 38% (10/26) of patients. Of the patients who tested positive or equivocal for HIT antibodies, only 18% (3/16) were confirmed by SRA and three other were deemed true positive due to high optical density or known history of multiple positive HIT antibody screens. Of the patients with a low 4T score who tested positive or equivocal, 75% (3/4) were false positive by SRA. The other one patient had a known history of positive HIT antibody and SRA was never requested to confirm. Of all the patients whose 4T scores were not documented and then tested positive or equivocal for HIT antibodies, none were determined to be true HIT by SRA.

**Conclusion:** A 4T score was not documented in 29% of HIT antibody requests, and HIT antibody testing was performed on all patients regardless of the degree of pretest probability. These findings suggest that, by strictly adhering to the current clinical guideline, at least 21% of HIT antibody testing would have been avoided. The HIT antibody assay and SRA are time consuming tests that should be reserved for appropriate clinical situations, especially with the current supply chain issues and staffing shortages.

## B-179

### Improving the Interpretation of Viscoelastic Test Results in the Critical Care Setting

D. A. Winegar, C. Gillespie, M. Sanchez-Illan. *HemoSonics LLC, Durham, NC*

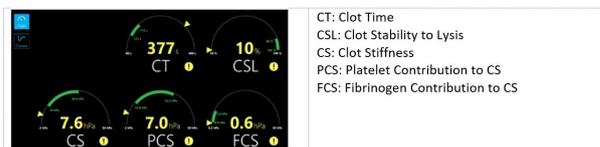
**Background:** The Quantra® System is a novel viscoelastic testing (VET) device developed to address barriers limiting the adoption of VET for guiding resuscitation of severe bleeding in critically ill patients. One barrier is the difficulty in interpreting results from existing VET devices. The Quantra System is a cartridge-based, fully automated system that was FDA-cleared for point-of-care. The results are provided quickly, via intuitive dials display. The Quantra QStat® Cartridge is intended for use in trauma and liver transplant surgery. The QStat Cartridge outputs five parameters to assess for coagulopathies that may result from depletion of coagulation factors, platelet dysfunction, fibrinogen depletion, and/or hyperfibrinolysis (see Figure below). The objective of this study was to assess the ability of potential QStat Cartridge users to correctly interpret test results.

**Methods:** Study participants included 10 medical professionals (anesthesiologists and surgeons) from 5 US institutions who regularly assess blood coagulation in critically ill patients. Participants represented a range of experience in their clinical specialty and most had experience with VET. A 30-minute virtual training session was conducted with the participants to introduce the QStat Cartridge. Participants were then presented with a series of results representing outcomes that may be observed in trauma and liver transplant settings. Participants completed a quiz to test their ability to interpret the results.

**Results:** Each participant viewed 12 QStat test results and answered a total of 131 multiple-choice questions. For all 5 of the QStat parameters, >97% of questions pertaining to each of the results and their clinical interpretation were answered correctly.

**Conclusions:** This study demonstrated that clinicians could be quickly trained to correctly interpret QStat test results presented via a simple intuitive results output. This suggests the potential for VET to be operationalized in critical care settings enabling hospitals to efficiently deliver goal-directed therapy to critically bleeding patients.

Example QStat Results Display:



## B-183

### Micro-mechanical PT/INR testing using smartphones

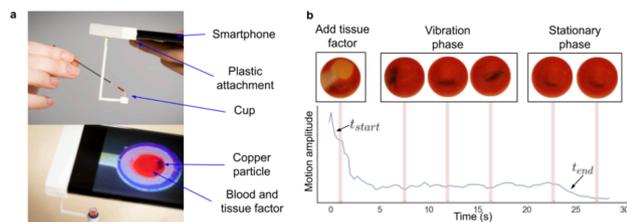
K. Michaelsen, J. Chan, J. Estergreen, D. Sabath, S. Gollakota. *University of Washington, Seattle, WA*

**Background:** Despite the increased use of non-vitamin K antagonist oral anticoagulants, frequent PT/INR testing remains critical for millions of people on lifelong anti-coagulation with warfarin. Currently, testing is performed in hospital laboratories or with expensive point-of-care devices limiting the ability to test frequently and affordably, particularly in low-resource environments. We present a proof-of-concept system using the vibration motor and camera on smartphones to track micro-mechanical movements of a copper particle in a drop of blood to determine PT/INR quickly and inexpensively.

**Methods:** A plastic attachment including a small sample cup was 3D printed and connected to the phone which directly transmitted the force from the vibration motor to the cup. Video processing algorithms were used to track the movement of the copper particle in the sample and determine when motion ceased due to the development of clot. This time was converted to PT/INR for anonymous residual plasma samples and whole blood samples including a subset from a variety of coagulopathic conditions.

**Results:** PT/INR inter-class correlation coefficients were > 0.96, for the smartphone compared to a clinical-grade coagulation analyzer for 140 anonymized plasma samples and 79 samples with coagulopathic conditions. PT/INR inter-class correlation coefficients > 0.93 for the smartphone for 80 whole blood samples tested against an FDA cleared home testing device. PT/INR inter-class correlation for sub populations of DIC, liver disease, warfarin therapy and trauma patients, containing between 18 and 38 samples were calculated and ranged from 0.88 for DIC to 0.98 for liver disease

**Conclusions:** This technology may provide affordable and effective PT and INR testing in low-resource environments. Further testing in a home environment for patients on warfarin is planned.



**Fig. 1** (a) Schematic of the system: a plastic attachment containing a cup with 10  $\mu$ l of whole blood, 20  $\mu$ l of tissue factor, and a copper particle. (b) The phone captures the motion of the particle starting from when the tissue factor is added to the blood ( $t_{start}$ ). The particle moves freely in the blood when it is in a liquid state. When the blood coagulates, the particle's motion reduces ( $t_{end}$ ).

## B-187

### Novel Quantitative Serology Platform for the Detection of COVID-19 Neutralizing Antibodies

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Veravas has developed the 7C process for the creation sensitive and specific antibody detection assays. The 7C process uses a novel conditioning step which removes interfering biomolecules that could interfere with sample detection — such as problematic heterophilic and autoantibody interference. Once the sample is clean, it undergoes both an antibody capture and concentration step allowing for the detection of antibodies in low abundance. This platform was used to develop a test to detect neutralizing antibodies against SARS-CoV-2 spike protein RBD and NTD in serum. The Veravas Serum COVID-19 Neutralizing Antibody Test demonstrated an overall 98.0% PPA (95% CI 87.8 - 99.9%) with patients who tested positive for SARS-CoV-2 using the EUA Roche cobas SARS-CoV-2 RT-PCR. The PPA was 93.3% (95% CI 66.0 - 99.7%) 0-7 Days after PCR Positive Result, 100% (95% CI 73.2 - 100%) 8-14 Days after PCR Positive Result, and 100% (95% CI 80.0 - 100%)  $\geq$  15 Days after PCR positive result, and the PPA was 100% (95% CI 39.6 - 100%) 0-7 Days Post-Symptom Onset, 93.8% (95% CI 66.7 - 99.7%) 8-14 Days Post-Symptom Onset, and 100% (95% CI 85.4 - 100%)  $\geq$  15 Days Post-Symptom Onset. The VERAVAS COVID-19 Neutralizing Antibody Test demonstrated 97.8% NPA (95% CI 93.2 - 99.4%) with samples collected prior to November 2019. The VERAVAS COVID-19 Neutralizing Antibody Test was used on the 37 NISBC Verification Panel serum samples and showed a sensitivity of 100% (95% CI 82.2 - 100%) and a specificity of 92.8% (95% CI 64.2 - 99.6). The clinical performance of the VERAVAS COVID-19 Neutralizing Antibody Test and SERUM COVID-19 Neutralizing Antibody Test Protocol was further assessed using the GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit (EUA201427) as the comparator method. The VERAVAS COVID-19 Neutralizing Antibody Test demonstrated 94.1% PPA (95% CI 82.8 - 98.5%) and 99.3% NPA (95% CI 95.3 - 100%) as compared against the *GenScript cPass Neutralization Antibody Detection Kit* (EUA201427). The VERAVAS COVID-19 Neutralizing Antibody Test IgG results (IU/mL) have also demonstrated a correlation to the GenScript cPass Neutralization Antibody Detection Kit ( $r = 0.87$ ,  $p < 0.001$ ). These studies indicate that the VERAVAS COVID-19 Neutralizing Antibody Test using the Veravas' novel antibody detection platform has created a sensitive and specific test for detecting neutralizing antibodies against SARS-CoV-2 spike protein RBD and NTD in serum.

## B-188

### Novel Salivary Sample Test for Covid-19 Neutralizing Antibodies

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Veravas has created a process branded "7C" to develop a highly specific and sensitive antibody detection platform. The "7C" process cleans and conditions sample, uses the clean sample to capture and concentrate the target, uses conjugate for detection, and cleaves the target. The platform achieves this high specificity by conditioning the sample which pre-analytically removes non-specific interferences, such as heterophilic antibodies and autoantibodies. Upon removal of interferences, targeted human immu-

noglobulins can be captured and measured using paramagnetic beads coated with biotinylated antigen(s) of interest. Using this technology, targeted immunoglobulins are subsequently concentrated, allowing for increased sensitivity. Utilizing the increased sensitivity and specificity advantages of the Veravas "7C" process and the innovative Veravas Antibody Detection Platform, we have created a novel assay that can detect levels of neutralizing SARS-CoV-2 antibodies (IgM, IgG, and IgA) in saline oral rinse (SOR) samples. Using SOR as the sample type for the assay has advantages, as it can easily be collected. Another advantage of using SOR is the ability to detect secretory antibodies, specifically secretory IgA2, which is believed to play the largest role in protective immunity. The clinical performance of the novel Veravas SOR COVID-19 Neutralizing Antibody Test was evaluated prospectively using fresh saline oral rinse samples from patients that were SARS-CoV-2 positive by the Emergency Use Authorized OraRisk COVID-19 RT-PCR (EUA200464). The PPA for the SOR specimens collected 2 to 16 days post-symptom onset was 95.5% (67/70; 95% CI 83.3 - 99.2%). To show concordance between samples tested by the Veravas Serum COVID-19 Neutralizing Antibody Protocol and the SOR COVID-19 Neutralizing Antibody Protocol, matched serum and SOR samples were collected in parallel from 27 unique healthy donors. There was 100% positive percent agreement (26/26, 95% CI 84.0% - 100%) and 100% negative percent agreement (1/1, 95% CI 5.46% - 100%) between the 27 matched samples tested. The high specificity and sensitivity of this assay illustrates the potential of the Veravas "7C" process to support the development of any low abundance antibody detection platform. This increased sensitivity and selectivity allows novel sample types like SOR to be used for detection of secretory antibodies.

## Microbiology and Infectious Diseases

## B-192

### Feasibility Assessment of a New Automated VlsE1/pepC10 IgG/IgM Chemiluminescent Immunoassay for Detecting Borrelia-Specific Antibodies from North American and European Serum Donors

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**Background:** Lyme borreliosis is the most common vector-borne illness in North America and Europe. North American infections are primarily caused by *Borrelia burgdorferi*, whereas *Borrelia afzelii* or *Borrelia garinii* typically cause European cases. Regardless of the geographic region, measurement of *Borrelia*-specific antibodies via serology assays remains an important diagnostic aid. Early serology assays developed for use in North America utilize *Borrelia burgdorferi* lysates as the antigen source, and thus are undesirable for detecting antibodies against European *Borrelia* species. More recently, so called 'next generation' serology assays, such as the ZEUS Scientific VlsE1/pepC10 ELISA, were developed and FDA cleared within the United States (U.S.) as an alternative to *burgdorferi* lysate-based assays. Since next generation assays utilize recombinant proteins and/or peptides containing amino acid sequences conserved across *Borrelia* species, they theoretically represent an acceptable option for testing European patient populations as well. The goal of these studies was to assess the feasibility of detecting *Borrelia*-specific antibodies from North American and European serum donors, using a newly developed VlsE1/pepC10 IgG/IgM chemiluminescent immunoassay (CLIA) in conjunction with a new automated analyzer. **Methods:** 92 characterized samples from North American donors were provided from the U.S. Centers for Disease Control and Prevention (32 cases, 60 controls). The CDC provided EIA data and Western blot data derived from their own testing of these samples. 40 characterized samples from European donors were also tested and provided by Amsterdam University Medical Centers (20 cases, 20 controls). For the European cohort, existing C6 peptide-based EIA IgG/IgM data and recombinant antigen-based Western blot IgG and IgM data were documented for each sample. The two cohorts were assayed by the VlsE1/pepC10 CLIA, then putative sensitivity and specificity values were calculated for the following: VlsE1/pepC10 CLIA alone and existing EIA alone, or VlsE1/pepC10 CLIA + existing Western blot and existing EIA + existing Western blot, interpreted as standard two-tiered testing (STTT) algorithms. **Results:** North American cohort: [VlsE1/pepC10 CLIA, Sensitivity = (26/32) = 81.25%, Specificity = (7/60) = 88.33%; *burgdorferi* lysate-based EIA, Sensitivity = (26/32) = 81.25%, Specificity = (17/60) = 71.67%], [STTT - VlsE1/pepC10 CLIA + Western blots, Sensitivity = (21/32) = 65.63%, Specificity = (0/60) = 100.00%; STTT - *burgdorferi* lysate-based EIA + Western blots, Sensitivity = (18/32) = 56.25%, Specificity = (2/60) = 96.67%]. European Cohort: [VlsE1/pepC10 CLIA, Sensitivity = (20/20) = 100.00%, Specificity = (2/20) = 90.00%; C6 EIA, Sensitivity = (19/20) = 95.00%, Specificity = (5/20) = 75.00%], [STTT - VlsE1/pepC10 CLIA + Western blots, Sensitivity = (15/20) = 75.00%, Specificity = (2/20) = 90.00%; STTT - C6 EIA + Western blots, Sensitivity = (15/20) = 75.00%, Specificity = (2/20) = 90.00%] **Con-**

**clusion:** These studies demonstrate that the newly developed VlsE1/pepC10 IgG/IgM CLIA, in conjunction with a new automated analyzer, yields sensitive and specific results from North American and European serum donors. Additional studies using larger sample cohorts from each geographic region are warranted towards further validating the performance of this new assay alone, as well as part of various two-tiered testing approaches.

**B-193**

**Importance of cotesting for HPV in Cervical Cancer risk assessment: tests sensitivity comparison of hrHPV vs high-grade cytology - Chilean outlook**

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**Background:** Cervical cancer is ranked as sixth in women cancer mortality and is the number one cause in reducing life span in Chile. This type of cancer is highly associated to human papillomavirus (HPV) infections from many HPV types, where virus types 16 and 18 represent a higher risk. Current local clinical guidance includes recommendation for high-risk HPV (hrHPV) testing for women over 30 only. The objective of this study was to compare clinical sensitivity of a hrHPV test with a high-grade cytology test (Pap), for providing clinical case evidence on improving local clinical guidance and create awareness.

**Methods:** We conducted a study with 288 women, ranging from 20 to 65 years old, collecting samples for cytology test and hrHPV test. For the latter, we used Onclarity HPV assay in Viper system from Beckton Dickinson.

**Results:** Only four samples shown abnormal cytology tests, while 44 samples were positive for hrHPV types (Table 1). Samples with abnormal cytology, corresponded to women over 37, except for one sample from a 22-year woman. From the overall positive samples, 16 (36.4%) were from women ≤ 30 (Figure 1), and 10 were positive for HPV type either 16 or 18, that were not detected by cytology test (Table 2).

**Conclusion:** Results for the hrHPV test shown better overall sensitivity for detecting HPV types associated to Cervical Cancer. Also, hrHPV test was able to detect HPV types 16 and 18, while Pap was not able to detect infection in any of these patients. This evidence is critical for including hrHPV tests at early ages and foster the contesting with cytology for better defining treatment next steps for patients.

	Total Patients tested	Positive	Negative	Positive %
hrHPV test	288	44	244	15,30%
high-grade cytology test (Pap)	288	4	284	1,40%

Table 1. Positive sample comparison between hrHPV vs high-grade cytology tests

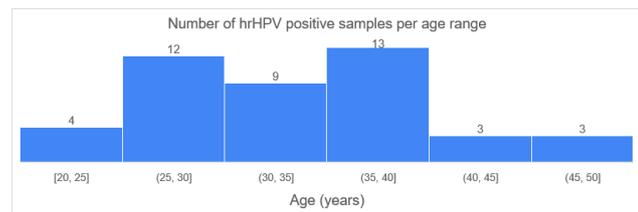


Figure 1. Age distribution for positive HPV samples detected by hrHPV test

HPV type	hrHPV test (+)	Pap test (+)
16	8	0
18	2	0
Other hrHPV	34	4
<b>Total</b>	<b>44</b>	<b>4</b>

Table 2. HPV types detected by methodology

**B-195**

**Correlation of the Rapid, Point-of-Care LumiraDx SARS-CoV-2 Antibody Test to Other SARS-CoV-2 Antibody Tests and to Viral Neutralization**

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**Background:** A rapid, point-of-care (POC) SARS-CoV-2 antibody test that could effectively predict an individuals’ immune status to SARS-CoV-2 infection could be

used to indicate when vaccinations and boosters should be administered. Currently however, no rapid SARS-CoV-2 antibody test can predict immunity to SARS-CoV-2 infection. If this clinical utility is to be realized, SARS-CoV-2 antibody tests will likely have to be able to detect antibodies that are capable of conferring immunity to SARS-CoV-2 (eg. neutralizing antibodies). It is known that almost all neutralizing antibodies present after SARS-CoV-2 infection are elicited to the S1 and receptor binding domain (RBD) portions of the spike protein. The Mt. Sinai Hospital Covid Seroklir SARS-CoV-2 IgG Antibody assay has FDA Emergency Use Authorization (EUA), uses both full-length spike antigen and RBD proteins, and the results of this test have been shown to correlate with *in vitro* Microneutralization assay. In this study we show the interpretative result correlation of the rapid, POC LumiraDx SARS-CoV-2 Antibody Test to the Mt. Sinai Hospital COVID-19 ELISA IgG Antibody Test and to viral neutralization. **Methods:** The rapid, POC LumiraDx SARS-CoV-2 Antibody Test has EUA for the qualitative detection of total antibodies to SARS-CoV-2. The LumiraDx test utilizes both S1 and RBD proteins for the detection of total antibody to SARS-CoV-2. Although the EUA version of the test reports a single qualitative result, this study utilizes a research software application (Connect Manager) to access separate results to antibodies binding to S1 and RBD SARS-CoV-2 proteins. The study was conducted utilizing a set of community derived serum samples (N=253) obtained from Mt. Sinai Hospital’s biorepository. Samples were interrogated with the LumiraDx SARS-CoV-2 Antibody Test and the Mt. Sinai orthogonal Covid Seroklir SARS-CoV-2 ELISA, both assays were performed according to the manufacturer’s instructions. Microneutralization assay (MNA) ID50’s were also reported for these samples. Qualitative results from these tests were compared using contingency tables and concordances calculated. **Results:** The SARS-CoV-2 S1 antibody results yielded by LumiraDx and Mt. Sinai Hospital Covid Seroklir assay demonstrated 96.1% (243/253) concordance. Discordant S1 results consisted of 1.6% (4/253) and 2.4% (6/253) of results that were Mt. Sinai (-)/LumiraDx (+) and Mt. Sinai (+)/LumiraDx (-) respectively. The SARS-CoV-2 RBD antibody results yielded by LumiraDx and Mt. Sinai Hospital tests demonstrated 94.9% (240/253) concordance. Discordant RBD results consisted of 0.8% (2/253) and 4.4% (11/253) of results that were Mt. Sinai (-)/LumiraDx (+) and Mt. Sinai (+)/LumiraDx (-) respectively. In addition, the LumiraDx SARS-CoV-2 antibody test results demonstrated 90.1% (228/253) concordance with MNA assay. Discordant results consisted of 2.4% (6/253) and 7.5% (19/253) of results that were MNA (-)/LumiraDx (+) and MNA (+)/LumiraDx (-) respectively. **Conclusion:** Qualitative results yielded by the rapid, POC LumiraDx SARS-CoV-2 Antibody Test show strong concordance to MNA results and to other SARS-CoV-2 antibody test results that are known to correlate with viral neutralization. These results suggest that the rapid, POC LumiraDx SARS-CoV-2 Antibody Test detects antibodies that may be capable of conferring immunity to SARS-CoV-2 infection.

**B-196**

**Comparative Study of Vega200 antigen platform to RT-PCR for Saliva Covid-19 Samples**

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Kaya17 has developed Vega200, a unique rapid point of care platform for antigen testing. This game changing Fluorescent Immuno Assay (FIA) platform is designed to have high sensitivity and specificity, comparable to RT-PCR. The first product, named nCoVega, is a saliva COVID-19 test. This test has an analytical sensitivity with LoD of 20 TCID<sub>50</sub>/mL, wide dynamic range up to 1.5x10<sup>6</sup> TCID<sub>50</sub>/mL and shows no Hook effect. This study compares nCoVega saliva results with RT-PCR saliva and swab from 465 subjects. The results show 98% overall accuracy achieved by the nCoVega test. In addition, a strong inverse correlation between RFU and Ct values is seen the graph below. Thus, the rapid and accurate nCoVega saliva test on this new FIA platform could be a replacement for a RT-PCR test. The Vega200 platform is versatile and has shown promising results for various cancer markers, other infectious agents and allergens.

**B-197****Vaccination against Covid 19 leads to an efficient immune response lasting at least up to three months after the third dose**

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**Background:**

SARS-CoV-2 mRNA vaccines have proven to have high efficacy. In a previous study we showed that a strong antibody response was induced by the vaccine BNT62b2 against different variants of concern (VoC). However, the current Omicron variant with its high number of mutations reveals immune escape in comparison to earlier VoCs. There are indications that three doses of mRNA vaccine are needed to induce neutralizing antibodies and to reduce the risk of hospitalization. Most of the respective studies have focused on the immediate response after the 3rd dose of the vaccine. Less is known about the duration of the response. We studied a cohort of Estonian medical workers. In a longitudinal serum sample analysis, we analyzed the immune response to BNT62b2 over one year, at the given vaccination scheme three months after the third dose of the vaccine.

**Methods:**

We studied a cohort of diagnostics lab personnel vaccinated with three doses of BNT162b2. The cohort had earlier been followed for their first and second dose vaccine response. The cohort received the first and the second vaccination dose in January 2021, and the third nine months after the start of the trial in October 2021. Their last blood sample was collected in the end of January 2022. The collected samples included a total of nine different time points collected before and after the three-dose vaccination. Antibody levels were analyzed by a chemiluminescent micro-particle immunoassay (CLIA) on an Abbott ARCHITECT i2000SR analyzer. Trimeric-spike and ACE2 interaction blocking that indicates presence of neutralizing antibodies was analyzed using a commercial IVD-CE ELISA kit ("SARS-CoV-2 Neutralizing antibody ELISA kit", Icosagen, Estonia), and versions of this in order to assess different VoCs. Furthermore, for CD4+ and CD8+ T cell response analysis flow cytometry was performed.

**Results:**

After the second dose, the antibody levels to the unmutated spike protein were significantly decreased at three months, and only 4% of the individuals were able to inhibit Omicron spike interaction compared to 47%, 38%, and 14% of individuals inhibiting wild-type, delta, and beta variants' spike protein. Nine months after the second vaccination, the antibody levels were similar to the levels before the first dose and none of the sera inhibited SARS-CoV-2 wild-type or any of the three VoCs. The third dose remarkably increased antibody levels and their ability to inhibit all variants. Three months after the third dose, the antibody levels and the inhibition activity were trending lower but still up and not significantly different from their peak values at two weeks after the third dose. Although responsiveness towards mutated spike peptides was lost in less than 20 % of vaccinated individuals, the wild-type spike-specific CD4+ and CD8+ memory T cells were still present at three months after the third vaccination in the majority of studied individuals.

**Conclusion:**

Our data show that two doses of the BNT62b2 vaccine are not sufficient to protect against the Omicron variant. However, the spike-specific antibodies and T cell responses are strongly elicited and well maintained three months after the third vaccination dose.

**B-198****Elevated rates of syphilis RPR biological false-positivity correlate to increasing SARS-CoV-2 antibody prevalence**

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**Background:** In December 2021, the U.S. FDA issued an alert reporting that biological false positive syphilis RPR test results can occur with the Bio-Rad Laboratories BioPlex 2200 Syphilis Total & RPR kit in some individuals who received a COVID-19 vaccine. Biological false-positive RPR test results have historically been observed in individuals with autoimmune disorders, systemic infections unrelated to syphilis, (e.g., tuberculosis, rickettsial diseases, HIV, and endocarditis) as well as following immunization (e.g., smallpox) and during pregnancy. While our laboratory does not utilize this assay, concerns about increases in RPR positivity led us to review syphilis

testing, including the RPR positivity rates in our laboratory for the 2-year period from March 2020 through February 2022. **Objective:** To determine whether the increased RPR positivity rate is due to the ongoing syphilis epidemic or due to an increase in biological false positive test results. **Methods:** We performed a retrospective analysis of syphilis RPR test results generated in 11 of our regional laboratories between March 2020 and February 2022, representing 350,000- 430,000 specimens per month. Test results for specimens submitted for the traditional syphilis reflex algorithm (RPR-Gold Standard Diagnostics) with reflex of positive specimens to treponemal antibody (TPAb) testing (DiaSorin) were analyzed. In addition, SARS-CoV-2 antibodies that target the spike (S) protein (Roche) and the nucleocapsid (N) protein (Roche) were measured in specimens submitted for syphilis testing between February 9 - 28, 2022 that were RPR positive and TPAb negative (n=1518) in selected laboratories.

**Results:** The overall RPR positivity rate was 4.0-4.6% from March 2020 to September 2021 with a marked increase to 5.2% in October 2021 and 6.6% in January 2022. Regional variability in RPR positivity rates was observed, consistent with regional variability in syphilis prevalence in the United States. Our analysis identified an increase in the percent of positive specimens that were RPR positive but negative for TPAb, suggesting the increase was due to biological false positives rather than true syphilis infections. The false-positivity rate was higher at the lower RPR titers (1:1) and decreased with increasing RPR titer. The increase in RPR positivity rates coincided with a rise in antibody positivity rates in specimens submitted for SARS-CoV-2 S Ab which increased from 75.5% in September 2021 to 91.4% in February 2022. Of 1518 patient specimens submitted to our laboratories for syphilis testing that were RPR positive/TPAb negative, 97.7% were positive for SARS-CoV-2 S and/or N Abs (80.1% were positive for both SARS-CoV-2 S and N Abs, 17.3% were positive for S Ab only, and 0.3% were positive for N Ab only) and 2.3% of patients were negative for both SARS-CoV-2 S and N Abs.

**Conclusion:** Our data suggest that an increase in RPR positivity rates is due to biological false positives and that the increased biological false positivity rate correlates with increasing SARS-CoV-2 antibody prevalence in the U.S. population tested by our laboratories. This work highlights the importance of reflexive testing for screening and diagnosis of syphilis infections especially in light of the current syphilis epidemic and SARS-CoV-2 pandemic.

**B-199****Development of a Prototype SARS-CoV-2 Antigen ARCHITECT Automated Immunoassay**

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**Background:** Abbott Laboratories' response to the COVID-19 pandemic included several diagnostic assays being approved for testing. A prototype ARCHITECT automated SARS-CoV-2 Antigen immunoassay was developed to address the potential need for the Core Laboratory. Initially, external antibodies were available for prototype development and later internal antibodies became available after an accelerated timeline. Combinations of antibodies were used to make a sensitive and specific prototype SARS-CoV-2 Antigen ARCHITECT immunoassay.

**Methods:** Thirty-eight antibodies produced in-house were screened on the ARCHITECT, an automated immunoassay instrument, with a total of approximately 350 antibody pairs. A one-step assay format was found to give the best sensitivity where the sample, capture and detection reagent were all added together, then washed extensively prior to signal generation. The primary format was selected based on sensitivity, specificity and preliminary testing of 20 positive and 20 negative VTM (viral transport medium) patient swab specimens with known Ct values from PCR testing on Abbott M2000.

**Results:** The sensitivity measured in BEI lysate for the primary prototype format (Ab1/Ab2) was 1.4 TCID<sub>50</sub>, 0.5 pg/mL and the backup prototype format (Ab3 /Ab2) sensitivity was 5.6 TCID<sub>50</sub>, 1 pg/mL. The LoBDQ estimates for Ab1/Ab2 format in calibrator diluent was 0.057pg/mL, 0.132pg/mL, 0.466 pg/mL, respectively. There was no cross reactivity with purchased culture fluids and viral lysates (OC43, NL63, 229E) or with recombinant viral proteins made internally in *E. coli* (OC43, HUK1, NL63, MERS and 229E). Both the primary and the backup prototype formats detected both SARS-CoV and SARS-CoV-2. Preliminary testing of 20 Positive/20 Negative specimens showed 14/20 Pos and 20/20 Neg for the primary prototype format and 10/19 Pos and 19/20 Neg for the backup prototype format.

**Conclusion:** These studies indicate the prototype ARCHITECT SARS-CoV-2 automated immunoassay was capable of sensitive and specific determination for SARS-CoV-2 in patient swabs extracted with VTM.

**B-200****Performance Evaluation of the ADVIA Centaur EBV-VCA IgM, EBV-VCA IgG and EBV-EBNA IgG Assays**

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**Background:**

Epstein-Barr virus (EBV) is one of the most common viruses in humans. Most individuals become infected during childhood, and it is estimated that in adults the seroprevalence is close to 95%. EBV is the causative agent of infectious mononucleosis (IM) but is also associated with other non-malignant and malignant diseases such as Burkitt's lymphoma and nasopharyngeal carcinoma. Using only three parameters (viral capsid antigen (VCA) IgM, VCA IgG and EBV nuclear antigen (EBNA) IgG) it is normally possible to distinguish acute from past infection.<sup>1</sup>

ADVIA Centaur EBV-VCA IgM, EBV-VCA IgG and EBV-EBNA IgG assays are indirect sandwich two-step immunoassays using chemiluminescent technology. They are designed for the qualitative detection of VCA IgM and IgG, and EBNA IgG antibodies in human pediatric and adult serum and plasma (EDTA and lithium heparin) for use as an aid in the diagnosis EBV infection, such as infectious mononucleosis, in individuals for whom an EBV test was ordered. The aim of this study was to evaluate the performance of the three assays on the ADVIA Centaur XP System

**Methods:**

Prospective samples were collected at 3 US sites and 1 site in Spain. Retrospective samples (VCA IgG negative, EBNA IgG negative and VCA IgM positive samples) were purchased from 8 different vendors from US and Europe. Samples were tested at 4 US sites. Prospective samples met criteria for inclusion by being leftover samples with an EBV test ordered by a physician. At least 1585 samples were analysed for each assay. Positive Percent of Agreement (PPA%) and Negative Percent of Agreement (NPA%) for ADVIA Centaur EBV-VCA IgM, ADVIA Centaur EBV-VCA IgG and ADVIA Centaur EBV-EBNA IgG assays were assessed against LIAISON EBV. In addition to method comparison study, performance characteristics evaluations such as reproducibility, interferences, and cross-reactivity by other disease conditions were also evaluated

**Results:**

Evaluation of the prospective population indicated a PPA% of 99.4% for ADVIA Centaur EBV-EBNA IgG, a PPA% of 98.9% for ADVIA Centaur EBV-VCA IgG and a NPA% of 96.6% for ADVIA Centaur EBV-VCA IgM assays compared to the LIAISON EBV devices. Evaluation of the retrospective population indicated a NPA% of 98.2% for ADVIA Centaur EBV-EBNA IgG, a NPA% of 90.1% for ADVIA Centaur EBV-VCA IgG and a PPA% of 100% for ADVIA Centaur EBV-VCA IgM assays compared to the LIAISON EBV devices. The assays demonstrated good reproducibility with CV of 3.4-10.2% for the 3 assays. High correlation between lots and high concordance using the SeraCare Mixed Performance Panel (PME202) were demonstrated. Neither endogenous interferences nor cross reactivity with around 30 different disease conditions (including HSV, VZV, CMV, Hepatitis, and HIV) were observed for any of the 3 assays.

**Conclusion:**

Study results demonstrate acceptable performance characteristics of the ADVIA Centaur EBV-VCA IgM, ADVIA Centaur EBV-VCA IgG and ADVIA Centaur EBV-EBNA IgG assays on the ADVIA Centaur XP System. These products are under development and not commercially available. Future product availability cannot be ensured

<sup>1</sup> Hsu JL, Glaser SL. Epstein-barr virus-associated malignancies: epidemiologic patterns and etiologic implications. *Crit Rev Oncol Hematol.* 2000;34:27-53.

**B-201****Evaluation in Fundamental Performance of a Newly Developed Combo Rapid Antigen Test Kit for SARS-CoV-2 and for Influenza A/B Virus**

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**Background:** A person infected with SARS-CoV-2 and other respiratory viruses, as influenza virus, often exhibits similar symptoms and it is hard to distinguish causative agents for diagnosis for proper medication. Usage of multiple diagnostic reagents for a specific pathogen requires extra-cost and is inconvenient especially if each test kits require different specimens, conditions, operations, and equipment. In order to save these troubles, we recently developed a combo rapid antigen test kit for SARS-CoV-2

and influenza A/B virus. In this study, we evaluated this combo-kit in its fundamental performance by comparing it with each rapid antigen test kits for SARS-CoV-2 and Influenza virus.

**Methods:** We developed an immunochromatographic test kit, ESPLINE SARS-CoV-2&Flu A+B (EL-combo), having two test lines for each antigen detection coated with antibodies for SARS-CoV-2 nucleocapsid antigen and antibodies for influenza A (FluA) and B (FluB) virus antigens, respectively. After taking the nasopharyngeal or nasal sample with a swab, we dipped it into pretreatment solution and squeezed it. Then we dropped the retreated sample into reaction cassette and pushed the convex button immediately to start the reaction. When the antigens are in the sample, the antigens and antibodies labelled with alkaline phosphatase (ALP) form complexes. These complexes are captured by the antibodies on test line, and blue line is detected by ALP enzymatic reaction with colorimetric substrate. It takes 20 minutes for the judgement. For evaluation of the EL-combo, we used ESPLINE SARS-CoV-2 (EL-CoV) and ESPLINE Influenza A+B (EL-flu): rapid antigen kits for SARS-CoV-2 and influenza viruses (A and B), as references respectively.

**Results:** Regarding the detection limits of SARS-CoV-2, it was 25pg/mL of the purified SARS-CoV-2 recombinant nucleocapsid protein, which was equal to EL CoV-2. Regarding influenza viruses, they were half and equivalent concentration of FluA and FluB antigens to EL-flu, respectively. Positive percent agreements (PPA) were 100% (100/100) for SAR-CoV-2 with EL-CoV and 100% (99/99) for FluA and FluB with EL-flu. Negative percent agreements (NPA) were 100% (100/100) for SARS-CoV-2 with EL-CoV and 99% (100/101) for FluA and FluB with EL-flu. Highly sensitive chemiluminescent enzyme immunoassay for FluA/B (Lumipulse Flu-A&B, Fujirebio) showed positive result with this discrepant sample, suggesting true positive for FluA/B. PPA of EL-combo for SAR-CoV-2 with RT-PCR was 58% (29/50), while it was 76% (29/38) when limited to samples of Ct values under 30. NPA of EL-combo was 100% (50/50) with RT-PCR. EL-combo gave negative judgement for the recombinant antigens of human coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-KHU1) and MERS-coronavirus, while SARS-coronavirus antigen was reactive. Dozens of purchased nasopharyngeal specimens for SARS-CoV-2 variants of concern (alpha, beta, gamma, delta and omicron) detected as same as wild type SARS-CoV-2. Samples including whole blood or several medical ingredients did not affect the judgements.

**Conclusion:** The fundamental performance of EL-Combo examined in this study was equivalent to those of EL-CoV-2 and EL-Flu, it suggested that EL-Combo would contribute to reduce of medical staff burden during the future Flu-seasons.

**B-202****SARS-CoV-2 Neutralizing antibody test to identify vaccinated patients**

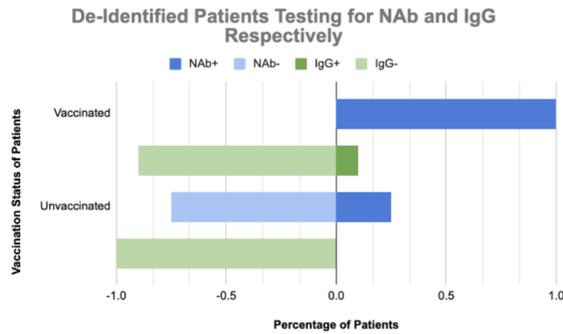
C. Hua, P. Nguyen, J. Hua, M. Nguyen, B. Le, J. Lanuzo, A. Chan, C. Bui, K. Bach, D. Sabio, D. Bui, E. Samaniego, D. Bach. *Bach Diagnostics, Irvine, CA*

**Background:** Studies show that serology assays measuring titers of non-specific Immunoglobulin G (IgG) antibodies against spike and nucleocapsid proteins of SARS-CoV-2, while immunogenic, are not sufficient for identification of immunity in vaccinated individuals. IgG neutralizing antibodies (nAbs) target an antigenic region on the spike protein called the receptor binding domain (sRBD) and block its interaction with angiotensin-converting enzyme 2 (ACE2) receptors, preventing viral entry and subsequent infection. In our study, we compare titers levels of nonspecific IgG against nAb IgG in vaccinated and unvaccinated patients to identify which assay is more sensitive at measuring antibody levels.

**Methods:** A cross-sectional study was performed using de-identified patient serology samples provided by Loma Linda University Medical Center. After validating the performances of the Diazyme assays for nonspecific IgG and nAb IgG, we compared the IgG and nAb titers levels from 28 de-identified patients.

**Results:** Sensitivity and specificity for the nonspecific IgG assay are 8.33% and 100%, respectively, and 95.83% and 75% for the nAb IgG assay, respectively.

**Conclusion:** Based on the results, the neutralizing antibody IgG assay is more sensitive than the nonspecific IgG assay, as it interacts with the antigenic sRBD region, showing detectable levels even in unvaccinated individuals. This suggests a better screening method for determining whether vaccinated individuals continue to have sufficient levels of immunity. Incorporation of neutralizing antibody assays in diagnostic testing can determine whether it is necessary for individuals to receive the second booster shot, especially if they pose a higher risk of severe acute infection if exposed to SARS-CoV-2. To minimize the discrepancies arising from time sensitive variables affecting antibody concentrations, further examination over a longitudinal study with a larger sample set is necessary.



**Figure 1.** 100% of vaccinated patients in the study tested positive for NAb, while 10% tested positive for IgG. 25% of unvaccinated patients tested positive for NAb, while no one in this sample tested positive for IgG.

**B-203**

**Host-Protein Score (Integrating TRAIL, IP-10, CRP) Distinguishes Between Viral and Viral-Bacterial Co-infection in Adult Patients Testing Positive for Viral Detection**

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**Background:**

Difficulty in determining respiratory tract infection (RTI) etiology can lead to antibiotic misuse. A limitation of viral testing is that it does not rule out bacterial co-infection. This study evaluates a recently FDA-cleared host-protein score's (MeMed BV score) ability to discriminate bacterial co-infection in patients testing positive for viral PCR and/or antigen.

**Methods:**

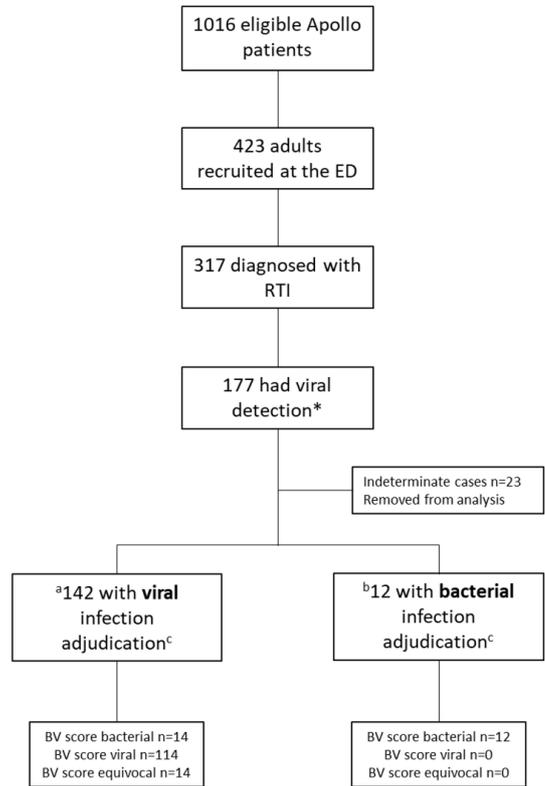
This was a sub-analysis of adult Emergency Department (ED) patients enrolled in the Apollo study with discharge diagnosis of RTI (NCT04690569). Reference standard diagnosis was adjudicated by independent experts based on comprehensive patient data. BV results are defined as viral or other non-bacterial etiology (0 ≤ score < 35), equivocal (35 ≤ score ≤ 65), and bacterial or co-infection (65 < score ≤ 100). BV performance was assessed against the reference standard. To estimate BV's potential influence on antibiotic use, it was assumed antibiotic prescription in the medical record indicated diagnosis of a bacterial infection and that if a BV score (bacterial vs. viral) had been available at the point-of-need, it would have influenced the physician's decision-making. Equivocal results were assumed not to change practice.

**Results:**

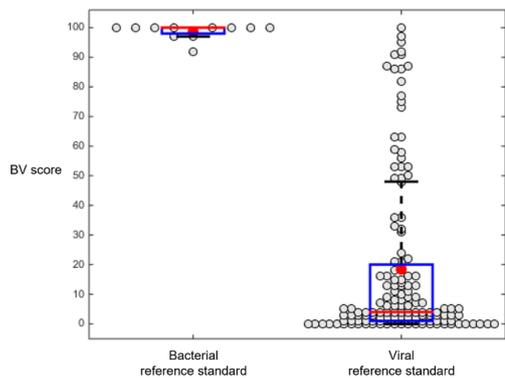
Of 423 adults (median age, 50.7 years, interquartile range 35.8-63.9; 47% female) recruited at the ED, 154 were diagnosed with RTI (58.4% upper, 41.6% lower) and had at least one viral detection. Twelve were reference standard bacterial, all correctly receiving bacterial BV scores. The 12 bacterial patients were more likely to be admitted (66.7% vs. 12.7%; p<0.001). BV yielded sensitivity 100% (95%CI: 73.5-100.0) and specificity 89.1% (95%CI: 82.3-93.9), with 9.1% equivocal cases. BV is estimated to potentially reduce antibiotic treatment of viral infections 2.8-fold (from 50% to 17.6%; p<0.001), without causing antibiotic withholding from patients with bacterial infections.

**Conclusion:**

BV discriminated bacterial co-infection in adult patients with viral PCR/antigen detection and has potential to reduce antibiotic overuse up to 3-fold.



<sup>a</sup>Viral PCR/antigen detection: Adenovirus n=4; Coronavirus HKU1, NL63, 229E, OC43 n=6; Rhinovirus/Enterovirus n=28; Influenza n=100; Parainfluenza 1/2/3/4 n=6; Respiratory syncytial virus n=7.  
<sup>b</sup>Viral PCR/antigen detection: Adenovirus n=1; Rhinovirus/Enterovirus n=5; Influenza n=4; Respiratory syncytial virus n=2.  
<sup>c</sup>A bacterial or viral reference standard diagnosis required at least 2/3 experts adjudicate the same etiology with confidence ≥90% or all 3 with confidence ≥70%.



**B-204**

**Evaluation of a new, Near-Patient Testing (NPT) IL-6 Assay on Symphony Immunoanalyzer**

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**Background:**

Dysregulated levels of interleukin-6 (IL-6) exerts several pathological effects such as inflammation as in sepsis, progression of cancer, rheumatoid arthritis, and many other conditions. Several studies indicate that high levels of IL-6 can serve as an early warning of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced hyperinflammation, disease severity, and multiorgan failure. Current EUA IL-6 assays are laboratory-based tests that require expensive equipment, high maintenance, sig-

nificant laboratory infrastructure, and skilled technicians thus limiting its widespread application. Therefore, in the current study, we have evaluated a new Symphony IL-6 assay (Bluejay Diagnostics, MA, USA) for near-patient testing (NPT) that can be utilized in Emergency Department (ED) and Intensive Care Unit (ICU) setting to provide a more rapid diagnostic platform.

#### Methods:

Symphony IL-6 assay is a fluorescence based-direct sandwich enzyme linked immunosorbent assay for the quantitative determination of IL-6. This IRB-approved clinical validation of IL-6 assay followed the Clinical and Laboratory Standards Institute (CLSI) guidelines using human whole blood EDTA samples on Bluejay Diagnostics immunoanalyzers. Five-day precision was performed using 2 levels of quality control (QC) materials (QC1 & QC3) (Randox Diagnostics, WV, USA). Linearity was determined using serially diluted patient sample with high IL-6 value. Limit of quantitation (LOQ) was performed using commercially available linearity standard material (Audit Microcontrols Inc, GA, USA). For establishing the IL-6 reference cut-off, 72 adult healthy volunteers were recruited after obtaining consent. In addition, the IL-6 cut-off values for disease severity was determined using 162 PCR-confirmed remnant Coronavirus disease 2019 (COVID-19) symptomatic samples from ICU and in-patient COVID wards. Interference was tested using 10 pg/mL and 100 pg/mL of IL-6 and interferent concentrations up to 50,000 pg/mL were evaluated. The statistical analysis was performed using Graphpad Prism 9.3.1 and EPA Evaluator v12.

#### Results:

Five-day precision study yielded a coefficient of variation (CV) of 20% (Mean±SD, 4.0±0.8 pg/mL) for QC1 and 11.4% (Mean±SD, 126±14.4 pg/mL) for QC3. The assay was linear between 0 to 6000 pg/mL with limit of quantitation (LOQ) of 4 pg/mL. Reference cut-off of IL-6 in healthy volunteers was found to be 23.0 pg/mL. The IL-6 cut-off to rule out COVID-19 disease severity based on mechanical ventilation was determined to be 34.0 pg/mL with a negative predictive value (NPV) of 0.97. A strong correlation (n=23; Deming r=0.9560; p<0.0001) with a positive bias of 16.64% by Bland-Altman method was noted for Symphony IL-6 against the EUA-approved Roche IL-6 assay. The bias could be due to the whole blood sample used in our assay compared to the recommended EDTA plasma for the Roche assay. The assay was found to have acceptable cross-reactivity (<10%) with IL-1a, IL-1b, IL-2, IL-4, IL-8, IL-10, MCP-1, TNF-a, INF-g, VEGF, and EFG.

#### Conclusion:

The NPT Symphony IL-6 assay has comparable performance to EUA-approved lab-based IL-6 assays. With its operational simplicity, reliability, portability, and fast turnaround time of less than 19 mins, the Symphony assay could be readily implemented for rapid identification of inflammation and disease severity in ED and critical care setting.

## B-205

### 18-Month Trends of SARS-CoV-2 Anti-Spike IgG Antibodies Among Healthcare Workers

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**Background** Healthcare workers (HCWs) are at an increased risk for exposure to SARS-CoV-2. Serological data can help provide insight into humoral immunity, but there is limited information currently available describing long-term serum antibody trends. Here we evaluate these trends among a cohort of HCWs at Boston Medical Center (BMC) over 18 months using a semi-quantitative SARS-CoV-2 IgG assay.

**Methods** Eligible HCWs at BMC were enrolled in July 2020 and followed for 18 months, providing survey data and serum samples at approximately 3-month intervals. Serum samples were analyzed using the Abbott Architect i2000sr for SARS-CoV-2 antibodies (anti-spike1-RBD IgG and anti-nucleoprotein IgG). Positive anti-n IgG results were used to assist in identifying cases of infection (index value  $\geq 1.4$  AU/mL). COVID-19 infection history and vaccination status were confirmed where possible using EMRs and participants were grouped accordingly in December 2021 for analysis of anti-s IgG (positivity threshold  $\geq 50$  AU/mL). Data were collected and managed in REDCap (CTSI-IUL1TR001430) and statistical analyses were run using SAS version 9.4. **Results** Of the 527 enrolled in July 2020, most were female (77.0%), had a normal BMI (47.4%), and identified as White (81.6%). Participants were largely nurses (41.2%) or physicians (31.5%). Most (87.7%) were fully vaccinated by March 2021 and 73.6% had received a booster dose by December 2021. Full vaccination was principally captured in March 2021 and participants' median serum anti-s IgG antibody concentrations ([SAb]) largely remained above the positivity threshold but declined until COVID-19 infection or booster dose uptake. Those with infection before vaccination saw an 82.2% decline by September 2021, HCWs infected after vac-

ination showed an 86.1% decline, and those with no infection saw a 90.1% decline. Booster uptake was captured in December 2021. Post-booster median [SAb] amongst HCWs infected before vaccination reached 53.8% of levels observed in March 2021. Conversely, HCWs that were infected after vaccination and those without a history of infection observed a 218.0% and 192.3% increase in median [SAb], respectively, compared to March 2021. **Conclusion** Antibody concentrations among vaccinated HCWs at BMC remained above the positivity threshold for anti-s RBD IgG up to 18 months post-infection and 15-months post vaccination. A heterogeneous response was observed in correspondence with infection and booster dose uptake. Observing serological trends is vital for understanding the durability of antibody response, ultimately helping to inform public health decisions.

## B-206

### SARS-CoV-2 Direct Antigen Rapid Test Performance

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#### Background:

There are significant limitations with RT-PCR testing for SARS-CoV-2 diagnosis. In a public health crisis, it is imperative to make testing available to an increased number of people. The Direct Antigen Rapid Test (DART) for SARS-CoV-2 has been developed to provide fast results, enabling anyone from the community to obtain a rapid test result. Achieving widespread use of rapid tests can help inform individuals on their infectious status and promote quarantine, thereby lessening the public health burden. We aim to assess the test performance of the DART device at an academic institution.

**Methods:** Eligibility criteria included any patient over 18 years old who presented to the emergency department at Boston Medical Center with six days or fewer of COVID-19 symptoms. Enrolled patients provided an additional NP or AN swab for the DART. DART, a lateral flow immunoassay that provides qualitative results of SARS-CoV-2 status. Medical record review was conducted to obtain the following data: demographics, RT-PCR results, inflammatory biomarkers, and co-morbidities. All data was collected and stored in REDCap. All analyses were performed in R (R-1.3.1056). P-values < 0.05 were considered significant.

**Results:** A total of 39 participants were enrolled, but analysis was limited to 35 due to laboratory error. 33 of 35 participants had a negative RT-PCR result. DART exhibited a negative agreement of 81.8%. However, DART did show discordant results for two RT-PCR positive specimens. Only one of the two RT-PCR positive participants was positive for DART, corresponding to a positive agreement of 50.0%. Neither demographic variables nor inflammatory markers were associated with SARS-CoV-2 RT-PCR results.

**Conclusion:** Due to the community prevalence at the time of the study, most participants had a negative RT-PCR result thus limiting our conclusions. Even with less-than-ideal performance characteristics, antigen testing can be utilized for epidemiological surveillance which can help contain the pandemic. Larger studies are needed to further characterize DART performance characteristics.

## B-207

### Comparison of COVID-19 Positivity Rate between Long-Term Care Facilities and Outpatients During Omicron Variant Surge: Long-Term Care Facility is the Place to be!

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

**Background:** Covid-19 is pandemic infection that claimed the life of over 900,000 patients. Long-term Care Facilities (LTCF) residents were the first to get affected in the beginning of the pandemic because of their setting and their residents' morbidity and fragility. During the period from November 2021 to January 2022 a sharp rise in Covid-19 cases was noted throughout the country due to Omicron variant; in this study we looked at the prevalence of Covid-19 in Long-term Care Facilities and outpatient; the outcome was compared to the State average. **Method:** 75,000 specimens collected for SARS-CoV-2 RT-PCR from November 2021 to January 2022. Patients were separated as Long-Term Care Facility residents or outpatient and they were further separated based on the State they resided. Percentage positivity for LTCF residents and outpatient were compared to the State positivity rate. Statistical analysis was done using Analyse-it.

	LTCF	Outpatient	Average NJ positivity rate
12/12-12/18	1.8%	9.5%	10-20%
12/19-12/25	6.5%	24.1%	>20%
12/26-1/1	15.2%	36.5%	>30%
1/2-1/8	20.03%	32.2%	>30%
1/9-1/15	13.2%	20.9%	>20%
1/16-1/22	9.4%	7.3%	10-20%
1/23-1/29	5.3%	4.3%	10-20%
1/30-2/5	2.7%	2.2%	<10%

**Results:** The average positivity rate was 7.5% for LTCF residents and 24.2% for outpatients which was very closer to NJ positivity rate. The highest positivity rate for the outpatients was the week between Christmas and New Year (positivity rate was 36.5%); the highest positivity rate for the LTCF resident was for the week after New Year (20%). The positivity rate for LTCF residents and outpatients were statistically similar beginning of November 2021 and end of January 2022. **Conclusion:** LTCF had the highest prevalence and death from COVID-19 in the first few months of the pandemic. However, and based on our finding the LTCF had better control of the COVID-19 due to strict policy on visitation, frequent testing which lead to earlier isolation and quarantine, better infection control monitoring, and higher than average vaccination and booster rates.

## B-208

### Overcoming Reagent Shortage for SAR-CoV-2 testing for Patients residing in Long-Term Care Facilities: Performance Evaluation of Allplex™ 2019-nCoV Assay using pooling Procedure.

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

**Background:** Covid-19 is pandemic infection caused by SARS-CoV-2 virus; over 400,000,000 cases were reported worldwide and over 77,000,000 confirmed in the USA. Long-Term Care Facilities were hit very hard during the pandemic which led to the urgent need to test their patients to be able to isolate and treat their patient. The challenge wasn't only to identifying the infected patient which is the most important step to fight the pandemic and stop the spread of the disease but to have enough supplies to accommodate huge increase in testing and screening demand. Pooling strategy was introduced to help test shortage; however, that requires the search for assay that has enough sensitivity for pooling to avoid false negative reporting for weakly positive samples. **Methodology:** The Allplex™ 2019-nCoV assay is a real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 virus. The assay detects and bases its determination on 3 different genes (N, RdRp, and E). This increases the sensitivity and allow for better detection. The assay was evaluated for: precision, reproducibility, limit of detection (serially diluting quantified inactivity SARS-CoV-2 whole virus), and accuracy. 126 samples were collected from patient in Long-Term Care Facilities; all samples were run as single sample using Allplex™ 2019-nCoV. 105 confirmed negative were used to make 21 of 5-sample negative pools; 21 pools, each consisting of 1 positive sample and 4 negative samples were used to make positive pools. Over 50,000 samples were run using pool methodology, period checking on positive and negative pool were done to ensure accuracy. Statistical analyses were done using Analyse-it. **Results:** The percentage agreement for precision, reproducibility was 100%. The limit of detection was 400 copies/mL. Negative samples pools were all negative; the pools with positive samples were all positive. The patients' correlation was 100% even with patients with high CT values. All individual positive samples produced positive results when pooled with negative samples (N=5); all positive pools done since the implementation of polling produced at least one positive sample. Periodic check for negative pools produced negative results. **Conclusion:** Based on the assay interpretation (as defined by their EUA submission) our positive and negative predictive values were 100%. The ability for Allplex™ 2019-nCoV to detect 3 targets genes (E, RdRp, and N genes) gives the assay better sensitivity which will allow pooling. Utilizing a pool strategy increased testing efficiency, helped maximizing throughput, and avoid the delays associated with referencing work to another laboratory. We are current average turnaround time from receipt in laboratory to report is 12 hours Using a pool strategy will also allow us to maximize reagents when positivity rates permit

## B-209

### Prevalence of SARS-CoV-2 IgG in Patients Residing in Long-Term Care Facilities: A One Year follow-up!

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

**Background:** Covid-19 is pandemic infection caused by new strain of coronavirus, SARS-CoV-2. The United State alone has more than 77 million confirmed cases and more than 900,000 deaths; the number of cases might be higher because some of the cases are mild or with no symptoms which make testing for past infection will give the seroprevalence and the real estimate of the infection. Although we don't have enough knowledge about the immunity nor the body response to the infection, a serology tests are very important to understand the past exposure or to check the immunity toward SARS-CoV-2 is an important key to check the seroprevalence in the population and impact and the future of the disease. **Methodology:** 1,091 samples collected from June 2020 to May 2021 from residents in Long-Term Care Facilities were SARS-CoV-2 IgG IgG Abbot Architect. Samples were separated based on gender and age. 101 patients had additional sample collected up to six months after the initial test. Statistical analyses were done using Analyse-it. **Results:** women accounted for 63.2% of the specimens tested. The overall positivity rate was 58.7 %; men had slightly higher positivity rate than women. The highest positivity rate (86.9%) was in June 2020; 25.8% of the patients who were positive turned negative upon follow up.

	% Positive
June 2020	86.9%
July 2020	75.0%
August 2020	62.1%
September 2020	26.4%
October 2020	56.5%
November 2020	40.0%
December 2020	51.8%
January 2021	50.9%
February 2021	42.9%
March 2021	42.9%
April 2021	35.7%
May 2021	33.3%

**Conclusion:** Our data showed that the majority of the people tested had positive results which could be due to a higher exposure among Long-Term Care Facilities residents and employees. One fourth of the patients who were positive initially turned negative upon follow-up. More studies are needed to determine the serological and immunity response after SARS-CoV-2 infection; do we lose immunity with time? Is serology marker good indication of immunity? Questions still need to be answered!

## B-210

### Validation of the STANDARD M10 SARS-CoV-2 RNA Assay

H. Kim. *Hallym Univ. Medical Center, Anyang, Korea, Republic of*

**Background:** Rapid and sensitive assays for SARS-CoV-2 detection are necessary in emergency settings. The STANDARD M10 SARS-CoV-2 (SD Biosensor, Korea) is a rapid real-time PCR assay using single disposable cartridge. We validated the STANDARD M10 SARS-CoV-2 using a conventional real-time RT-PCR (rRT-PCR) in emergency settings.

**Methods:** A total of 425 nasopharyngeal swabs were collected in a regional emergency center. The specimens were tested with the STANDARD M10 SARS-CoV-2 assay and rRT-PCR assay (Real-Q 2019-nCoV Detection, BioSewoom, Korea). The viral targets are the E gene and the OFR1ab gene in the STANDARD M10 assay, and the E gene and the RdRP gene in rRT-PCR assay.

**Results:** The overall sensitivity and specificity of the STANDARD M10 assay were 92.2% and 99.97% compared to rRT-PCR, respectively. Fifty-one (12.0%) specimens were positive for rRT-PCR. Of the 51 specimens, 47 were positive and 4 were inconclusive for the STANDARD M10 assay. The overall concordance rate between STANDARD M10 and rRT-PCR was 98.8%.

**Conclusion:** The STANDARD M10 assay rapid antigen test showed a good correlation with rRT-PCR. The test will be useful in emergency settings.

**B-211****Biomarkers in ICU-septic patients to predict bacteremia**

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**Background:** The objective of this study was to construct the new prediction model for determining septic patients with and without bacteremia. Not limited to analyzing c-reactive protein (CRP) or procalcitonin (PCT), we evaluated and used any biomarkers with remarkable diagnostic utilities to enhance the model's prediction power.

**Methods:** Total 233 septic adult patients admitted to intensive care unit in 2019 were enrolled in the study. SEPSIS-3 criteria (sequential organ failure score (SOFA) score  $\geq 2$ ) was applied to select only septic patients as our research subjects. Also, blood culture contamination was rectified by clinically evaluating each positive culture result that some false-positive culture results got reclassified as non-bacteremia. **Results:** Of 233 patients recruited for the analysis, 47 were bacteremic and 186 were non-bacteremic. Both CRP and PCT showed a substantial area under the curve (AUC) value for discriminating bacteremia among septic patients (0.783 and 0.854, respectively). The prediction model for bacteremia was further optimized through a synergy effect of clinical markers that multiple logistic regression (MLR) model with AUC of 0.901 was constructed by combining neutrophil lymphocyte ratio (NLR), bilirubin, SOFA score, CRP, and PCT as the independent predictors. Also, a high association between bacteremia and mortality rate was identified through a survival analysis ( $P=0.004$ ).

**Conclusions:** While CRP and PCT are useful indices for diagnosing bacteremia among septic patients, our MLR model indicates that the accuracy of bacteremia diagnosis could be further improved by the combined use of NLR, bilirubin, SOFA, CRP, and PCT.

**B-212****Antibody Response of SARS-CoV-2 mRNA Booster Vaccination in Solid Tumor Cancer Patients**

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**Background:**

Cancer patients are known to have significantly worse outcomes from COVID-19 infection, and those undergoing active chemotherapy treatment remain at higher risk for decreased immunity. Limited studies to date have quantitatively assessed the correlation between mRNA vaccine boosters and COVID-19 antibody titers in solid tumor patients undergoing active chemotherapy treatment, particularly within U.S. community hospital settings.

**Methods:** To investigate how the concentrations of SARS-CoV-2 anti-spike antibodies differ in chemotherapy-treated solid tumor cancer patients before and after mRNA booster vaccination, concentrations of anti-spike and anti-nucleocapsid antibodies were measured in 19 solid-tumor cancer patients undergoing treatment before and after booster vaccination. Inclusion criteria for the study were a completed mRNA vaccination (Pfizer or Moderna) series followed by a booster for SARS-CoV-2, an expected survival of at least six months, and no record of immunosuppressive therapy apart from primary chemotherapy. Each study participant underwent blood specimen collection at the start of the study and then approximately at six weeks and three months.

Testing for antibodies against SARS-CoV-2 involved the use of two methods: SARS-CoV-2 Total Anti-Nucleocapsid Antibody Assay (Anti-N), and SARS-CoV-2 Total Anti-Spike Antibody Assay (Anti-S) (Roche Diagnostics, Indianapolis, IN). The two electrochemiluminescence sandwich immunoassays use the appropriate SARS-CoV-2 antigen to detect the presence of antibodies. The Anti-N is a qualitative assay providing a positive/negative result. The Anti-S provides a semi-quantitative result with a clinically reportable range from 0.4 to 25,000 U/mL and any results  $>0.8$  U/mL considered positive.

This study was approved by the NorthShore IRB as part of a larger, ongoing investigation.

**Results:**

Concentrations of Anti-S after two doses of mRNA vaccine were highly variable, with concentrations ranging from 9 to 1888 U/mL and with a median and interquartile range of 351 U/mL and 639 U/mL, respectively. Anti-S concentrations after the third vaccination dose (aka "booster") showed dramatic increases in antibody levels in almost all patients. For the 19 where this data was available, the median concentration after booster rose to 11241 U/mL ( $p<0.05$ ). This increase was observed in all cancer

groups. Additionally, poor responders to the first 2-dose vaccination series, defined as those with an Anti-S titer of  $<300$  U/mL, also showed a robust response with a pre-booster median concentration of 147 U/mL and a post-booster of 9307 U/mL ( $p<0.05$ ). Eight of the patients in this study had a poor response to the initial vaccine series with Anti-S concentrations less than 300 U/mL. Encouragingly, all poor initial responders had increased Anti-S concentrations after the booster.

**Conclusion:** Overall, the study shows that Anti-S concentrations increase in solid tumor cancer patients after vaccination. Findings were also consistent in poor initial responders, demonstrating the importance of mRNA boosters in protecting potentially vulnerable solid tumor cancer patients from infection by the SARS-CoV-2 virus. Findings strengthen the recommendation for booster vaccination in this population when eligible. As part of a large ongoing study, future inquiry will investigate the effect of cancer type, treatment type, and dosing regimens on antibody rise and persistence over time.

**B-213****Evaluation of the new ARCHITECT Toxo IgG and Alinity i Toxo IgG Assays for the US Market**

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**Background:** Infections during pregnancy caused by Toxoplasma, Rubella, Cytomegalovirus and Herpes simplex viruses (TORCH is often used as an acronym for these infections) can result in serious consequences for the fetus. To ensure accurate diagnosis and correct clinical patient management the performance of serological tests is highly important. The ability to distinguish between acute and past infection by using IgG and IgM antibody detection as an aid in diagnosis helps to avoid unnecessary treatment of pregnant females. **Methods:** Evaluation of the new ARCHITECT and Alinity i Toxo IgG assay's for the US market included a clinical study (method comparison) at 4 clinical sites in the US based on guidance from CLSI EP12-A2. A total of 1614 specimens were collected to evaluate the negative and positive percent agreement between the ARCHITECT Toxo IgG assay and a current FDA cleared commercially available anti-Toxo IgG assay; this includes 777 routine order and 84 preselected positive specimens collected in the US and 482 routine order and 71 preselected positive specimens from outside of the US. In addition, the clinical study included 200 specimens collected from pregnant females in the US. System reproducibility was determined based on guidance from CLSI EP05-A3. Testing was conducted using 3 lots of the ARCHITECT Toxo IgG reagents, 2 lots of the ARCHITECT Toxo IgG Calibrators, and 2 lots of the ARCHITECT Toxo IgG Controls and 1 ARCHITECT i2000SR instrument at each of the 3 clinical sites in the US. Two controls and 5 human serum panels were assayed in 4 replicates at 2 separate times per day on 5 different days. Within-laboratory precision was determined based on guidance from CLSI EP05-A3. Testing was conducted in-house using 1 lot of the Alinity i Toxo IgG reagents, 1 lot of the Alinity i Toxo IgG Calibrators, and 1 lot of the Alinity i Toxo IgG Controls on 1 Alinity i analyzer. Two controls and 5 human serum panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

**Results:** Agreement between the new ARCHITECT Toxo IgG assay and the tested FDA cleared commercially available anti-Toxo IgG assay showed for all specimens a negative percent agreement of 98.75% (1268/1284) and a positive percent agreement of 95.68% (310/324). System reproducibility on the ARCHITECT i2000SR was determined to be 3.5% CV for the Positive Control and ranging from 5.6 - 6.7% for the 3 positive human serum panels. Within-laboratory precision on the Alinity i Toxo IgG assay was determined to be 2.1% CV for the Positive Control and ranging from 2.3 - 3.2% CV for the 3 positive human serum panels. **Conclusion:** The new ARCHITECT Toxo IgG assay showed negative and positive percent agreement greater than 95% compared to the used FDA cleared commercially available anti-Toxo IgG assay on the intended use population. The ARCHITECT Toxo IgG and Alinity i Toxo IgG assay's demonstrated satisfactory precision on controls and human serum samples.

**B-216****Performance Evaluation of AdvanBio ABcare® SARS-CoV-2 Ag Test for Rapid Point of Care Testing**

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**Background:**

The ABcare® SARS-CoV-2 Ag home test is intended for self-testing at home and as an aid in the diagnosis of SARS-CoV-2 infections. The assay is based upon lateral flow

one-step sandwich assay format with colloidal gold for the rapid qualitative determination of SARS-CoV-2 (Severe, Acute, Respiratory, Syndrome) nucleocapsid protein antigen in interior nasal swab specimens. This test contains a membrane that is pre-coated with SARS-CoV-2 nucleocapsid protein (NP) antibody on the test line and goat anti-mouse IgG on the control line. An anti-SARS-CoV-2 NP antibody that is labeled with colloidal gold is sprayed on a conjugate pad. If the viral antigen is present in the sample, it will be captured by the anti-SARS-CoV-2 antibody on the conjugate pad. As the complex continues to travel up the card, the viral antigen is detected by the SARS-CoV-2 antibody coated on the membrane to form a sandwich format, and a visible pink line is shown to indicate a positive result. The assay is rapid, providing a result within 15 minutes. With the benefits of fast testing turnover time and cost-effectiveness without expensive instruments as a reader, this test serves as a rapid point of care test (POCT) for the detection of SARS-CoV-2 antigens. Here, we report the performance of the ABcare® SARS-CoV-2 Ag test. Methods: Performance evaluation of the ABcare® SARS-CoV-2 Ag test for the detection of SARS-CoV-2-specific NP antigen was performed on a well-characterized set of community derived samples (N=566), inclusive of both symptomatic and asymptomatic patients. Of these 110 samples from patients confirmed to be PCR positive for SARS-CoV-2 infection were used to assess the sensitivity of the assay. 456 other samples confirmed to be PCR negative were used to determine the specificity. Positive predictive value (PPV) and negative predictive value (NPV) were also determined.

#### Methods:

Performance evaluation of the ABcare® SARS-CoV-2 Ag test for the detection of SARS-CoV-2-specific NP antigen was performed on a well-characterized set of community derived samples (N=566), inclusive of both symptomatic and asymptomatic patients. Of these 110 samples from patients confirmed to be PCR positive for SARS-CoV-2 infection were used to assess the sensitivity of the assay. 456 other samples confirmed to be PCR negative were used to determine the specificity. Positive predictive value (PPV) and negative predictive value (NPV) were also determined.

#### Results:

The sensitivity and specificity of the ABcare® antigen assay observed was 94.55% (95% CI: 88.51%-97.97%) and 100% (95% CI: 99.19%-100%), respectively. Additionally, the analytical performance showed an excellent limit of detection (LOD) with no hook effect, no cross-reaction with other major viruses nor other interferences with blood and nasal discharges were observed.

#### Conclusion:

SARS-CoV-2 Ag detection by the ABcare® test offers rapid self-test results with higher sensitivity and 100% specificity in a cost-effective manner at home.

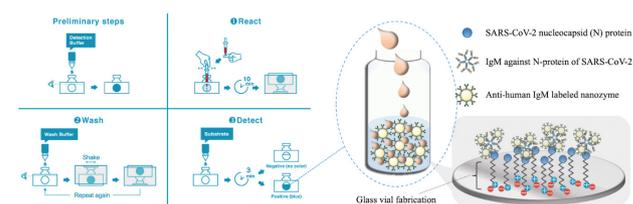
## B-217

### Evaluation of a Novel Ultra-Sensitive Immunosensor for SARS CoV-2 Diagnosis

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**Background:** From the onset of the current global pandemic hundreds of millions of reverse transcription-polymerase chain reaction (RT-PCR) tests have been completed representing the gold standard for SARS CoV-2 diagnoses. Nonetheless, this test doesn't exist without any criticism or disadvantages. A number of people have expressed feelings of discomfort resulting from nasopharyngeal swabs and hours of turnaround time (TAT) to receive RT-PCR results. Self-linkable nanozyme was utilized in a colorimetric immunosensor for signal amplification to increase the sensitivity for serological IgM detection. Unlike the manual enzyme-linked immunosorbent assay (ELISA), this novel immunosensor will reduce TAT to 15 minutes. Our study aims at assessing the analytical and clinical performance of the novel immunosensor that detects IgM against SARS CoV-2 nucleocapsid (N) protein. **Methods:** Thirty-six samples from RT-PCR confirmed SARS CoV-2 positive patients were used for sensitivity analysis and among those sixteen samples were collected within 7 days after symptom onset. Also, one hundred and eighty-six non-SARS CoV-2 sera with a potential cross-reaction to SARS CoV-2 immunoassays were used for specificity analysis. **Results:** The novel colorimetric immunosensor showed a sensitivity and specificity of 100% (95% CI: 87.9% - 100%) and 100% (95% CI: 97.4% - 100%) in those two hundred and twenty-two samples for detecting anti-SARS CoV-2 N protein IgM. The immunosensor could detect SARS CoV-2 infection as early as the first day after symptom onset without any cross-reaction from vaccine elicited antibodies. **Conclusion:** The analytical performance of the novel colorimetric immunosensor is satisfactory. The immunosensor was designed to run equipment-free and be user-friendly.

With a clinical performance on par with the current gold standard RT-PCR, this novel immunosensor will prove beneficial in communities where health care personnel are lacking and medical resources are scarce.



## B-218

### Community-based SARS-CoV-2 testing using saliva or nasopharyngeal swabs to compare efficacy of weekly COVID-19 screening to wastewater SARS-CoV-2 signals

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**Background:** Wastewater-based epidemiology has long been suggested as a sensitive method to monitor pathogen occurrence in a community. Multiple studies worldwide confirmed that SARS-CoV2 RNA can be detected in wastewater. However, no direct evaluation of the prevalence of COVID-19 with people living in the wastewater sewer-shed areas and SARS-CoV-2 viral RNA concentration is established. Here we aimed to define the correlation between wastewater SARS-CoV-2 signal and individual COVID-19 status, as well as compare positivity rates in two underserved communities in Portland, Oregon to those reported in the greater Multnomah county. **Methods:** This study was approved by the institutional review board at Oregon Health & Science University. We recruited 403 individuals via two voluntary walk-up COVID-19 testing sites in disproportionately affected areas in northeast and southeast Portland over a period of 16 weeks (11/16/2020 - 3/8/2021). Testing was open to asymptomatic or symptomatic individuals. SARS-CoV-2 infection rates in our cohort were compared to wastewater PCR signals and general positivity rate in Multnomah county. **Results:** The weekly SARS-CoV-2 positive rate ranged from 0% to 21.7% and trended significantly higher than symptomatic positivity rates reported by Multnomah county (1.9% - 8.7%). Among the 362 individuals who reported symptom status, 76 (21.0%) were symptomatic and 286 (79.0%) were asymptomatic. COVID-19 was detected in 35 (8.7%) participants; 24 were symptomatic, 9 were asymptomatic and 2 were unknown (Fig 1). Wastewater testing found 0.33 - 149.9 viral RNA genomic copies/L/person over this time period and paralleled community COVID-19 positive test rates. **Conclusion:** Wastewater sampling accurately identified increased SARS-CoV-2. Importantly, the rate of SARS-CoV-2 positivity in underserved areas was significantly higher than general county-reported rates, suggesting a disproportionate effect of SARS-CoV-2 in these communities.

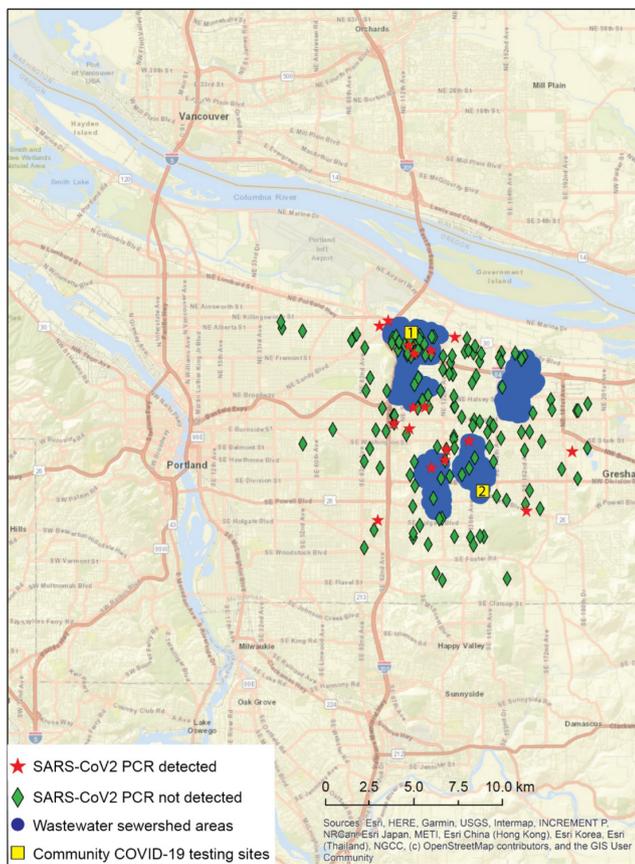


Fig 1: Location of the wastewater sewershed areas, community testing sites and individuals from our cohort in Portland, OR.

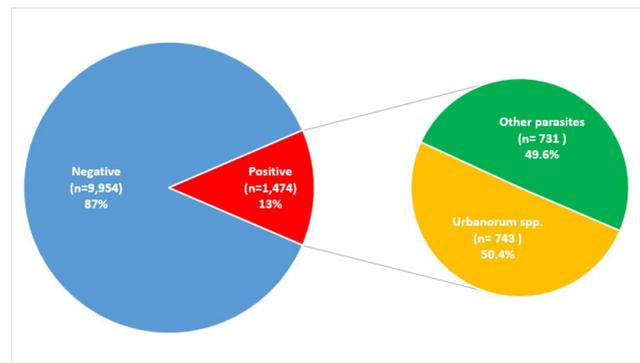
**B-219**

**Occurrence of *Urbanorom Spp.* in the Parasitology Sector in Medical Laboratory from Blumenau - SC, Brazil**

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**Background:** The protozoan *Urbanorom spp.* has presented an expressive prevalence in the positive findings of the parasitological feces exams in the regions affected and it seems to be related to meaningful gastrointestinal symptoms. As it was recently depicted, its identification can be underestimated, and, consequently, its diagnosis, appropriate treatment and prevention. This study aims to determine the prevalence of *Urbanorom spp.* in parasitological feces exams, in private medical laboratory in the city of Blumenau-SC, Brazil; in addition, it aims to describe the morphological characteristics of the parasite; and to provide demography data summary of the listed patients. **Methods:** Exploratory study from the results of the parasitological feces exams in private medical laboratory in the city of Blumenau-SC. The prevalence of the protozoan *Urbanorom spp.* was calculated in relation to the feces samples submitted to analysis in the years of 2020-2021. The demographic data related and the clinical indication were obtained from the medical request. **Results:** From the 11,428 feces parasitological analysis, processed in 7,117 patients from 10 months to 99 years old, 86% of Blumenau residents, being detected mainly the protozoan *Urbanorom spp.*, with a prevalence of 7% (n=743 samples), representing half of the positive findings. From the patients with positive results for the new protozoan, 14% were from 0 to 12 years old; 41% reside in the central region of Blumenau; and 13% are from occupational origins. The described symptoms in the medical indications were: dyspepsia; changing in the bowel habits, abdominal pain, malaise, nausea, vomit, diarrhea and weight loss for no apparent reason. **Conclusion:** This current study has found a surprising prevalence of the *Urbanorom spp.* in the laboratorial parasitological analysis,

which in fact, coincides with the literature about the occurrence and physiopathogenesis of this new endoparasite. This is an alert to expand its diagnosis, treatment and prevention.



Picture 1 – *Urbanorom spp.* occurrence in fecal samples in the parasitological sector of Medical Laboratory from Blumenau – SC

**B-220**

**Performance of the Multiplex TaqPath™ COVID-19, FluA, FluB Combo Kit for SARS-CoV-2 Screening**

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**Background** Detection and differentiation of SARS-CoV-2, Influenza A and Influenza B infections is necessary to select the appropriate management especially during flu season in the background of the COVID-19 pandemic. While diagnostic testing in symptomatic patients is the mainstay from a patient management perspective, screening in asymptomatic subjects is critical from a public health standpoint. We evaluated performance of the multiplex TaqPath™ COVID-19, FluA, FluB Combo Kit in detecting SARS-CoV-2 infection in, both, asymptomatic and symptomatic individuals. **Methods** Northeastern University (NU) conducts routine COVID-19 testing for its student, faculty and staff to support in-person instruction. A one-week prospective study in January 2022 was conducted where samples were collected from symptomatic and asymptomatic individuals. Samples were analyzed in parallel using the multiplex TaqPath™ COVID-19, FluA, FluB Combo Kit as well as the TaqPath™ COVID-19 Combo Kit in a blinded fashion. Positive percent agreement (PPA) and negative percent agreement (NPA) were calculated. **Results** 17193 were analyzed during the one-week duration of the study. The multiplex kit demonstrated good concordance with the COVID-19 only kit for detection of SARS-CoV-2 with PPA of 92.05% and NPA of 99.83%. Of the 38 samples that were positive on COVID-19 only kit and negative on multiplex kit, 1 sample was from a symptomatic subject and 37 discordant samples were from asymptomatic individuals. Average Ct values for these cases was 33.28. S-Gene Target Failure (SGTF) was detected in 77% (370/478) of cases, potentially indicating presence of Omicron BA.1 variant. No FluA or FluB infections was detected among the tested samples. **Conclusion** The TaqPath™ COVID-19 FluA, FluB Combo Kit has acceptable performance as a screening test for SARS-CoV-2. Discordant samples were mainly from asymptomatic individuals with low viral burden. Although the study was conducted during expected peak period, seasonal flu was significantly milder than expected in January 2022.

	TaqPath™ COVID-19 Combo Kit			
	Pos	Neg	Total	
TaqPath™ COVID-19, FluA, FluB Combo Kit	Pos	440	29	469
	Neg	38	16686	16724
	Total	478	16715	17193
<b>Positive Percent Agreement (PPA)</b>	<b>92.05%</b>	<b>95% CI (XX-YY)</b>		
<b>Negative Percent Agreement (NPA)</b>	<b>99.83%</b>	<b>95% CI (XX-YY)</b>		

## B-221

**Multi-center Performance Evaluation of the HSV-1 IgG and HSV-2 IgG Assays\* on the ARCHITECT i2000SR System**

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**Background:** Herpes Simplex Virus (HSV) is present worldwide and humans are the only natural reservoirs. HSV transmission occurs by close person-to-person contact with a person who is actively shedding the virus. Infection occurs when the virus enters susceptible mucosal surfaces or breaks in the skin. HSV-1 is traditionally associated with orofacial disease, while HSV-2 is traditionally associated with genital disease. HSV infections can cause various types of diseases such as mucocutaneous, ocular, central nervous system (CNS) infections, disseminated disease in the immunocompromised, and neonatal herpes. Neonatal herpes, a potentially fatal infection, is one of the most serious complications of genital herpes. Type-specific serology tests can help with the identification and diagnosis of HSV infection. HSV-1 IgG and HSV-2 IgG assays are fully automated, two-step immunoassay for the qualitative determination of specific IgG antibodies to HSV-1 and HSV-2 in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. The objective of this multi-center study is to evaluate the performance of the HSV-1 IgG and HSV-2 IgG assays on the ARCHITECT i2000SR system.

**Methods:** A total of 915 samples from unique subjects including 297 serum samples from pregnant females were evaluated at 3 different sites, 2 sites in the US and 1 site in Spain. All samples were analyzed with the HSV-1 IgG and HSV-2 IgG assays on the ARCHITECT i2000SR system for qualitative method comparison with HerpeSelect 1 and 2 Immunoblot IgG. Western Blot (University of Washington, US) analysis was used to resolve the status of the samples that exhibited equivocal profile with HerpeSelect 1 and 2 Immunoblot IgG. Sensitivity and Specificity were calculated, including their confidence interval (CI) at 95% level. Additionally, 3 serum and 3 plasma pooled samples at different concentration of HSV-1 IgG and HSV-2 IgG were tested at 3 sites over 5 days to evaluate the reproducibility of the HSV-1 IgG and HSV-2 IgG assays.

**Results:** For the HSV-1 IgG assay, the evaluation indicated a Sensitivity of 95% (95% CI: 93% - 96%) and a Specificity of 98% (95% CI: 95% - 99%) when compared to HerpeSelect 1 and 2 Immunoblot IgG. For the HSV-2 IgG assay, the Sensitivity was 94% (95% CI: 91% - 96%) and Specificity was 97% (95% CI: 96% - 98%). Both assays demonstrated good reproducibility, with a coefficient of variation (%CV) of 3.6 - 5.1% for HSV-1 IgG assay and a %CV of 4.0 - 5.4% for HSV-2 IgG assay.

**Conclusion:** The HSV-1 IgG and HSV-2 IgG assays demonstrated good performance on the ARCHITECT i2000SR system and compared favorably with HerpeSelect 1 and 2 Immunoblot IgG method, supporting their use as an aid in the presumptive diagnosis of HSV-1 and HSV-2 infection.

\* The products have not been cleared/approved by the FDA.

## B-222

**A Novel, Easy to Use, Non-invasive Alternative to Nasopharyngeal Swabs that Provides Equivalent or Better Sensitivity for Nasopharyngeal Specimen Collection**

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For upper respiratory tract (URT) infections (e.g. influenza, COVID-19, and Pertussis), the specimen of choice is a nasopharyngeal (NP) specimen collected either by a swab, suction, or wash done by a clinician. Because these procedures are invasive and uncomfortable, a "2nd best specimen" such as mid-turbinate or nasal swab is often used. We present an alternative method for obtaining the NP specimen of choice (the gold standard) that is non-invasive, provides far more material for analysis, is highly sensitive, and provides results at least equivalent to the NP swab.

The NEMo (Nasopharyngeal Extraction MethodOlogy) NP specimen collection device provides a simple, accurate, comfortable, and easy to use solution for collecting URT specimens. With a quick squeeze of its reservoir bottle, the NEMo collects an NP wash from the nasal cavities directly into a tube ready for analysis. The resulting liquid NP specimen represents a site with the highest viral load and thus increases sensitivity of testing for PCR and rapid antigen tests alike.

We compared the clinical accuracy and user experience for the NEMo versus NP swabs, mid-turbinate swabs, and saliva specimen collection. The study enrolled 42 patients with symptoms of or possible exposure to COVID-19 across two sites with 23

of the patients testing positive (PCR) for COVID-19. For those patients, NEMo had a 100% detection rate compared to 90% for both NP and mid-turbinate swabs with one patient testing positive using the NEMo and negative for both swabs (see table). Ct counts found similar viral loads for these three collection methods, while the saliva collection performance was substandard. The majority of patients preferred using the NEMo as compared to saliva and the two swabs.

The NEMo device has been shown to be as sensitive as the NP swab for sample collection for COVID-19 and other URT pathogens.

WASH-IT NEMO Study Clinical Results (100% detection & highest ranking in survey)			
Collection Method	COVID Detection	Patient Preference (ranked as most preferred method)	Order of Collection
NEMo	23/23 (100%)	25/42 (60%)	4
NP swab	21/23 (91%)	4/42 (10%)	1
Mid-turbinate swab	21/23 (91%)	3/42 (7%)	2
Saliva	3/23 (13%)	10/42 (24%)	3

## B-223

**Prevalence of antibodies to SARS-CoV-2 in Maryland patients following the emergence of the Omicron variant**

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**Background:** As the SARS-CoV-2 pandemic enters its third year and the Omicron variant case numbers wane in Maryland and across the US, vaccine induced immunity and immunity due to natural infection are still being investigated. Studies have shown differential responses to vaccination and natural infection based on disease severity, numerous co-morbidities and causes of immunocompromised states. Population level studies of antibody status can aid in understanding overall risk and levels of immunity. Serosurveys in 2020 and early-mid 2021 (prior to widespread vaccination) showed low levels (~5%) of anti-SARS-CoV-2 antibodies in most of the US and worldwide populations. With vaccines now widely available in the US and breakthrough infections seen with the Omicron variant, current rates of antibodies to SARS-CoV-2 are not well characterized. In this study, we aimed to determine the prevalence of antibodies to SARS-CoV-2 in patients presenting to University of Maryland Medical Center (UMMC) in Baltimore, Maryland.

**Methods:** Remnant plasma and serum samples collected from 836 in-patients and out-patients at UMMC were tested for total (IgA/IgM/IgG) anti-SARS-CoV-2 antibodies. Assays targeting antibodies to SARS-CoV-2 full-length Spike, Receptor Binding Domain (RBD) of Spike, and Nucleocapsid proteins were employed to determine seroprevalence of SARS-CoV-2 antibodies from vaccination and natural infection.

**Results:** The Anti-Spike (97.6% sensitivity; 100% specificity) and Anti-RBD (96.8% sensitivity; 99.2% specificity) enzyme-linked immunosorbent assays (ELISA) were validated previously. An anti-nucleocapsid ELISA was developed and determined to be 95% (36/38) sensitive in SARS-CoV-2 infected patients (>15 days to 8 months since symptom onset) and 98.7% (157/159) specific in samples collected pre-2019. Four in-house prepared controls were included on each run (%CV: 3.9-6.0). Testing found that 90.1% ±2.7 (411/456) of samples had antibodies targeting Spike, 86.8% ±2.3 (718/836) to RBD, and 36% ±3.2 (298/836) to Nucleocapsid. Spike and RBD assays showed 95% concordance. Nucleocapsid antibodies were found in absence of RBD or Spike in 5.3% ±2.6 (16/298) and 1.1% ±1.6 (2/175) nucleocapsid positive samples, respectively. Five samples were positive by Nucleocapsid and Spike, but negative for RBD. In the absence of Nucleocapsid antibodies, 83% ±4.33 (235/281) and 81% ±3.31 (436/538) of samples were positive for antibodies to Spike and RBD, respectively.

**Conclusion:** Slight discordance was expected due to differences in epitopes, sensitivity, and specificity, however, higher concordance between Spike and Nucleocapsid testing may indicate reduced RBD sensitivity in the context of natural infection. Overall, results indicate that >80% of the patients at UMMC have antibodies due to vaccination and over 1/3 of patients have antibodies indicating natural infection. Maryland vaccine statistics indicate 90% (72% Baltimore City) of 18+ individuals are fully vaccinated as of February 2022. Our findings are in-line with state statistics, indicating a robust immune response to vaccination with minimal waning of antibodies below detectable levels, likely due to expanded access to boosters/3<sup>rd</sup> doses. Natural infection rates have climbed, likely due to breakthrough infections, however, overall prevalence of anti-nucleocapsid antibodies maybe lower in the general population due possible sampling of patients hospitalized for SARS-CoV-2 infections.

**B-224**

**A Rapid Cellular Host Response Test May Aid ED Decision Making for Sepsis Care**

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**Background:** Emergency department (ED) physicians tasked with sepsis diagnosis often face a dearth of information that could lead to over-/undertreatment. The IntelliSep test is an investigational host response test assessing leukocyte biophysical properties from a routine blood sample in under ten minutes. The test provides a single score, the IntelliSep Index (ISI; 0.1-10.0), stratified into three discrete interpretation bands (Green, Yellow, Red) of increasing sepsis likelihood. This study investigates whether the ISI may provide additional information to aid ED diagnosis.

**Methods:** Adults presenting to the ED with clinical suspicion of infection were prospectively enrolled at multiple sites (USA; Apr. 2019 - Feb. 2020). Anticoagulated blood from each patient was tested with the IntelliSep test. We compared the ED Diagnosis of sepsis and the ISI with retrospective physician adjudication (with knowledge of ED diagnosis, blinded to ISI) for severe sepsis. SOFA scores and ED diagnoses were abstracted from the medical record.

**Results:** 301 subjects in the analysis had 12.6% severe sepsis prevalence. The ISI had a significantly higher PPA (Sensitivity; 97.4%) and NPV (99.3%) with a Green Band cutoff compared to ED Diagnosis at 50.0% and 92.7%. The ISI Red Band and ED Diagnosis had comparable PPV at 34.9% and 48.7%, respectively. Importantly, 18 subjects with severe sepsis and elevated ISI (Yellow or Red) did not have an ED Diagnosis of sepsis, 44% of which worsened (increased SOFA score in 2 days subsequent to presentation). Additionally, 7 subjects with ED Diagnosis of sepsis and low ISI (Green) were adjudicated as not having severe sepsis, yet 57% received broad-spectrum antibiotics and orders for blood cultures and lactate.

**Conclusion:** The ISI, a rapid, quantitative measure of host immune response, may have the potential to improve ED decisions for patients with signs or suspicion of infection.

Distribution of patients stratified by ED diagnosis, ISI Bands, and severe sepsis incidence.						
	ISI Green Band	ISI Yellow Band	ISI Yellow Band	ISI Red Band	ISI Red Band	ISI Red Band
Retrospective Physician Adjudication	ED Diagnosis Sepsis	ED Diagnosis Not Sepsis	ED Diagnosis Sepsis	ED Diagnosis Not Sepsis	ED Diagnosis Sepsis	ED Diagnosis Not Sepsis
Severe Sepsis-2 (+)	0	1	5	8	14	10
Severe Sepsis-2 (-)	7	157	8	55	5	31

**B-225**

**Humoral immunity assessment after vaccination using six serologic assays as SARS-CoV-2 Surrogate for plaque reduction neutralization test**

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**Background:** Detection of SARS-CoV-2 neutralizing antibodies (NAbs) is critical to evaluate herd immunity and monitor vaccine efficacy against SARS-CoV-2.

**Methods:** Quantitative SARS-CoV-2 antibody levels after vaccination were measured in two chemiluminescent immunoassays (CLIAs) (Roche, Siemens), two enzyme immunoassays (EIAs) (LG RBD, LG S1) and two surrogate virus neutralization tests (sVNTs) (GenScript, Genbody), and compared to plaque reduction neutralization test (PRNT) results. Sequential blood samples were collected before and after 1 month and 3 months vaccination in 30 healthy participants (two-dose of Oxford-AstraZeneca (AZ) (n = 15) or Pfizer-BioNTech (BNT)(n = 15).

**Results:** All sera (n=60) collected after two-dose vaccination were positive for PRNT with NAb titers ranging from 1:10 to 1:723. BNT vaccine group had higher median NAb titers compared to AZ vaccine group at both 1 month (P = 0.002) and 3 months after vaccination (P = 0.003). Overall concordance rates between serological assays and PRNT were excellent with 97.8 - 100% (p < 0.01). In correlation analysis using quantitative results, sVNTs showed strong correlation with PRNT (GenScript r=0.773, Genbody r=0.739, P <0.0001) and there was also a strong correlation between two assays (r=0.853, P<0.0001). Four binding antibody assays (2CLIA,2 EIA)

showed also significant correlation with PRNT (r=0.571 - 0.690, P<0.001) and between them (r =0.619 -0.904, P<0.001). In ROC curve analysis to detect PRNT50 1:10 positivity, all six assays showed good performance (AUC >0.900) and sVNTs showed higher AUC values.

**Conclusion:** The serologic assays presented in this study could be used as surrogate neutralization test to evaluate the immunization effect of SARS-CoV-2 vaccine.

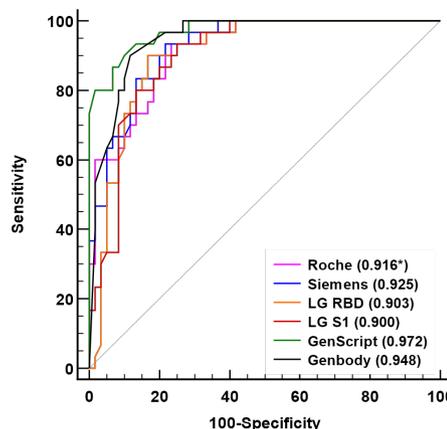


Figure 1. Comparison of ROC curve analysis to predict PRNT positivity (>=1:10) for six serological assays \*AUC results

**B-226**

**Identification and validation of Flagellar attachment zone 1 protein as a potential biomarker for Human African Trypanosomiasis**

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**Objective:** Neglected tropical diseases are a distinct group of tropical infections that occur commonly in economically depressed regions. Human African Trypanosomiasis (HAT) a parasite, also known as African sleeping sickness, is prevalent in the Sahara region of Africa. As of the most recent survey in 2018 an estimated 1,000 people suffered from this disease sometimes resulting in death. The objective of this current research is validation of proteins identified that are essential for HAT.

**Methods:** Previously a pull-down assay using a biotin-conjugated drug identified a set of proteins that belong to the parasite Trypanosoma brucei. These proteins were identified by an in-solution trypsin digestion and mass spectrometric identification of peptide sequences using an orbitrap trap mass spectrometer. The pull-down assay used a parasite cell line (lister 427) at an amount of 1 X 10<sup>7</sup> per milliliter. The cells were sonicated for 10 cycles at 5 seconds a cycle with a 30 second cycle break at 0° C. After disruption of the membrane by sonication the lysate was incubated with the biotinylated conjugate for one hour. Followed by introduction of streptavidin beads acting as the stationary phase allowed non-covalent interactions between the biotinylated targets and the streptavidin beads. A series of wash took place to remove any non-specific binding and was followed by introduction of pure biotin to competitively knock off the biotinylated ligand protein complex. The proteins where reduced and alkylated with DTT and iodoacetamide then underwent a in solution trypsin digestion. The sample was then ran on a Orbitrap elite mass spectrometer system. In confirmation of one of the proteins that were pulldown, a transfected cell line was generated using PCR cloning. The pulldown was repeated, and western blotting was used to confirm the protein interaction via HA antibody to the generated transfected cell line. Additionally, an FITC-generated conjugate was synthesized to visually the interaction *in vivo* via immunofluorescence. **Results:** Proteins were identified by an in-solution trypsin digestion and mass spectrometric identification of peptide sequences using an orbitrap trap mass spectrometer. Flagellar attachment zone protein 1 was confirmed to have an interaction with the compound of interest. **Conclusion:** A pull-down assay is a valid method to understand interactions at the molecular level allowing for targets of interest to be identified. Pairing the method with mass spectroscopy allows for the detection of proteins and in this study, we have identified 7 parasite specific proteins and validated one of the 7 proteins. These proteins are potential plasma biomarkers for diagnosing a HAT infection.

## B-227

## Longitudinal post immunisation serology of covid 19 using the Abbott Alinity SARS-CoV-2 IgG II quantitative test

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**Background:** Since the pandemic started in late 2019, serological tests have been developed to detect antibodies against the virus. Authorized COVID-19 mRNA vaccines also induce the production of antibodies against the RBD domain of the spike protein. These Antibodies can therefore be detected using a specific assay for this domain while assays directed against antibodies to the nucleocapsid are likely detected only after natural infections.

**Methods:** Health care workers (n=97) at a tertiary care centre (Norwich, UK) who were scheduled for vaccination with 2 doses of BNT162b2 were invited to participate in the CALM (COVID Antibody Longitudinal Monitoring, IRAS No. 292799) prospective cohort study. Serologic testing was performed prior to vaccination as well as weekly after the first dose and monthly after the second dose (between Jan 2021 and March 2022). Abbott Alinity i system Immunoassays (Abbott Park, IL, USA) were used to measure SARS-CoV-2 anti-spike IgG (IgGII Quant, quantitative) and anti-nucleocapsid IgG (qualitative) in serum. Both assays were fully validated for performance prior to starting the study. Of critical importance for longitudinal studies, the spike assay is a quantitative assay that is linear over a wide dynamic range of 21 to 40,000 AU/mL. Positivity cut-off as per manufacturer's instruction is 50 AU/mL. Qualitative results are positive for index results above an Optical Density Index (S/C) of 1.4.

**Results:** The Abbott IgGII Quant assay permitted the longitudinal serological evaluation of SARS-CoV-2 immunisation. A complementary test for nucleocapsid antibodies confirmed the presence of the anti-spike IgG to be due to immunisation and not natural infection. We observed a significant increase in anti-spike IgG antibody titer at 2 weeks with a peak serological response at about week 3 (peak median of about 1300 AU/mL) after the first vaccine dose. Antibody titers then decreased progressively but did not reach negativity prior the second vaccine dose (approx. 12 weeks) was applied per UK guidelines. The second dose of vaccine elicited a far stronger immune response (peak median of 22,000 AU/mL) which lasted for a far longer period. After 6 months, the levels of anti-Spike IgG are still higher than the peak response of the first dose (median at 6 months of about 2500 AU/mL). The response to immunization was affected by Vitamin D status, especially in young adults after the first dose suggesting a beneficial effect of Vitamin D.

**Conclusion:**

The Abbott IgGII Quant assay for SARS-CoV-2 is useful for population-level postimmunization surveillance. Associated with an anti-nucleocapsid antibody test, kinetics of the immune response can be used to evaluate reinfections, ie long COVID syndrome.

## B-228

## Bacterial identification and antimicrobial susceptibility testing directly from positive blood culture vials using two commercial platforms

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**Background:** Blood cultures (BC) remain the gold-standard method for the microbiology diagnostic of bloodstream infections. The aim of this study was to evaluate the performance of a commercial automated system using two panels to define the antimicrobial susceptibility profile of Gram-negative and Gram-positive pathogens directly from the BC vial applying *in-house* protocols. **Methods:** From September to December 2021, we analyzed aerobic BC reported as positive by the Bactec 9240 automated system (BD). The sample preparation for bacterial identification on the Vitek MS® and the direct inoculation to automated susceptibility testing (AST) on the Vitek®2 systems were performed using two *in-house* protocols based on trifluoroacetic acid 0.1% and three-step centrifugation methods. In parallel with the direct inoculation, an aliquot of each positive BC was used to prepare a Gram stain, as well as to inoculate in two culture mediums. All the BC samples subcultured on agar plates were also processed by the conventional protocols to ID and AST using the Vitek®2 system, according to the manufacturer's recommendations. To compare the results from direct with conventional AST protocols, the MIC value obtained from both

methods were reclassified as susceptible, intermediated, and resistant according to CLSI clinical breakpoints. The categorical agreement rates, very major error (VME), major error (ME), and minor error (mE) rates were calculated for each antibiotic. **Results:** One hundred twenty-four positive BC bottles were evaluated in this study. Out of these, 61 were identified as Gram-negative and 63 as Gram-positive pathogens, consisting of 36 Coagulase Negative *Staphylococcus* (CNS), 17 *Staphylococcus aureus*, 9 *Enterococcus faecalis*, 1 *Enterococcus faecium*, 8 *Acinetobacter baumannii* complex, 1 *Acinetobacter junii*, 7 *Pseudomonas aeruginosa*, 1 *Pseudomonas putida*, 1 *Pseudomonas oryzae*, 15 *Klebsiella pneumoniae*, 2 *Klebsiella aerogenes*, 13 *Escherichia coli*, 6 *E. cloacae*, 4 *Proteus mirabilis*, 2 *Providencia stuartii* and 1 *Serratia marcescens*. The rapid AST protocol by direct inoculation was highly correlated with the conventional protocol for 27 antibiotics, exhibiting 95.34% (981/1029) of the agreement rate. VME, ME and mE were detected in 1.36% (14/1029), 2.04% (21/1029) and 1.26% (13/1029) of the antibiotics analyzed, respectively. The VME and ME were detected mainly among Gram-negative and Gram-positive pathogens, respectively. **Conclusion:** The preliminary results showed high feasibility of the rapid susceptibility testing by automated system directly from positive BC using a simple, fast and inexpensive *in-house* protocol able to shorten the time-to-result for up to 24 hours in cases of BC diagnostics.

## B-229

## Epidemiological Surveillance of New Delhi Metallo-Beta-lactamase-producing Enterobacteriales at Brazilian hospitals in pre and Covid-19 period

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**Background:** New Delhi Metallo-β-lactamases (NDM)-producing has been progressively identified in carbapenem-resistant Gram-negative bacteria on all continents, representing a significant challenge for clinical management and public health worldwide. In this scenario, the inappropriate use of antibiotics intensified by the COVID-19 pandemic has opened a great warning about the real impact that this pandemic can have on long-term antimicrobial resistance rates. The aim of this study was to describe the increase of NDM-producing *Enterobacteriales* clinical isolates recovered from Brazilian hospitals comparing pre-Covid-19 (2018-2019) period versus Covid-19 (2020-2021) period.

**Methods:** This study was a retrospective analysis from January 2018 to December 2021 based on microbiological and molecular data of carbapenem-resistant *Enterobacteriales* (CRE) isolates, detected in several Brazilian tertiary hospitals. The AFIP Laboratory has detected CRE isolates with positive screening assay of Metallo-β-lactamase producer, using commercially available disks containing ertapenem, imipenem and meropenem with and without EDTA (0.1 M). The bacterial identification was performed by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) and the minimum inhibitory concentration (MIC) of all antibiotics was determined using Vitek®2 System, except for polymyxin B which was by broth microdilution method. The detection of the carbapenemase (*bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>GES</sub> and *bla*<sub>OXA-48-like</sub>) and ESBL genes were determined by Real-Time PCR.

**Results:** A total of 450 NDM-producing *Enterobacteriales* were analyzed at the period. Then, the frequency of isolates detected in pre-Covid-19 period (100/450; 22.2%) and Covid-19-period (350/450; 77.8%). The top five microorganisms' prevalence detected in pre-Covid-19 and Covid-19 period were as follow: 316 *Klebsiella pneumoniae* (24.6%; 75.3%), 64 *Proteus mirabilis* (6.3%; 97.7%), 21 *Providencia rettgeri* (33.3%; 66.7%), 16 *Enterobacter cloacae* (18.8%; 81.2%) and 10 *Escherichia coli* (10%; 90%). Twenty-three samples belonged from multiple *Enterobacteriales* species (30.4%; 69.6%). These microorganisms were isolated from rectal swab (n=143), urine (n=128), blood (n=72), catheter tip (n=44), fluid secretion and bronchoalveolar lavage (n=23), skin and soft tissue (n=20) and others (n=20). In all of them, high-level of resistance were detected to ceftazidime, ceftriaxone, cefepime, ertapenem, imipenem and meropenem. Between *K. pneumoniae* isolates, 42.6% and 53.7% were resistant to amikacin and gentamicin, respectively. All 450 *Enterobacteriales* isolates showed screening as possible MβL producers. All of them harbored *bla*<sub>NDM</sub> gene and one *K. pneumoniae* also harbored *bla*<sub>KPC</sub> gene. The presence of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> genes were detected in 86%, 77.2% and 83% of the strains tested, respectively.

**Conclusion:** All the strains harbored *bla*<sub>NDM</sub>, associated with one or more ESBLs genes and were considered multidrug-resistant. The findings suggest the occurrence and dissemination of *bla*<sub>NDM</sub>-harboring plasmid in Gram-negative bacilli isolated from Brazilian hospitals have increased sharply since 2020. The early detection of the mechanism of resistance could be very helpful for clinical outcomes, surveillance, and infection control measures.

**B-230**

**Analysis of the humoral immune response of health professionals after SARS-CoV-2 virus vaccines**

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**Background:** Given the global scenario in the fight against the COVID-19 pandemic, vaccines were developed and approved for use in the general population quickly. This has been one of the most important allies in reducing infection, viral spread, and increasing individual and group protection, especially as COVID-19 has become more severe. The most widely used vaccines in this context were CoronaVac (Sinovac), Oxford (AstraZeneca), Pfizer, and Janssen, particularly in the Brazilian population. As a result, assessing the population's immune response during the post-immunization period with each of these immunizers is critical. The purpose of this study was to evaluate the humoral immune response of health professionals in Brazil 60 days after receiving the second dose of two different vaccines.

**Methods:** For this study, 195 health professionals of both genders were voluntarily recruited before receiving AstraZeneca/Oxford or CoronaVac/Sinovac vaccines. All of them signed the informed consent and periodic blood collections were made 15 days (15D1) after the first dose of vaccines and 30 (30D2) or 60 days (60D2) after the second dose. After, we evaluated the levels of IgG and IgM antibodies of participants' serum using chemiluminescent microparticle assay (CMIA). To do so, SARS-CoV-2 IgG II Quant assay (CE marked) or SARS-CoV-2 IgM was performed at the Abbott Alinity i platform according to the manufacturer's instructions. Antibody levels were compared by Kruskal-Wallis analysis of variance (ANOVA) with Dunn's multiple comparisons test and the same time point comparison among vaccines was made by two-sided Mann-Whitney U-test. \*p<0.05 were considered statistically significant.

**Results:** Oxford vaccinated individuals had higher serological IgG titers during 15D1 than 30D2 (17254 ± 7414 vs 2538 ± 286,9), (AU/mL, mean±SEM). On the other hand, Coronavac vaccinated participants significantly increase IgG titers 30 days after the second dose (1756 ± 195,8 vs 1029 ± 235). When we compared both vaccines' IgG serological induction, it was observed that Oxford vaccinated participants showed increased titers of serum IgG production during all three periods evaluated. Finally, we found that Coronavac IgM titer statistically differs from Oxford IgM during 30 and 60 days after the second dose. No differences in serum antibody production were observed in our gender-based evaluation.

**Conclusion:** We can build an overview of the longevity of vaccine protection by monitoring the parameters of the immune response, which implies changes in the vaccine application protocol and the need for booster doses. Furthermore, the decline in specific antibody titers exposes the population to the risk and promotes the spread of new variants that may evade the vaccine immune response. However, antibody long period evaluation could be important to overcome this issue.

**B-231**

**The respiratory syncytial virus and Sars-cov-2 diagnostic pattern in children from São Paulo, Brazil**

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**Background:** The COVID-19 pandemic continues to be a source of concern. As the number of infected and immunized people declines, society becomes more vulnerable, and the spread of other infectious diseases such as a respiratory syncytial virus (RSV) and influenza (IAV) increases following the resumption of social activities. The goal of this study was to determine the change in frequency and seasonality of RSV, Sars-CoV2, and Influenza by analyzing data from one of the largest laboratories that attends pediatric samples collected from private and public services in So Paulo (Brazil) during 2021.

**Methods:** A total of 21.717 samples from children aged 0 to 12 years old (y.o.) were sent in for testing for three different respiratory viruses. Real-time PCR was used to detect Sars-Cov2 and chromatographic assays were used to detect RSV and influenza. We also analyzed blood samples to determine the total number of leukocytes, lymphocytes, and erythrocytes.

**Results:** 2480 samples of children ranging from 0 up to 12 years old were detected as positive for RSV, 1494 for Sars-CoV2, and 12 for 974 Influenza type A. Most of the positive results for RSV were observed in February (n=472) and March (n=771). After May, the Sars-Cov-2 positive tests begin to overlap the RSV cases until September, most of them in children from 5 up to 12 y.o. Differently, the high positive result for

RSV was in children from 0 up to 2 years old. The total leucocytes count of the RSV infected group showed a significant reduction compared to the non-infected children (10.14 mil/mm3 vs 12.52 mil/mm3 respectively) p<0.0001.

**Conclusion:** Local acute lower respiratory tract infection (ALRTI) studies are critical because they can be used to track global RSV, SarsCoV2, and Influenza outbreaks. Understanding seasonality, age dominance, clinical and immunological parameters in children under the age of 12 may aid in public health campaign strategies and the rapid development of vaccines for children.

**B-232**

**The susceptibility of the HIV seropositive population living with HBV in São Paulo, Brazil**

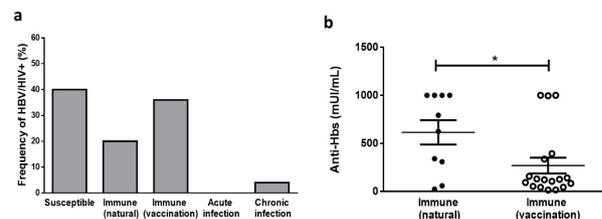
G. Costa de Carvalho, J. Terzi Maricato, J. Nogueira Martins Rodrigues, D. Ribeiro Ramadan, S. Tufik. *Associação Fundo de Incentivo a Pesquisa, São Paulo, Brazil*

**Background:** It is estimated that 257 million people worldwide were infected with the chronic Hepatitis B virus (HBV). Hepatitis viruses are responsible for liver inflammation, and people who have HBV are more likely to die from cirrhosis and hepatocellular carcinoma. HBV patients who are also infected with the Human immunodeficiency virus (HIV) have higher levels of hepatitis B viremia and develop chronic hepatitis B at the same rate as HBV patients who are only infected with HBV. The purpose of this study was to better understand the susceptibility of the HIV seropositive population living with HBV.

**Methods:** During 2020 and the first semester of 2021, we conducted an analytical and retrospective review study on a large laboratory databank in So Paulo (Brazil). The population serological profile against hepatitis and other opportunistic agents in HIV-positive adults was examined in this study.

**Results:** Using a biomarker evaluation strategy of anti-HBsAg, total anti-HBc, anti-HBc IgM, and anti-HBs in 149 HIV seropositive individuals, we discovered that 40% of subjects were susceptible to HBV infection (Figure 1a). We also found that the natural immunized HIV seropositive population had 2.28 times more Hbs antibodies than the vaccinated group (mean=614.8 mUI/MI 127.1 vs mean =269 mUI/MI 82.40) p<0.05. (Figure 1b). The most common IgG+ diagnostics discovered for another virus were for CMV (n=68).

**Conclusion:** We believe that the HBV biomarkers panel screening is critical for a comprehensive study in HIV-susceptible populations.



**B-233**

**Novel Herpes Simplex Virus, Varicella Zoster Virus, and Treponema pallidum Swab Formulation for Use with Genital Lesion Molecular Detection Assays as a Prospective Quality Control**

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**Background:**

The emergence of genital ulcer disease is becoming a global health concern, with over 20 million new cases diagnosed each year<sup>1</sup>. Molecular assays that detect Herpes Simplex Virus (HSV) 1&2, Varicella Zoster Virus (VZV), and *Treponema pallidum* (TP) - the most common etiological agents of genital ulcer disease—are evolving in clinic; however, there is limited quality control material available to support the use of these tests. While native pathogens are available for HSV 1&2, VZV, and TP, the samples are not formulated to mimic patient specimen formats, as genital lesion detection is usually performed with swab-based collection methods. Additionally, the few TP quality control materials on the market are comprised of “naked” synthetic DNA, and thus are not sufficient to control whole workflows, such as sample extraction. To our knowledge, we are the first to develop a prospective multiplex whole-workflow

control for genital lesion panels. The sample is formulated on a Copan FLOQSwab®, not only to mimic patient specimen formats, but also to ensure sample compatibility with all assay workflows and Sample Transport Mediums. The objective of this study is to confirm the performance of the multiplex HSV1&2, VZV, and TP swab formulation (herein referred to as a VHS sample) using genital ulcer detection assays.

#### Methods:

Microbix designed an inactivated multiplex VHS sample, consisting of HSV1, HSV2, VZV, and TP, desiccated on a Copan FLOQSwab®. The VHS samples were tested using the SpeedX PlexPCR VHS assay, the Seegene Allplex™ Genital Ulcer Assay, and other commercial tests currently in development. Furthermore, the VHS swab samples were used in an External Quality Assessment pilot run to verify the performance of molecular assays that detect at least one of HSV1, HSV2, VZV, and TP.

#### Results:

The VHS samples showed acceptable performance when tested with commercial genital lesion assays, commercial assays in development, and molecular assays used by clinical laboratories participating in the External Quality Assessment pilot run.

**Conclusion:** To our knowledge, we were the first to design a multiplex VHS sample desiccated on a Copan FLOQSwab®. The VHS samples showed acceptable performance on multiple platforms, thereby demonstrating their potential use as cross-platform compatible, whole-workflow quality controls, verification panels, and External Quality Assessment samples. Overall, the sample's unique composition and format ensures that clinical laboratories and original equipment manufacturers have access to high-quality whole-workflow third party control material and ultimately, can be confident in the performance of their genital lesion assays.

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## B-234

### Nationwide Surveillance Network Design and Operation for Emerging Resistant *Neisseria gonorrhoeae* in Korea

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**Background:** Gonococcal infection caused by *Neisseria gonorrhoeae* is a major public health. Keeping track of a resistance trend of *N. gonorrhoeae* is very important for choosing an appropriate antibiotics treatment and lowering antimicrobial resistance rate (AMR). However, molecular diagnostic method is now widely performed for cases of sexually transmitted infection (STI) and has replaced a traditional culture. Consequently, surveillance of antimicrobial resistance of *N. gonorrhoeae* has been extremely limited and it is very difficult to state country report to WHO Global Antimicrobial Resistance Surveillance System (GLASS). We planned a sustainable Gonococcal Isolation Network (GIN) in Korea.

**Methods:** The proposed GIN was organized by Research Institute of Bacterial Resistance (RIBR) in Yonsei University College of Medicine. This GIN had two different systems for collection of *N. gonorrhoeae*. First, genital specimens were obtained from STI clinics, which were widely distributed across Korea, and were transported to RIBR by courier service. Second, big independent clinical laboratories (ICLs) which contracted with private clinics and having own specimen transfer logistics were enrolled. Location of sentinel STI clinics were adequately allocated by population and number of patients from national health insurance data. Gonococcal cultures were done on modified Thayer Martin agar and Chocolate agar for 72 hours in RIBR and ICLs. Antimicrobial susceptibility of *N. gonorrhoeae* isolated was done by CLSI disk diffusion test (penicillin G, ceftriaxone, spectinomycin, tetracycline, ciprofloxacin, nalidixic acid, and cefixime). E-test was used for ceftriaxone and azithromycin. Molecular epidemiologic tests were done with *penA* genotyping, MLST and NG-MAST.

**Results:** Twelve sentinel STI clinics were allocated as 1-4 clinics in six metropolitan cities and provinces, 4 clinics in Gyeonggi, 3 in Seoul, 2 in Daegu-Gyeongbuk, each 1 clinic in Incheon, Chungcheong and Jeolla. Six ICLs (2 in Seoul and 3 in Gyeonggi, and 1 in Incheon) were selected for collaboration of GIN. During preliminary operations, we tried 734 gonococcal culture from various provinces in Korea. A total of 125 *N. gonorrhoeae* isolates were collected from patients with urethritis (91 males and 34 females). The resistance rate of penicillin was 52.8% and penicillinase-producing *N. gonorrhoeae* was 14%. The resistance rate of tetracycline and ciprofloxacin was 94.4% equally. In 2020 ceftriaxone-resistant strains were not found among Korean gonococci, but 1.6% organisms showed intermediate resistance by European EU-

CAST guideline. Common *penA* type was 10.001 (40.8%) and 34.001 (18.4%). The frequent types of MLST were ST1921 (23 isolates) and ST8123 (21 isolates). The overall performance was excellent and it showed sustainability of GIN.

**Conclusion:** Based on this nationwide GIN operation experience, we could be able to collect more than 200 isolates annually. AMR trends, emerging resistant gonococcal surveillance and molecular epidemiologic study will also be available with this network. As a result of this study, antibiotic resistance to major sexually transmitted infections in Korea is expected to increase significantly in the future and continuous monitoring is necessarily expected. In addition, these data will be reported to GLASS as a Kor-GLASS report.

## B-235

### Relationship of Time Interval from “Fully Vaccinated” and “Breakthrough” SARS-CoV-2 Infections: Observational Study in a Highly Vaccinated Population.

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**Background:** With the emergence of new SARS-CoV-2 variants combined with waning of vaccine-induced immunity, there remains a concern of “breakthrough infections” occurring in vaccinated individuals. In Fall 2021, Northeastern University (NEU) adopted a policy of mandatory COVID-19 vaccination of its students, faculty, and staff as part of return to in-person instruction. In addition, weekly PCR testing is performed on its entire population. In this observational study, we looked at the incidence of “breakthrough” SARS-CoV-2 infections over a 7-month period since the first vaccine breakthrough infection. **Methods:** Vaccination status and dates were recorded based on volunteered data. 99.6% of the student population and 97.7% of the faculty/staff were considered “fully vaccinated” as defined by the CDC guidelines (2 weeks after their second dose in a 2-dose series, such as the Pfizer or Moderna vaccines, or 2 weeks after a single-dose vaccine, such as Johnson & Johnson’s Janssen vaccine). For the 7-month period between July 2021 and January 2022, ~30,000 individuals underwent weekly SARS-CoV-2 RT-PCR testing using the TaqPath™ COVID-19 Combo Kit. “Breakthrough” infection was defined as any positive test for SARS-CoV-2 infection occurring in an individual who is “fully vaccinated” based upon SARS-CoV-2 diagnostic testing data from the Life Science Testing Center. **Results:** During the 7-month period, a total of 446 “breakthrough” SARS-CoV-2 infections were recorded. 2 cases were recorded in July 2021, and from August-November 2021 (<6 months post “fully vaccinated”), which coincided with dominance of the Delta variant, a monthly average of 22 cases were recorded. For the months of December 2021 and January 2022 (≥6 months post “fully vaccinated”), 185 and 171 cases were recorded respectively. Approximately 50% of all breakthrough cases were observed between 180 and 240 days post full vaccination. All vaccines displayed the same trend of efficacy with infections spiking after 6 months. This was also associated with concurrent rise in SARS-CoV-2 cases with S-Gene Target Failure indicating the predominance of Omicron. However, majority of the cases were either asymptomatic or associated with mild disease. **Conclusions:** The increased transmissibility of Omicron may be primarily responsible for the significant increase in “breakthrough” infection rates with waning immunity post-vaccination playing a secondary role.

## B-236

### SARS-CoV-2 Seroprevalence in Rhode Island pregnancies in early 2020

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**Background:** The purpose of this study was to estimate the prevalence of antibodies against SARS-CoV-2 in pregnant women in Rhode Island during early 2020 and to examine whether the prevalence differed by month of collection, age, county, or economic status as estimated by zip code. The hypothesis was that the incidence of SARS-CoV-2 seropositivity on the assays utilized would increase over the study period and would be highest in Providence County, the most densely populated county in the state of Rhode Island, when compared to other RI counties, and that seroprevalence would be highest in the zip codes with lowest income levels.

**Methods and Study Design:** Residual serum samples collected during prenatal screening between 15 and 22 weeks of gestation in the Division of Medical Screening and Special Testing were available in freezer storage for testing. Samples were linked to select demographic variables including age at collection, zip code and county of

residence. Seven hundred and fifty-six pre-pandemic samples (collected Feb – June 2019) and 787 pandemic period (2020) samples (collected March – June 2020) were analyzed. Two distinct SARS-CoV-2 immunoglobulin G (IgG) automated assays, that targeted either the viral nucleocapsid or spike (receptor binding domain) proteins, were used. A “seropositive” result was defined, for the purposes of this study, as a positive finding for both IgG assays. False positive rates were estimated by comparing to seropositivity rates from residual sera collected in early 2019, prior to the circulation of SARS-CoV-2. Results were stratified by month of collection, Providence or other county, and by income as estimated by zip code of residence. IgM and repeat IgG testing of select samples was also performed.

**Results:** Seven hundred and fifty-six pre-pandemic prenatal specimens from 2019, and 787 prenatal specimens from 2020 were analyzed. Among 756 samples from 2019, 1 was positive for anti-spike protein receptor binding domain (anti-S) IgG antibody and 13 were positive for anti-nucleocapsid antibody (anti-N) IgG. No samples were positive for both anti-S and anti-N antibodies. Two specimens were positive for anti-N IgM, however these values were close to the cut-off value at Among 787 specimens from 2020, 16 (2.03%) were positive for both anti-N IgG and anti-S IgG. When stratified by month of collection, there was a significant increase in seropositivity rate ( $p=0.023$ ). There was no significant trend in seropositivity by age of subject ( $p=0.70$ ). There was a trend toward higher seropositivity rate in lower income levels as estimated by zip code ( $p=0.08$ ) but this did not reach statistical significance. Providence County had a significantly increase seropositivity rate when compared to non-Providence County ( $p=0.03$ ).

**Conclusions:** Serology can play a role at the population level in monitoring the rates of infection. There is a lack of information on the performance of IgG assays in pregnancy. When a positive result was defined as having both anti-N IgG and anti-S IgG, the test performed well. Serology could be utilized to monitor infection trends in a population, including during pregnancy.

### B-238

#### Vitamin A and vitamin E in SARS-CoV-2 Infection: An Overview

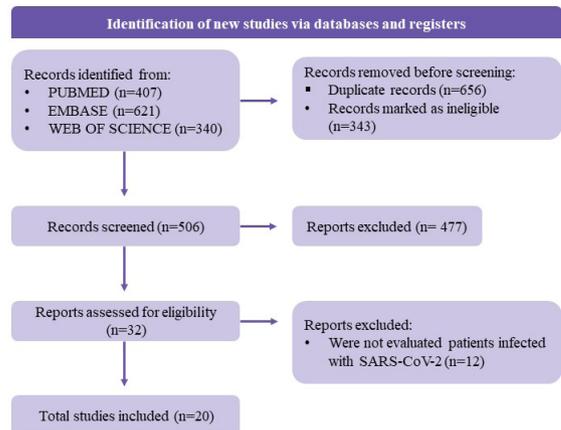
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**Background:** COVID-19 is a disease caused by SARS-CoV-2, a new coronavirus first identified in Wuhan (China) and has spread rapidly worldwide, affecting the immune system, and causing exacerbated inflammation. Viral infections can deplete vitamins such as vitamins A and E, and inadequate nutrient levels can be harmful to health. The aim of this study is to investigate whether COVID-19 affects the levels of these vitamins, and how they can help in fighting the infection.

**Methods:** A systematic search was performed according to the PRISMA statement using three different databases. The data from the articles were collected and analyzed by two independent researchers. The quality of in vivo studies was also assessed.

**Results:** A total of 1,505 articles were retrieved, of which 20 met all of the inclusion criteria. Eleven of these articles were in silico and/or in vitro studies, and nine were in vivo studies. Adult and older adult patients, including pregnant women with COVID-19, had decreased vitamin A and E concentrations, being below or close to the reference values for deficiency, but not in children. Supplementation of these vitamins was associated with better patient prognosis. Experimental studies have shown that these vitamins may be targets against SARS-CoV-2 infection and Retinoic Acid-inducible gene I (RIG-I) has an important role in combating the disease by different mechanisms.

**Conclusion:** Although associations between vitamins A and E and SARS-CoV-2 infection were found, the therapeutic capacity of these vitamins requires additional biological validation through more robust studies, such as randomized clinical trials and assessment of food intake. Furthermore, studies that assess serum levels of these vitamins before infection are required.



### B-239

#### MeMed BV Distinguishes Between Viral and Bacterial Infection in Sepsis Patients

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#### Background:

Sepsis is a life-threatening organ dysfunction syndrome caused by the body's response to infection. Timely and appropriate sepsis management, including appropriate treatment of bacterial infection, improves outcomes. MeMed BV (BV), a test for differentiating between bacterial and viral infection, is based on computational integration of the circulating levels of three proteins (TRAIL, IP-10, CRP). Here we evaluate its ability to differentiate bacterial from viral infection in sepsis patients.

#### Methods:

This was a sub-analysis of sepsis patients recruited prospectively in the Apollo study (NCT04690569). Apollo eligibility required the attending physician's clinical suspicion of acute infection and reported fever. Sepsis was defined as two or more SIRS criteria and a suspected bacterial or viral infection classified by expert adjudication. A bacterial or viral classification required at least 2/3 experts to assign the same etiology label with confidence  $\geq 90\%$  or all 3 assign with confidence  $\geq 70\%$ . BV was measured using a platform generating a bacterial likelihood score (0-100). Based on pre-defined thresholds, scores 0-34 indicated viral (or other non-bacterial) infection, scores 35 to 65 were equivocal and 66-100 indicated bacterial infection (or co-infection). BV performance was assessed against expert panel classifications.

#### Results:

Seventy-nine out of 1016 eligible Apollo patients had missing heart rate and respiration rate data and a further 136 could not be classified by the experts. Out of the remaining 801 patients, 217 adults with median age of 41.8 years (IQR: 29.2-61) and 149 children with median age of 2.4 years (IQR: 1.4-5.4) had 2 or more SIRS criteria. 119 patients had at least 3 SIRS criteria and 39.6% (145/366) of the patients were hospitalized with a median duration of 4 days (IQR: 3-6 days). In the sepsis cohort, 91 patients were classified as bacterial and 275 as viral. BV yielded sensitivity and specificity of 98.8% (95%CI: 93.6-100) and 89.7% (95%CI: 85.3-93.2) and NPV of 99.6% (95%CI: 97-99.9), outperforming PCT (cut-off 0.5 ng/mL; sensitivity 52.8% (95%CI: 42-63.3); specificity 86.2% (95%CI: 81.5-90); NPV 84.6% (95%CI: 81.5-87.3)).

**Conclusion:**

BV accurately distinguished bacterial from viral infection in sepsis patients. This new triage tool has potential to help with timely identification of bacterial infection, enabling prompt treatment.

**B-240****Simultaneous Detection of Respiratory Infectious Diseases using Immunoprecipitation and Liquid Chromatography-Tandem Mass Spectrometry**

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**Background:** With recent emergences of new infectious diseases and their variants, there is a need to develop a faster and more accurate analytical tool that can differentiate infectious diseases such as SARS-CoV-2 and influenza viruses. Among different viral components, nucleocapsid protein or nucleoprotein (NP) is highly conserved and specific for infectious disease virus types. Therefore, targeting NP could provide a more robust and simpler way for disease identification. This study describes a targeted approach for simultaneous detection of NPs from different respiratory infectious diseases using immunoprecipitation (IP) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

**Methods:** Equal amounts of all biotinylated antibodies were pooled together as one antibody panel for this study. The biotinylated antibody panel was added to samples collected via nasopharyngeal swabs in viral transport media (VTM) followed by incubation for 15 minutes at 25 °C with rotation at Multimix Tube Rotator. The antigen-antibody complex in VTM was directly subjected to IP using Thermo Scientific™ Pierce™ MS-Compatible IP Kit (Streptavidin). The IP purified samples were then digested for 15 minutes at 70 °C with vortexing at 1000 rpm using SMART Digest™ Trypsin Kits and analyzed by Thermo Scientific™ Vanquish™ MD HPLC system hyphenated to Thermo Scientific™ TSQ Altis™ MD mass spectrometer. Data processing was performed using TraceFinder™ LDT 1.0 software.

**Results:** Multiple viruses, SARS-CoV-2, influenza virus A and B types, respiratory syncytial virus, and human coronavirus (HCoV-229E), were selected to show that this method can distinguish different disease viruses. The IP uses a specific antigen-antibody interaction and provides a highly targeted and confident detection of each disease. The workflow was optimized from sample preparation to LC-MS analysis. The protein precipitation and post sample clean-up were eliminated. From IP procedure, two incubation steps for antigen-antibody complex formation and immobilization on the magnetic beads were reduced to 15 minutes each (originally 1 hour each). Trypsin digestion incubation time was optimized to 15 minutes (previously 90 minutes). Particularly, the reduction of trypsin digestion time was achieved owing to a generation of much cleaner sample matrix by IP. The entire process was finalized to less than 1 hour from 6 hours. LC-MS run time was also optimized to 5 minutes. A total of 12 peptides were successfully monitored (2 to 3 peptides per disease) by SRM. Calibration curve was generated with stable isotope-labeled standards (Thermo Scientific™ HeavyPeptide AQUA Ultimate). With criteria of % RSD < 15, % CV < 15 and R<sup>2</sup> > 0.99, the LOQs were determined to be between 0.05 to 1 fmol of peptides on LC column with retention time variation ± 0.01 minutes. The method significantly improved the sensitivity and turn-around time, compared to other digestion or peptide enrichment methods. For Research Use Only - Not For Diagnostic Procedures

**Conclusion:** The IP generated clean and MS-compatible sample matrix and provided reliable quantification of 0.05 to 1 fmol of the peptides on column. This optimized and fast process increases sample throughput and ultimately expedites turn-around time.

**B-241****Direct RT-LAMP Test for the Detection of SARS-CoV-2**

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**Introduction** SARS-CoV-2 is the causative agent of the global pandemic that has incited in early 2020. COVID-19 has placed a huge burden on healthcare systems around the world. Fast, accurate, and economical diagnosis is an extremely important aspect aiding the diagnostic effort in the fight against COVID-19, reducing the transmission of the virus by efficiently capturing infected patients. To accelerate the diagnostic process, at a time of significantly increased demand for genetic diagnosis, Genomtec optimized a highly specific and sensitive, isothermal direct kit detecting SARS-CoV-2 in saliva and throat/nasopharyngeal swab specimens, omitting the standard laboratory RNA purification method enabling shorter turnaround time in a standard molecular laboratory setting.

**Materials and methods** The test methodology is based on a real-time multiplex Loop-mediated isothermal amplification preceded by a reverse transcription reaction (RT-LAMP). Three sets of primers were used in the reaction by detecting accordingly highly conservative fragments of *N* and *S* genes of SARS-CoV-2 as the multiplex LAMP, and a fragment of the *RPP30* human gene, as an inhibition control in a separate well. The assay is optimized for use directly with biological material that is saliva and/or throat/nasopharyngeal swab specimens, with minimal sample processing resulting in RNA-rich supernatant suitable to be used for directly for the amplification.

**Results** The test was fully optimized with confirmed diagnostic parameters. Particularly noteworthy is the limit of detection of the assay test that was determined as low as 2 virus copies per reaction for saliva and 10 virus copies per reaction for swab (of either S or N gene fragments targeted in the assay, with the recognition of detected gene based on HRM). Investigation of analytical reactivity and specificity was also performed. The assay successfully detects all identified variants of SARS CoV-2 classified by WHO to date (including Omicron variant). The clinical evaluation performed on clinical saliva samples from 63 patients confirmed that the assay exhibits 100% specificity and 93.75 % sensitivity compared with a standard nasopharyngeal-based laboratory Real-Time RT-PCR diagnostic test. The PPV (positive predictive value) and NPV (negative predictive value) were 100% and 93.94% respectively, whereas the test accuracy was obtained at 96.83%.

**Conclusion** Despite minimal sample handling procedures and relatively fast time to results, competing to other diagnostic SARS-CoV-2 tests, especially isothermal ones, Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit provides a fast, sensitive, and highly specific tool for SARS-CoV-2 diagnosis.

**B-242****Quantitative Multiplex SARS-CoV-2 IgG Antibody Test for Measuring Natural and Vaccine-Induced Immune Response**

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**Background:** COVID-19 vaccination has been vital to control the pandemic; however, several studies have reported a waning immunity to vaccines over time. Circulating IgG antibody levels are a good indicator of vaccine-induced immunity. The development of reliable methods for quantifying circulating IgG in serum and plasma is critical to monitoring the durability of the immune response. Currently available serological tests provide a qualitative result for the presence or absence of antibody reactivity to a single antigen in a sample. It is essential to quantify the antibody levels against more than one antigen of the SARS-CoV-2 virus to understand the kinetics of antibody response. We have developed a multiplex assay to simultaneously quantify antibody responses to 4 different antigens of SARS-CoV-2 in a single test.

**Methods:** This multiplex assay utilizes Luminex® xMAP® technology, where we 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. This assay includes four internal controls to monitor each step of assay performance. Using the WHO quantification standard, we have quantified IgG response to the four antigens, RBD, NP, S1, and trimeric spike protein. We assessed 94 serum or plasma samples from 32 vaccinated individuals immunized by different vaccines, including Moderna mRNA-1273, Pfizer/BioNTech BNT162b2, Janssen (Johnson & Johnson), Ad26.COV2.S. This sample set includes five samples from vaccine breakthrough infection cases. We also had 81 negative samples that were not vaccinated and collected between 2017-2018.

**Results:** Samples from uninfected or unvaccinated individuals showed negative to a low level of reactivity in the multiplex assay. We determined the threshold levels for each of the four antigens based on the uninfected sample set from 2017-2018. Varying levels of IgG antibody response were measured in vaccinated samples to RBD, S1, and trimer, with very little reactivity to nucleocapsid protein. Samples for natural infection showed significant levels of IgG antibody response to nucleocapsid protein, and these levels diminished over time faster than the three spike protein antigens.

**Conclusion:** This multiplex quantitative IgG test provides a valuable tool to quantify the circulating IgG antibodies in case of infection and vaccination. This assay may measure vaccine immunogenicity and assess the durability of immune response after vaccination.

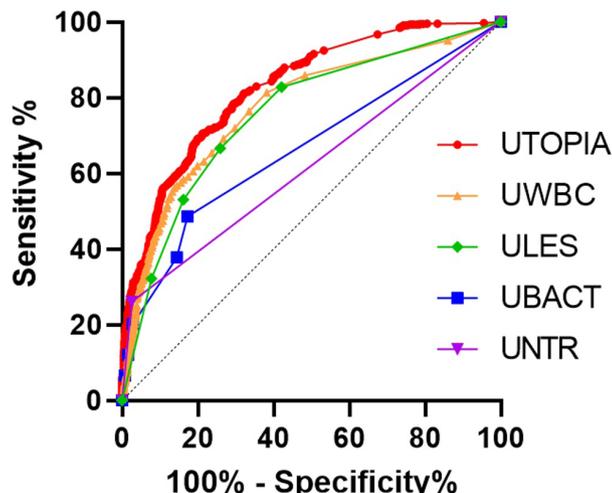
**B-243**

**Evaluation of a prediction algorithm value in predicting positive urine culture in pediatrics: a retrospective cohort study at Nationwide Children’s Hospital**

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**Background:** Urinary tract infection (UTI) is a common public health issue. The gold standard for diagnosing UTI is urine culture, which is labor intensive and time consuming. Many clinicians therefore rely on urinalysis results as predictors of urine culture positivity. This study aimed to investigate the association between urinalysis results and those of concurrent urine cultures, and to assess the accuracy of a published prediction model for the results of urine culture in a pediatric population. **Methods:** This was a retrospective chart review of 5,535 patients carried out at Nationwide Children’s Hospital from January 2019 to December 2020. Dipstick and microscopic sediment urinalysis were performed on iQ200. Positive urine culture was defined as  $\geq 50,000$  colony-forming units per milliliters of a urinary pathogen. A novel prediction algorithm called the UTOPIA value =  $1 \div (1 + e^{(2.803456 - 0.727126x1 - 0.336935x2 - 1.325869x3 - 0.531561x4 - 0.000121x5 - 0.004186x6)}) \times 100$ , initially proposed by Kim, et al, was assessed based on an association between the results of urinalysis and those of concurrent urine culture (Kim D et al., Sci Rep. 2021 Mar 16). Six variables were selected for the UTOPIA equation: x1:age UTI risk, x2:sex, x3:nitrite, x4:leukocyte esterase (LE), x5:white blood cell count (WBC) and x6:grade of bacteria with x2-x4 and x6 being categorical. **Results:** The study population had a median age of 7 years (mean=9), was 69% female, and of those with positive urine cultures, 85.2% were female. The UTOPIA value exhibited an area under the ROC curve value of 0.825, which is significantly higher (P<0.001) than the individual components for ages 0.546 (0.52-0.57), sex 0.575 (0.56-0.59) nitrite 0.618 (0.60-0.63), LE 0.757 (0.74-0.78), the grade of bacteria 0.664 (0.64-0.69), and the WBC count 0.776 (0.75-0.80). **Conclusion:** The UTOPIA value demonstrated good diagnostic performance for predicting urine culture results and possibly reducing unnecessary urine culture and antibiotic use in a retrospective pediatric population.

**Comparative ROC Plots**



Test	AUC	Std Er	95% CI
UTUPIA	0.825	0.009	0.81 to 0.84
UWBC	0.776	0.011	0.75 to 0.80
ULES	0.757	0.011	0.74 to 0.78
UBACT	0.664	0.011	0.64 to 0.69
UNTR	0.618	0.009	0.60 to 0.63

**B-244**

**Performance of ADVIA Centaur SARS-COV-2 Antigen Testing Compared to Nucleic Acid Testing Across Patients with Multiple Variants in an Urban Medical Center**

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**Background:** The ongoing COVID-19 pandemic has created supply chain deficits and the need to rapidly alter testing strategies, including use of SARS-COV-2 antigen (COV2Ag) testing to supplement nucleic acid testing (NAAT) and expand screening efforts. We leveraged banked samples to assess the performance and understand the limitations of high-throughput COV2Ag testing compared to nucleic acid testing in a large urban hospital system.

**Methods:** A cohort of 346 nasopharyngeal samples was stored at -80°C in viral transport media and collected in time periods from January–October 2021 (pre-alpha, alpha B.1.1.7, delta variants). The cohort had 207 (60%) female patients, 290 (84%) symptomatic patients, and the median age was 51 years. 272 (78%) samples were positive by NAAT as part of standard of care testing using the EUA CDC LDT or the GeneXpert System (Cepheid, Sunnyvale, CA). All testing was performed in compliance with local regulations for patient testing. COV2Ag presence was determined using the ADVIA Centaur CoV2Ag assay (Siemens Healthineers, Tarrytown, NJ). The automated sandwich immunoassay uses five mouse monoclonal antibodies to detect SARS-CoV-2 nucleocapsid antigen and provides an index value with a threshold of 1.0 being reactive. Specimens were thawed, aliquoted and neutralized per manufacturer instructions. Briefly, 1 mL of sample was incubated with 2 drops of lysis reagent for 15 minutes before storage at -20°C for 3 weeks prior to testing. All samples were thawed and tested on the same day. Calibration and quality control materials were within manufacturer’s specifications. Note that this study was performed prior to the US EUA and reflects a workflow used internationally, however the assay is the same.

**Results:** We found that COV2Ag had 85.6% agreement with NAAT (n=346). Among symptomatic patients COV2Ag agreed with NAAT 90.0% (n=290), and among asymptomatic patients 63.0% (n=54) agreement. Among NAAT positive patients we found 82.7% agreement, in NAAT negative patients we found 97.3% agreement, with the two COV2Ag positive specimens having index values <1.5 (positive is  $\geq 1.0$ ). We found no significant differences in percent agreement among three different time periods that exhibited separate variants. Agreement between positive NAAT and COV2Ag increased at lower cycle threshold (Ct) values, which is generally indicative of the presence of more viral material. In NAAT positive patients with a Ct value <25 COV2Ag had 100% agreement (n=176), Ct <30: 98.1% (n=213), Ct <35: 90.8% (n=240), and Ct >35: 19.4% (n=31).

**Conclusion:** We found that the COV2Ag assay exceeded the WHO minimum performance requirements of  $\geq 80\%$  sensitivity and  $\geq 97\%$  specificity and assay agreement was higher at lower Ct values. There is ongoing debate around the use of high Ct values in SARS-COV-2 NAAT to indicate current SARS-COV-2 positivity because high Ct values can persist for months following SARS-COV-2 infection. COV2Ag has been considered helpful for large scale screening efforts due to high throughput and reduced wait times, as well as for procedural screening prior to patient surgery or transfers which benefit from faster turn-around times to free hospital space. Ultimate implementation with appropriate utility should be determined by local laboratory and clinical medical directors.

**B-245**

**Implementation of the Cue COVID-19 Test in Stat Lab and Point of Care Settings**

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**Background:** During the COVID-19 pandemic, a need for rapid nucleic acid amplification testing (NAAT) for SARS-CoV-2 was identified to facilitate procedures and hospital admissions in our institution. We implemented the Cue COVID-19 Test (Cue Health, San Diego, CA) in both a stat laboratory and point of care (POC) setting. The Cue COVID-19 Test is intended for the qualitative detection of nucleic acid from SARS-CoV-2 directly from proprietary nasal swabs or using nasal swab specimens in viral transport media (VTM), making it easily adaptable to both stat laboratory and POC workflows.

**Methods:**

Result options on the Cue COVID-19 test are positive, negative, invalid, or canceled. Potential causes for canceled or invalid tests are reader was moved during analytical run, test cartridge was removed before test was completed, the sample wand was

inserted into cartridge too soon or late, or internal quality control error. Separate workflows were created for stat laboratory testing (using specimens collected in VTM) and point of care testing (using direct dosing in an outpatient phlebotomy area). POC testing was performed using the proprietary Cue nasal wand loaded directly into the Cue cartridge. Positive, invalid, and test cancelled results were confirmed by calling patients back for recollection and testing by a different rapid NAAT, most often on the LIAT (Roche Diagnostics, Indianapolis, IN). The stat lab testing was performed using a nasopharyngeal swab collection processed in VTM. The Cue swab was then inoculated from the VTM and loaded into the Cue cartridge. Confirmatory testing (for positive, invalid, or cancelled tests) was performed by testing the same VTM sample on the LIAT NAAT. We compared overall positivity rate, rate of false positives (Cue positives that did not confirm on alternative NAAT), invalid rate, and test cancelled rates between stat lab (VTM) and POC (direct dosing) applications of the Cue test. **Results:** In the POC setting we performed 2407 tests from 1/1 to 12/31 in 2021: with a positivity rate of 0.33%, a false positive rate of 0.08%, invalid rate of 2.37%, and cancelled rate of 4.74%. Using VTM tested in our stat laboratory, we performed 20160 tests during the same time period in 2021 with a positivity rate of 1.6%, false positive rate of 0.40%, invalid rate of 4.77%, and cancelled rate of 3.47%.

**Conclusion:** Testing in the stat lab using VTM samples resulted in a higher false positive rate and higher invalid rate compared to POC testing using direct dosing. However, in the POC setting the test cancelled rate was slightly higher. Given that test cancellation can occur from either an error with the cartridge or moving the reader, the higher cancellation rate in the POC setting is likely due to devices being in a less protected environment where they are more prone to rough handling and/or movement while the sample is being analyzed. The Cue Health analyzer performed well in both settings in providing a rapid result to facilitate procedures and admissions for most patients tested.

## B-246

### Research to establish a model for measuring and refining lot to lot variability specifications for a rapid SARS-CoV-2 RNA test

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**Background:** The Fluxergy (Irvine, CA) rapid SARS-CoV-2 RNA test is a qualitative real-time reverse transcription polymerase chain reaction (RT-qPCR) test designed for use in point-of-care settings. After an initial evaluation of a prototype device revealed substantial lot to lot variability in the rates of discrepant results and error codes on initial runs; we developed a research protocol to determine whether end user feedback and data provided to the manufacturer could lead to rapid improvement in testing card and reaction mixture lot to lot variability.

#### Methods:

This was a prospective collaboration between three laboratories (end users) and Fluxergy. The first portion of the study was conducted using 3 test card lots manufactured using Fluxergy's original lot performance specifications. Each of the three end user sites analyzed 10 residual viral transport medium (VTM) specimens per test card and reaction mixture lot (5 previously positive for SARS-CoV-2 by another EUA approved method and 5 specimens previously negative for SARS-CoV-2). Data gathered included raw fluorescence signal, C<sub>t</sub> number, incidence of presumed false positive or false negative results, and incidence and types of error codes generated. After receiving data on the initial 3 lots, Fluxergy identified several areas that could be improved: modifications in the system sample loading, improvements in sample well fluidics, and adjustments to test software for more efficient consumable loading and robust test result determination. Fluxergy was also able to refine the internal quality control requirements for new lot acceptance and improve logistical processes for cold shipping. After implementing these changes, Fluxergy shipped an additional 7 lots of tests cards to each of two end user sites (one site dropped out before changes were made). Each site analyzed 10 VTM specimens (5 positive and 5 negative) on the 7 additional lots. The study was supported by the CAP Foundation John H. Rippey Grant for Expedited Research.

#### Results:

For the first 3 lots tested, combined data from the 3 end-user sites showed 40/90 (44%) error codes on initial runs, and 5/90 (6%) discrepant results. Discrepant results included 4/45 presumed false negatives (comparator EUA method positive, Fluxergy negative) and 1/45 presumed false positives (comparator EUA method negative, Fluxergy positive). For the 7 lots following Fluxergy's implementation of enhanced manufacturing specifications and refined internal quality control requirements, we ob-

served 6/140 (4%) error codes on the first run, 1/140 (0.7%) discrepant results. The discrepant result was a presumed false positive (comparator EUA method negative, Fluxergy positive).

#### Conclusion:

Using data from end users (laboratories), Fluxergy was able to make improvements in product design and reduce lot to lot variability in test card and reaction mixture performance. One limitation to our approach was that testing of lots occurred with the use of previously tested residual (not freshly collected) samples, in a controlled laboratory environment, which may not capture variations seen from samples or users in a point-of-care environment.

## B-248

### A Simplified, Flexible, and Sensitive Saliva-based PCR Test For Accessible SARS-CoV-2 Testing

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**Background:** Quickly detecting and isolating individuals who can transmit SARS-CoV-2, the virus that causes COVID-19, is essential for limiting further spread of the virus. Policy makers on the local, state, and federal levels rely on the detected number of active cases to make decisions, and individuals use this information to evaluate the risk of virus spread should they return to workplaces, attend school, or visit family and friends. Robust testing strategies have been plagued with limited authorized assays and high test prices, with large-scale implementation hampered by worldwide supply chain issues.

**Methods:** Having identified its potential early in 2020, we simplified saliva-based COVID-19 diagnostics by (1) not requiring specialized collection tubes or preservatives; (2) developing clear guidance for reliable self-collection; (3) validating a range of generic, low-cost collection devices; (4) replacing RNA extraction with a simple enzymatic step; and (5) testing specimens in dualplex RT-qPCR. Moreover, we validated this approach ("SalivaDirect") with materials from multiple vendors to permit flexibility and circumvent supply disruptions. We have continued to adapt the protocol and have validated additional workflows in response to varied laboratory-specific needs to further aid the implementation of saliva testing to local communities.

**Results:** SalivaDirect's simplified protocol does not compromise on sensitivity. We demonstrate stable detection of SARS-CoV-2 RNA in raw (unsupplemented) saliva for prolonged periods at elevated temperatures. The lower limit of detection ranges from 1.5-12 virus RNA copies/ul of saliva (workstream-depending), comparable to many other sensitive PCR tests. Using comparative assays and sample types, we have demonstrated SalivaDirect to efficiently detect SARS-CoV-2 in asymptomatic individuals. Importantly, we maintained the high level of sensitivity when adding heat pre-treatment options for safer sample handling and improving sample viscosity, and also when adding workflows permitting pooled testing of up to 5 samples. To date over 3.5 M SalivaDirect tests have been successfully conducted through 160+ designated labs in 40+ states with only 17 false-positive and 10 false-negative results reported. Low sample rejection (0.53%) and invalid test results (0.61%) demonstrate the reproducibility and robustness of the implementation of the test protocol across this unique laboratory network setting.

**Conclusion:** Saliva is a sensitive and reliable alternative to swabs for SARS-CoV-2 testing. SalivaDirect enables labs to utilize existing infrastructure, expediting test implementation and requiring minimal investment to scale-up to meet mass testing needs. With safe and reliable self-collection of saliva, our vision is to help provide accessible and equitable testing solutions, especially in low-resource and remote settings.

## B-249

### Spread dynamics of SARS-CoV-2 circulating variants in Brazil

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**Background:** Genomic surveillance is proving to be an important strategy to monitor in real-time the SARS-CoV-2 variants emerging. Next-generation sequencing (NGS) is the gold standard for describing new variants. Thus, a large amount of NGS data has been generated by different centers around the world. In this scenario, databases like GISAID present a great relevance in allowing metadata information sharing between scientific communities. The data can be visualized using numerous bioinformatics tools to build graphics, phylogenetic trees, and maps, that could give precious information on the virus spreading and evolution, aiding the decision-making in the

pandemic combat. **Objective:** To describe the distribution of SARS-CoV-2 variants from May 2021 to January 2022 in the Brazilian territory using public sequencing data. **Methods:** SARS-CoV-2 sequences were acquired from the GISAID EpiCoV Database. The search was restricted to samples from Brazil, collected between May 1st, 2021 to January 15, 2022. The final dataset consisted of 3,662 sequences including Alpha, Beta, Gamma, Delta, Omicron, Mu, and Lambda variants. The sequences were submitted to multiple alignment using MAFFT v7.480 and the phylogenetic inference was performed with IQTREE (maximum likelihood method/GTR+I+G4 nucleotide substitution model). The support value of the branches was evaluated using the Bootstrap test (1,000 replicates). To analyze the variants' frequencies, annotation data from all 99,142 sequences deposited in the database until January 23, 2022 were collected and processed. Python frameworks Pandas and Plotly were used for graphs generation. **Results:** The Gamma and Delta variants comprised more than 90% of the phylogenetic tree, indicating that these were the variants with the greatest circulation in Brazil within the analyzed period. According to phylogenetic inference, the Alpha variant was the first VOC introduced in Brazil. However, at the beginning of the pandemic, several variants were circulating in Brazilian territory, but none were effectively dominant; this changes with the Gamma variant emerging, responsible to promote the second wave of disease in January 2021. The Gamma variant was first detected in the Amazon state and spread to the others states, remaining dominant for a longer period. Following that, new waves of infection were marked by the predominance of new variants, which were, Delta and Omicron. Delta was initially detected in January 2021, reached its peak in the first half of September that lasted until mid-December 2021. The VOC Omicron has a high rate of transmissibility compared to other variants of the study and showed an accelerated growth pattern in the last days sampled. Beta, Mu, and Lambda variants did not settle in the country and their circulation was detected at a lower frequency. **Conclusion:** This study allowed to identify differences in the spread pattern of each variant. For example, VOC Omicron became the predominant variant in the last week of December in less than one month, with a higher transmission rate than other variants. In this sense, genomic surveillance and bioinformatics tools for data analysis are essential to understanding the dynamics of viruses and consequently the pandemic.

**B-250**

**The molecular detection of Cytomegalovirus by real-time PCR: a comparison of two commercial assays**

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**Background:** Human Cytomegalovirus (CMV), currently called Human betaherpesvirus 5 (HHV5), belongs to the Herpesviridae family and the Cytomegalovirus genus. CMV can be transmitted through sexual contact, saliva, perinatal blood contact, or blood transfusion. In most cases, the infection remains asymptomatic, however, it can cause serious illness in newborns and immunosuppressed patients, such as HIV, cancer, or transplant patients. During immunosuppressive therapy, latent virus reactivation or primary infection often occurs. In pregnant women, the transmission of CMV to the baby can occur before birth, during childbirth, or through breastfeeding. Congenital infections can cause serious abnormalities such as microcephaly, motor impairment, and mental retardation. Therefore, early detection of maternal infection and its distinction between primary or latent infection are important to determine treatment. Real-time PCR can predict viral reactivation even before the onset of symptoms, identifying subclinical infections. Ideally, quantitative tests for CMV should be used when the result is positive because the majority of the population has already been infected with CMV and a positive qualitative result can occur in asymptomatic individuals. The viral load reported by PCR can be correlated with the risk of developing the disease, guiding the professional in the monitoring of patients, and the need for early establishment of antiviral therapy. **Objective:** To evaluate and compare the performance of Real-Time Polymerase Chain Reaction (qPCR) kits for detection of Cytomegalovirus (CMV) in DNA biological samples: BioGene CMV PCR kit (Bioclin) and TaqMan assay (ThermoFisher). **Methods:** 93 samples between whole blood, urine, bronchial lavage (BAL), cerebrospinal fluid, and biopsy fragment were selected from routine detection of CMV by PCR-nested of Pardini Group. The samples were tested in the TaqMan assay and the BioGene Kit. The results of the tests were compared with each other and with the results of the Nested PCR, methodology currently offered for the qualitative CMV test by the Pardini Group. The BioGene kit and the TaqMan assay showed 100% agreement with the in-house method (PCR Nested) in samples of BAL, liquor, and other materials tested. For urine, the positive agreement was 100% and a negative sample in PCR-nested was positive in both kits. For whole blood, there were four false-negative results in the TaqMan assay and three in the BioGene kit, and a negative sample on nested PCR was positive for both the TaqMan assay and the BioGene kit. Probable DNA degradation of whole blood samples may have been the cause of the false-negative results. In the validation tests,

the TaqMan assay had a higher frequency of endogenous control amplification failure and one more false-negative result in whole blood than the BioGene kit. **Conclusion:** The BioGene Kit showed superior results when compared to the TaqMan assay. The BioGene Kit can be a good choice for the detection of Cytomegalovirus (CMV) DNA in biological samples and patient monitoring.

**B-251**

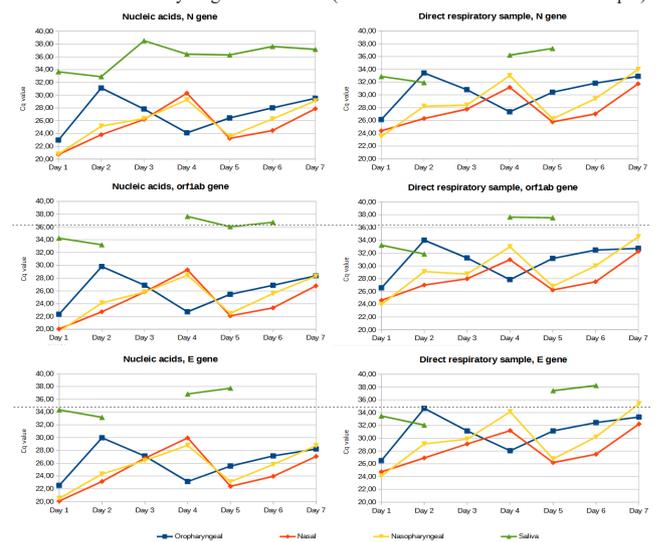
**Utility of a Direct Sample Real Time PCR for the Follow up of SARS-CoV-2 Infection with Different Respiratory Samples**

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**Background:** Throughout the COVID-19 pandemic, due to the high demand for diagnostics, there has been a shortage not only of specific diagnostic devices (Real Time PCR, rapid test), but also of complementary products needed for their execution, including nucleic acid extraction reagents. Recently, Operon has launched the kit Real SARS-CoV-2 PLUS for the detection of SARS-CoV-2 compatible with both direct respiratory specimen (nasal/nasopharyngeal/ oropharyngeal swabs and saliva) and nucleic acids. We have evaluated the usefulness of this test for the long-term monitoring of COVID-19 infection, comparing the results obtained with 4 different types of respiratory tract samples and their corresponding nucleic acids. **Methods:** All samples were taken early in the morning and over 7 consecutive days from symptom onset. Swab samples were preserved in virus transport medium (Operon, 1 ml) and saliva samples in a sterile tube. Nucleic acids were extracted from 200 µl of sample with "Maxwell 16 Viral Total Nucleic Acid Purification Kit" (Promega) (swab samples), and with "MinElute Virus Spin Kit" (Qiagen)(saliva). RT-PCR amplification (respiratory sample and nucleic acids) and analysis of results were performed according to the manufacturer's instructions (Operon).

**Results:** All three types of swabs tested (oropharyngeal, nasopharyngeal and nasal) remained positive for SARS-CoV-2 throughout the 7 days, both with direct and nucleic acid sampling. Saliva samples were only clearly positive during the first two days of infection, with greater variability of results observed on the following days.

**Conclusion:** The usefulness of direct sample analysis with the SARS-CoV-2 Real RT-PCR test for the diagnosis and monitoring of an infection over at least 7 days is demonstrated. The suitability of three of the four sample types tested (oropharyngeal, nasopharyngeal and nasal swabs) is also demonstrated. With saliva, the virus could only be detected in the early stages of infection (both with nucleic acid and direct sample).



Figures: Ct values obtained for every type of sample analysing nucleic acids or direct respiratory samples along 7 days after the onset of symptoms.

## B-253

Comparative Evaluation of HP DETECT™, a Stool Antigen ELISA Test for *Helicobacter pylori*

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**Background** *H. pylori* is a bacterium located in the upper gastrointestinal tract of humans which has been linked to gastric disease such as peptic ulcers and gastritis as well as stomach cancer. Histology and rapid urease test (RUT) on endoscopic biopsy specimens are considered the gold standard composite reference method (CRM) for detection of *H. pylori*. Non-invasive methods are desired as an alternative to endoscopy for the detection and post-eradication therapy monitoring of *H. pylori* infection. Non-invasive techniques include the urea breath test (UBT) and serological ELISA test for the detection of *H. pylori* antibodies. Detection of antibodies in human sera is no longer recommended as antibody levels remain high for a prolonged time after infection is treated. UBT may give false positive results in cases where other urease-producing bacteria are present in the stomach. Stool antigen testing is widely employed due to the non-invasive nature of the testing and high sensitivity and specificity relative to the gold standard CRM. This study describes the development of the HP DETECT™ Stool Antigen ELISA, which detects the presence of *H. pylori* antigens directly in human feces. No invasive sample collection is needed in this test and it provides a direct measure of *H. pylori* infection or effectiveness of treatment. **Methods** Samples: 227 human fecal specimens collected from patients undergoing endoscopy and biopsy of the antrum and corpus of the stomach for detection of active infection of *H. pylori*. Assays: Composite Reference Method (CRM): Histology of the biopsy samples was paired with results from rapid urease testing to define clinically infected subjects. Comparator ELISAs: Meridian Premier® Platinum HpSA® PLUS and TECHLAB H. Pylori CHEK™. The panel of 227 specimens were run on HP DETECT™ and Premier® Platinum HpSA® PLUS, and 206 of the specimens were run on TECHLAB H. Pylori CHEK™. Data from HP DETECT™ was compared to the comparator ELISAs and to the CRM. Cross-reactivity/Interference: 45 bacterial, fungal, and viral organisms and 17 potentially interfering substances were added to positive and negative samples for analytical specificity testing. Post-therapy: A panel of 14 paired pre- and post-eradication therapy specimens with paired CRM results was run on the HP DETECT™ ELISA. **Results** The sensitivity of HP DETECT™ vs. Premier® Platinum HpSA® PLUS was 96.4% (80/83), and the specificity was 96.5% (139/144). The sensitivity of HP DETECT™ vs. H. Pylori CHEK™ was 100.0% (81/81), and the specificity was 98.4% (123/125). The sensitivity of HP DETECT™ vs. the CRM was 95.4% (83/87), and the specificity was 98.6% (138/140), while the sensitivity of Premier® Platinum HpSA® vs. the CRM was 90.8% (79/87) and the specificity was 97.1% (136/140). The sensitivity of HP DETECT™ in post-eradication therapy samples was 100.0% (10/10), and the specificity was 100.0% (4/4). No cross-reactivity or interference was observed. **Conclusion** The HP DETECT™ Stool Antigen ELISA demonstrated excellent sensitivity, specificity, and overall agreement with both comparator ELISAs and with the gold standard CRM for *H. pylori* infection. Sensitivity and specificity in post-therapy treatment samples was also excellent.

## B-256

## Reactivity of LUMIPULSE G SARS-CoV-2 Ag Reagent to Mutant Virus

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**Background:** SARS-CoV-2 is an RNA virus that is prone to mutation, and several variants, including the variants of concern (VOC) and variants of interest (VOI), caused epidemic waves during the COVID-19 pandemic. Each variant has characteristic mutations (major mutations) through the genome including genes encoding spike protein, and nucleocapsid protein (NP). In addition, there are lots of mutations that occurred at low frequency (minor mutations). Since the SARS-CoV-2 antigen tests are based on the detection of NP in the specimens, mutations in NP, even minor mutations, might reduce the sensitivity of the tests. To address the issue, we evaluated effects of mutations on a quantitative antigen test kit, Lumipulse G SARS-CoV-2 Ag (LP-CoV-2), with recombinant NP carrying major and/or minor mutations selected by our original genome surveillance system. **Methods:** LP-CoV-2, chemiluminescent enzyme immunoassay (CLEIA), use multiple monoclonal antibodies recognizing N-terminal domain (NTD) and C-terminal domain (CTD) in NP, was used to quantitate NP concentration in a sample on a fully automated CLEIA system (LUMIPULSE G1200). Dozens of nasopharyngeal swab (NPS) specimens of VOC or VOI were purchased from Precision For Medicine (Bethesda, MD), Boca Biolistics (Pompano

Beach, FL), and Trina Bioreactives AG (Uster, Swiss). RT-PCR for SARS-CoV-2 was carried out according to the manual (v2.9.1) provided by National Institute of Infectious Disease, Japan. The genome surveillance system is described elsewhere (Fujimoto, et al.). This system identified the minor mutations that had been reported in increasing numbers over a period of time or were presumed to affect antibody epitopes. 18 of recombinant NP were purchased from Sino Biological (Beijing, China) and ACROBiosystems (Newark, DE). 12 of the recombinant NP were expressed in *E. coli*, and were purified by chromatography. **Results:** We analyzed correlation between antigen concentrations and RNA quantities determined by LP-CoV-2 and RT-PCR, respectively, in each NPS specimen of variants or wild-type (WT), indicating well-correlation and the correlation patterns were indistinguishable among variants, though details of mutations were not available these specimens. Genome analysis indicated that VOC and VOI had major mutations at a total of 20 amino-acid positions through genome surveillance. We found additional mutations at 30 amino-acid positions through genome surveillance. Based on these findings, we analyzed 30 recombinant NP carrying combination of the mutations from these 50 sites (9 in the N tail, 6 in the NTD, 20 in the Linker, 4 in the CTD, and 11 in the C tail) by Western-blot, and we found these mutant NP gave a band of similar intensity to those of WT. Quantitative results of the LP-CoV-2 measurements of these NP were equivalent to that of WT, indicating that major and minor mutations observed in VOC and VOIs did not affect the measurements by LP-CoV-2. **Conclusion:** These results suggest that LP-CoV-2 would be robust enough to detect several NP mutations reported as certain numbers in genome database. Based on the observed biased mutation sites in NP and the recognition regions of multiple monoclonal-antibodies used in LP-CoV-2, this reagent would be robust to the mutations observed in the next COVID-19 pandemic.

## B-258

## Candida Isolates From Blood Cultures: A Review of 2019 and 2020 Data From a Huge Laboratory in Northeastern Brazil

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**Background** *Candida* is an opportunistic pathogen that affects high risk patients who are either immunocompromised or critically ill and is associated with almost 80% of all nosocomial fungal infections, representing the major cause of fungemia with high mortality rates (40%). *Candida albicans* is the main cause of candidemia and among the non-*albicans* species *C. parapsilosis*, *C. glabrata* and *C. tropicalis* are the most frequent agents. The aim of this study was to evaluate the distribution of *Candida* species isolated from blood cultures at Dasa Laboratory, in Recife, Northeastern Brazil. **Methods** It began by surveying all positive *Candida* blood cultures processed by the Dasa microbiology laboratory from January 2019 to December 2020. The cultures, originated from blood samples, were identified by Vitek® MS system (Biomerieux™ Marcy-l'Étoile, France). **Results** A total of 239 (2019: n=109; 2020: n=130) sample isolates were positive for *Candida* (0,18%). The most frequent species were *C. non-albicans*. None *Candida auris* was isolated during the period of this study (Table1). **Conclusion** This study shows that *Candida non-albicans* was the main isolated agent and evidences the importance of *C. tropicalis* in nosocomial fungal infections.

Table 1. Identification of 239 *Candida* spp. isolates from blood cultures, between January, 2019 and December, 2020. Recife-PE, Brazil.

	2019	2020	TOTAL
Collected blood cultures	17,569	21,976	39,545
Positive blood cultures	2,394	2,080	4,474
<i>Candida</i> isolates	109	130	239
<i>C. albicans</i>	21	41	62
<i>C. tropicalis</i>	33	37	70
<i>C. parapsilosis</i>	16	34	50
<i>C. krusei</i>	17	3	20
<i>C. glabrata</i>	9	7	16
<i>C. guilliermondii</i>	2	7	9
Others	11	1	12

**B-260**

**CLSI Oxacillin in Combination with Cefoxitin Screen Breakpoints for MicroScan Dried Gram Positive MIC Panels from a Multicenter Assessment of Gram Positive Bacteria**

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**Objectives:** Oxacillin combined with cefoxitin screen performance were evaluated against data from a multicenter study with *Staphylococcus* on MicroScan Dried Gram-Positive MIC (MSDGP) Panel. MIC results were compared to Oxacillin results obtained with frozen broth microdilution panels prepared according to CLSI methodology. To evaluate methicillin susceptible or methicillin resistant isolates, oxacillin and cefoxitin CLSI categorization rules were applied.

**Materials/Methods:** MSDGP panels were evaluated at three clinical sites by comparing MIC values obtained using MSDGP panels to MICs utilizing CLSI/ISO reference panel. Study included 349 clinical isolates tested using turbidity and Prompt<sup>®</sup> inoculation methods. MSDGP panels were incubated at 35±1°C and read on WalkAway, autoSCAN-4, and visually. Read times for MSDGP panels were 16-20 hours. Frozen reference panels were prepared and read according to CLSI/ISO methodology. CLSI breakpoints (mcg/mL) used for interpretation of MIC results were: ≤2 S, ≥4 R for Oxacillin with *Staphylococcus aureus* and *Staphylococcus lugdunensis* and ≤0.5 S, ≥1 R for all other *Staphylococcus* spp. and ≤4 S, ≥8 R for CfxS with *Staphylococcus aureus* and *Staphylococcus lugdunensis*. Additionally, *mecA* testing was performed on *Staphylococcus* species (except *S. aureus*, *S. lugdunensis*, *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*) on isolates with MIC values of 1-2 mcg/ml.

**Results:** Essential and categorical agreement were calculated compared to frozen reference panel. Results for isolates tested during efficacy are found in the following table.

Read Method	Organism Group	Essential Agreement (EA) %		Categorical Agreement (CA) %		Very Major Error (VMJ) %		Major Error (MAJ) %	
		P	T	P	T	P	T	P	T
WalkAway	<i>S. aureus</i> & <i>S. lugdunensis</i>	99.3 (136/137)	100 (137/137)	100 (137/137)	100 (137/137)	0.0 (0/34)	0.0 (0/34)	0.0 (0/103)	0.0 (0/103)
	Other <i>Staphylococcus</i> spp.	91.5 (194/212)	97.2 (206/212)	96.2 (204/212)	97.2 (206/212)	1.7 (1/58)	3.5 (2/58)	3.3 (5/154)	0.7 (1/154)
autoSCAN-4	<i>S. aureus</i> & <i>S. lugdunensis</i>	99.3 (136/137)	98.5 (135/137)	100 (137/137)	100 (137/137)	0.0 (0/34)	0.0 (0/34)	0.0 (0/103)	0.0 (0/103)
	Other <i>Staphylococcus</i> spp.	94.8 (201/212)	94.3 (200/212)	98.1 (208/212)	96.2 (204/212)	0.0 (0/58)	10.3 (6/58)	2.0 (3/154)	0.7 (1/154)
Visually	<i>S. aureus</i> & <i>S. lugdunensis</i>	98.5 (135/137)	100 (137/137)	98.5 (135/137)	100 (137/137)	0.0 (0/34)	0.0 (0/34)	1.0 (1/103)	0.0 (0/103)
	Other <i>Staphylococcus</i> spp.	92.5 (196/212)	96.7 (205/212)	97.6 (207/212)	96.7 (205/212)	0.0 (0/58)	5.2 (3/58)	3.3 (5/154)	0.7 (1/154)

Calculations excluding 1 well errors due to lack of intermediate category

**Conclusion:** Oxacillin and Cefoxitin Screen MIC results for Gram-Positive bacteria obtained with MSDGP panels correlate well with MICs obtained with frozen reference panels using CLSI interpretive criteria in this multicenter study.

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**B-261**

**Updated CLSI Meropenem Breakpoints for MicroScan Dried Gram Negative MIC Panels from a Multicenter Assessment of *Acinetobacter* species**

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

**Materials/Methods:** MSDGN panels were evaluated at four clinical sites by comparing MIC values obtained using the MSDGN panels to MICs utilizing a CLSI broth microdilution reference panel. The study included a total of 56 *Acinetobacter* spp. clinical isolates tested using the turbidity and Prompt<sup>®</sup> methods of inoculation during the combined phases of efficacy and challenge. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually at 16-20 hours. Frozen reference panels were prepared according to CLSI/ISO methodology, incubated for 20-24 hours and read visually. CLSI breakpoints (µg/mL) used for interpretation of MIC results were: ≤ 2 S, 4 I, ≥ 8 R for *Acinetobacter* species.

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

Read Method	Essential Agreement (EA) %		Categorical Agreement (CA) %		Very Major Error (VMJ) %		Major Error (MAJ) %		Minor Error (MIN) %	
	P	T	P	T	P	T	P	T	P	T
WalkAway	91.1 (51/56)	96.4 (54/56)	98.2 (55/56)	92.9 (52/56)	0.0 (0/37)	0.0 (0/37)	0.0 (0/19)	0.0 (0/19)	1.8 (1/56)	7.1 (4/56)
autoSCAN-4	92.9 (52/56)	96.4 (54/56)	98.2 (55/56)	92.9 (52/56)	0.0 (0/37)	0.0 (0/37)	0.0 (0/19)	0.0 (0/19)	1.8 (1/56)	7.1 (4/56)
Visually	92.9 (52/56)	96.4 (54/56)	98.2 (55/56)	91.1 (51/56)	0.0 (0/37)	0.0 (0/37)	0.0 (0/19)	0.0 (0/19)	1.8 (1/56)	8.9 (5/56)

T = Turbidity inoculation method, P = Prompt inoculation method

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

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<sup>†</sup>Deceased

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**B-262**

**Updated FDA/CLSI Ceftazidime Breakpoints from a Multicenter Assessment for *Enterobacterales*, *Acinetobacter* spp. and *Pseudomonas aeruginosa* Using MicroScan Dried Gram Negative MIC Panels**

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**Background:** Updated FDA/CLSI ceftazidime breakpoints were evaluated against data from a multicenter clinical study with *Enterobacterales*, *Acinetobacter* spp. and *P. aeruginosa* on a MicroScan Dried Gram-negative MIC (MSDGN) Panel. MIC results were compared to results obtained with frozen broth microdilution panels prepared according to CLSI methodology.

**Materials/Methods:** Panels were evaluated at five clinical sites by comparing MIC values obtained using MSDGN panels to MICs utilizing CLSI reference panel. Data from efficacy and challenge combined included 1,351 *Enterobacterales*, *Acinetobacter*

spp. and *P. aeruginosa* clinical isolates tested using turbidity and Prompt® methods of inoculation. MSDGN panels were incubated at  $35 \pm 1^\circ\text{C}$  and read on WalkAway System, autoSCAN-4 instrument, and visually. Read times for MSDGN panels were at 16-20 hours. Frozen reference panels were prepared and read according to CLSI methodology. FDA and CLSI breakpoints ( $\mu\text{g/mL}$ ) used for interpretation of MIC results were: Enterobacterales  $\leq 4$  S, 8 I,  $\geq 16$  R; *Acinetobacter* spp.  $\leq 8$  S, 16 I,  $\geq 32$  R; *P. aeruginosa*  $\leq 8$  S,  $\geq 16$  R (FDA only).

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

Read Method	EA %	CA %	Very Major Error (VMJ) %	Major Error (MAJ) %
Enterobacterales	93.5 (1010/1080)	96.8 (1045/1080)	1.8 (3/165)	1.1 (10/896)
<i>Acinetobacter</i> spp.	96.6 (84/87)	94.3 (82/87)	2.0 (1/50)	2.8 (1/36)
<i>P. aeruginosa</i> *	93.5 (172/184)	97.3 (179/184)	0.0 (0/33)	2.0 (3/151)

\*One well errors were excluded from CE calculations due to the lack of intermediate breakpoint.

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

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## B-264

### Chronic Antral Gastritis With Cytomegalovirus-Associated Foveolar Hyperplasia

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**Background:** Chronic diarrhea is a syndrome of great clinical complexity that general practitioners, internists and gastroenterologists must frequently face. The diagnostic approach of children with diarrheal disease in most cases is based on the interpretation of complementary examinations performed on feces, which support the final decision-making.

**Methods:** A 2-month-old infant, with normal heel prick test and exclusive breastfeeding, goes to the emergency room due to diarrhea of 21 days of evolution, with low-grade fever and vomiting during the last 3 days. During this period, she presented an increase in the number of stools of liquid consistency, greenish color and with strands of blood. In a private hospital, stool and urine cultures were performed with negative results and it was recommended that cow's milk proteins be withdrawn from the maternal diet, with no improvement.

**Results:** During admission, fluid therapy was started, breastfeeding was replaced by hydrolyzed formula, and a blood test was performed, revealing leukocytosis with lymphomonocytosis and thrombocytosis, hypoproteinemia, and C-reactive protein and procalcitonin values of 31 mg/L and 0.31 ng/mL respectively. During the following days, she presented an increase in transaminases with values of GGT 171 U/L, AST 209 U/L and ALT 134 U/L with normal bilirubin. An abdominal ultrasound revealed no pathological changes except for the existence of abundant fluid content in the intestinal loops and the results of stool/blood culture and the presence of adenovirus, norovirus and rotavirus in feces were negative. From the laboratory, it was proposed to carry out a biochemical analysis of the stool after an absolute diet, which revealed that the diarrhea was of the secretory type with normal values of alpha-1 antitrypsin and calprotectin of 304.6  $\mu\text{g/g}$ .

Based on these data, it was decided to perform a gastrocolonoscopy with biopsies. The pathology study revealed a mononuclear infiltrate of CD3+/TCR beta+/CD8+ lymphocytes with a low CD4+ population and prominent endothelial vessels with immunorepression of cytomegalovirus (CMV). In addition, the gastric mucosa showed signs of atrophy of the lamina propria glands with marked apoptosis, foveolar hyperplasia and vessels with prominent endothelium with images of cytomegalic nuclear pseudo-inclusion. These results were confirmed with the presence of CMV DNA in blood (30,300 IU/mL) by quantitative PCR. The patient was treated with intravenous ganciclovir and evolved favorably.

**Conclusion:** In patients with chronic diarrhea, apart from performing a correct anamnesis and basic analytical studies in blood and stools, it is crucial to assess the type of diarrhea and if there is an indication for a colonoscopy. Furthermore, although CMV diarrhea is more common in immunocompromised patients, this infection should be considered in the differential diagnosis of chronic diarrhea regardless of immune status.

## B-266

### A Novel Rapid test for *Aspergillus* Galactomannan

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**BACKGROUND** Invasive Aspergillosis (IA) is a life-threatening opportunistic mycosis that occurs in some people with a compromised immune system. The detection of *Aspergillus* galactomannan (GM) rapidly gained widespread acceptance as part of the diagnostic workup of a patient suspected of IA. Recently, a novel QuicGM™ *Aspergillus* Galactomannan Ag Lateral Flow Assay (LFA) from Dynamiker Biotechnology (Tianjin) Co., Ltd. was released as a screening test of IA. It is a fluorescent immunochromatographic cassette test using a monoclonal antibody against GM and Europium nanoparticles (Eu NP) (Figure 1). It is a semi-quantitative test that was easy to operate, rapid with portable detection devices, and can be widely accepted by clinical and primary medical. We conducted a comparative study between the Dynamiker QuicGM™ *Aspergillus* Galactomannan Ag (LFA) and the Dynamiker *Aspergillus* Galactomannan Assay (ELISA) to validate the performance of the novel assay.

**METHOD** A total of 113 serum samples and 3 BALF samples were collected. The samples were tested in parallel using the *Aspergillus* Galactomannan Assay (ELISA) and QuicGM™ *Aspergillus* Galactomannan Ag (LFA). **RESULTS** The total coincidence rate, positive coincidence rate, and negative coincidence rate of the two assays were 93.1%(108/116), 87.8%(36/41), and 96%(72/75) (Kappa value was 0.83>0.75) in 116 samples respectively. **CONCLUSIONS** The performance of the QuicGM™ *Aspergillus* Galactomannan Ag (LFA) and the *Aspergillus* Galactomannan Assay (ELISA) were highly consistent, and the performances of the Dynamiker *Aspergillus* Galactomannan kit were all in line with the statement of the manufacturer. The results are accurate and reliable, which could be used as an aid for the early rapid screening test of IA.

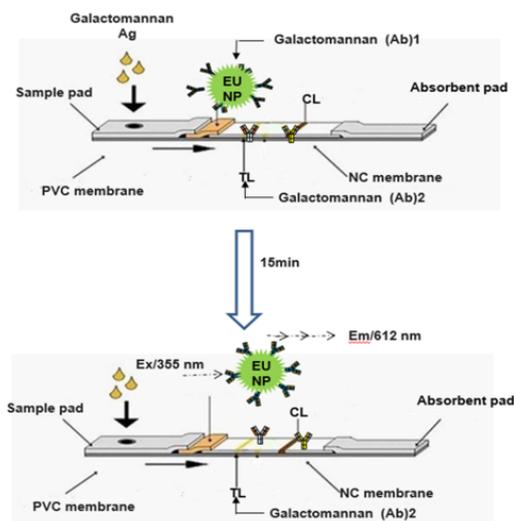


Figure 1 Diagram of the Dynamiker GM (LFA)

## B-267

### Early antibody responses to Pfizer vs Sinovac SARS-CoV-2 vaccines.

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**BACKGROUND:** In our country, the majority of the population were vaccinated with the BNT162b2 mRNA vaccine (Pfizer), however, there were a few individuals who opted for vaccination with the inactivated COVID-19 vaccine Sinovac-CoronaVac

(Sinovac). We compared the early total spike antibody (S-Ab) and nucleocapsid antibody (Nuc-Ab) responses after the first and second doses of mRNA and inactivated virus vaccines in COVID-naïve individuals. **METHODS:** 77 subjects (age range 24-70, mean 40.0 ± 11.9 years) were vaccinated with the Pfizer vaccine, and 32 subjects (age range 22-68, mean 46.7 ± 13.4 years) were vaccinated with the Sinovac vaccine. Samples were tested pre-vaccination, 10 days after the first vaccine dose and 20 days after the second dose on the Roche Elecsys Anti-SARS-CoV-2 S quantitative double-antigen sandwich electro-chemiluminescent immunoassay and Nuc-Ab on the Roche nucleocapsid anti-SARS-CoV-2 immunoassay (both run on the Roche Elecsys e801 auto-analyser). Roche total S-Ab was converted to WHO international units based on the following equation: (S-Ab) BAU/mL = 0.97 × Roche value in U/mL. **RESULTS:** Total S-Ab increased from 0.4 BAU/mL to a peak median of 97.9 BAU/mL after the second Sinovac dose and increased from 2.48 BAU/mL to a peak median of 2235 BAU/mL after the second dose of BNT162b2 vaccine. The BNT162b2 vaccine generated much greater S-Ab response than the Sinovac vaccine after both the first dose (median difference 1.41 BAU/mL, 95% CI 0.54 to 3.69, p = 0.0002) and the second dose of vaccine (median difference 2089 BAU/mL, 95% CI 1611 to 2429, p < 0.0001). The Nuc-Ab was non-reactive in all Pfizer vaccinees after both doses but increased from cut-off index 0.09 to 0.49 after the second dose of Sinovac vaccine (see Table 1). **CONCLUSION:** Vaccination with the Pfizer BNT162b2 generated a more robust total SARS-CoV-2 S-Ab response than the Sinovac vaccine.

**Table 1:** Total spike and nucleocapsid antibody responses after the first and second doses of Pfizer mRNA SARS-CoV-2 vaccine and Sinovac SARS-CoV-2 inactivated viral vaccine. Abbreviations: Dose 1 D10: 10 days after dose 1, Dose 2 D20: 20 days after dose 2, COI: cut-off index

Sinovac Nucleocapsid antibody			
Time period	n	Mean (COI)	Median (COI)
Baseline	20	0.08	0.08
Dose 1 D10	30	0.10	0.09
Dose 2 D20	26	0.71	0.49
Sinovac Spike antibody			
Time period	n	Mean (BAU/mL)	Median (BAU/mL)
Baseline	20	0.40	0.40
Dose 1 D10	30	0.55	0.40
Dose 2 D20	30	73.4	97.9
Pfizer Spike antibody			
Time period	n	Mean (BAU/mL)	Median (BAU/mL)
Baseline	70	0.40	0.40
Dose 1 D10	71	3.12	2.48
Dose 2 D20	67	2145	2235

**B-268**

**UTI in a Selected Outpatient Population of Sicily**

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**BACKGROUND:** Urinary tract infections (UTIs) are among the most common bacterial infections acquired in the community and in hospitals. UTI is diagnosed using a combination of urinary symptoms and urine culture demonstrating numbers of a known uropathogen above a given threshold (usually defined as >1,000 cfu/ml of urine, but thresholds as ≤100 cfu/ml and ≥100,000 cfu/ml). The goal of this study is analysed the epidemiology of our non-hospitalized population to observe and analysed the epidemiologic activity and antibiotic resistance in Misterbianco Humanitas Hospital (HCCO), in Sicily. **METHODS:** Clean catch mid-stream urine specimens were collected from all subjects. Uropathogens were isolated and identified by using conventional standard techniques: samples were cultured on Blood agar, MacConkey agar and Sabouraud Dextrose Agar. Identification and Antibiotic Susceptibility pattern was determined on VITEK 2 (Biomereux®) and confirmed on Mueller-Hinton using Kirby -Bauer disc diffusion method. **RESULTS:** In period between April 2020 and October 2021, using culture based methods, we have analysed 1536 ambulatory patients: 57% negative and 43% positive; antibiotic resistance occurred in 43 samples, 5%.

**Figure 1:** results of investigation. **DISCUSSION:** Of all positive samples, UTI is the most common bacterial infection. It is generally associated with minimal morbidity except among specific subpopulations. However, there is a significant absence of data regarding its accurate incidence; in particular, in non hospitalizes people. The financial implications of UTI are enormous, predominantly a result of the high incidence of UTI, cost of prophylaxis associated with antibiotic resistances, and indirect cost. In our study the antibiotic resistances are 6% totally (5% ESBL, 0% KPC and 1% VAN+) totally. There is a growing need to ensure appropriate therapy with agents that maximize success for community-acquired infection while minimizing risk of the development of antimicrobial resistance. Our commitment is to continue surveillance to support family doctors and prevent hospitalization

SEX	N	P	E. COLI	KLEBSIELLA SPP	PROTEUS SPP	S. AGALACTIAE	ENTEROCOCC SPP	OTHER	RESISTENCE	ESBL	KPC	VAN+
F	471	453	240	21	21	18	108	45	43	33	1	8
M	401	211	96	9	11	0	55	40	6%	5%	0%	1%
MM	46%	32%	29%	30%	34%	0%	34%	47%				

**Preanalytical and Postanalytical**

**B-269**

**Reducing Consumable Plastic Malonic Acid Interference in an LC-MS/MS Method to Quantify Serum Methylmalonic Acid**

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**Background:** Vitamin B12 is necessary for many processes in the body and as a cofactor in methylmalonic acid (MMA) metabolism. Vitamin B12 deficiency can be caused by poor diet or digestion related issues. Vitamin B12 deficiency may present with few symptoms, however chronic deficiency may lead to macrocytic anemia, peripheral nerve damage and memory impairment. MMA is measured in the differential diagnosis of vitamin B12 deficiency as elevations in MMA are associated with decreased vitamin B12 stores. Elevated levels of MMA may also be the result of an inherited defect of enzymes involved in MMA metabolism, called methylmalonic acidemia. Current spectroscopy-based MMA assays require sample extraction processes that place the sample in frequent contact with plastic consumables. Malonic acid is commonly present in plastic consumables used in laboratories and may interfere with quantitation of MMA. We set out to develop and optimize a highly specific method to quantify MMA that was free from malonic acid contamination. **Methods:** MMA purchased from Cerilliant (Round Rock, TX) was used to create calibrators and quality controls in deionized water and in pooled serum from BioIVT (Westbury, NY), respectively. The serum sample (100µL), internal standard (50µL), and 1% acetic acid in water (250µL) were loaded onto a pre-conditioned Phenomenex Strata X-AW-33 30mg 96-well plate. The plate was pre-conditioned using 2% ammonium hydroxide in methanol, methanol, and 1% acetic acid. After sample loading, the SPE plate was washed with 50:50 methanol: water and MMA was eluted with 2% ammonium hydroxide in methanol. Extracts were dried under nitrogen at 45°C and reconstituted in 0.4% formic acid in water. Using an ACQUITY UPLC Premier System, samples were injected onto a ACQUITY UPLC BEH C18 1.7µm column using a water, methanol, and formic acid gradient on a SCIEX QTRAP 5500 LC-MS/MS system. Plastics and consumables were rinsed with water or methanol before use in the extraction process. **Results:** The method displayed good resolution between MMA and succinic acid as well as excellent specificity when evaluated against other water-soluble vitamins. The SPE allowed for rigorous washing which resulted in extracts free of matrix effects. The extraction produced analyte recovery ≥90% and excellent linearity from 5-1000ng/mL with minimal carryover. The method showed inter- and intra-precision across three varying spiked samples with coefficients of variation (CV) below 6%. The lower limit of quantitation (LLOQ) was evaluated at 5.0ng/mL and resulted in a CV of 10.3%. Analysis of samples across instruments and by an independent LC-MS/MS method demonstrated a <5% bias. Consumable contamination was initially evaluated to be 2-3X the peak areas of the LLOQ and was reduced to less than 20% of the LLOQ allowing for excellent accuracy and precision at the LLOQ. **Conclusion:** We have presented an optimized method for MMA quantification by LC-MS/MS analysis. We found that common consumables containing malonic acid falsely inflate MMA results. To provide accurate MMA results, all consumables were rinsed with similar reagents prior to extraction. It is recommended that MMA methods be verified as contamination free in order to provide accurate and robust results.

**B-270**

**Impact of recentrifugation of blood collection tubes on chemistry and immunochemistry analytes after 24 and 72 hours of refrigerated storage on the Roche Cobas 8000 platform**

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**Background:** We discovered that blood collection tubes (BCTs) were inadvertently recentrifuged due to improper placement on our automated preanalytical system. This study was undertaken to determine the impact of recentrifugation of blood specimens collected in serum separator (SSTs) and plasma separator (PSTs) tubes after refrigerated storage for 24 and 72 h on the concentrations of chemistry and immunochemistry analytes.

**Methods:** Blood was collected from 20 volunteers in SSTs and PSTs, centrifuged, and 36 chemistry and 14 immunochemistry analytes were measured at baseline in single-centrifuged tubes on a Roche Cobas 8000 chemistry platform. After baseline testing, the BCTs were refrigerated for 24 or 72 h, re-centrifuged, and retested. The results were compared to the single-centrifuged tubes for statistical and clinical significance.

**Results:** Re-centrifugation of BCTs after 24 or 72 h of refrigerated storage showed clinically significant increases in lactate dehydrogenase activity and potassium concentration and clinically significant decreases in glucose (except in SSTs after 24 h of refrigerated storage) and CO<sub>2</sub> concentration, but no significant differences in immunochemistry analyte concentrations.

**Conclusion:** The results of this study demonstrate that it may be safe to report most routine chemistry and immunochemistry analyte concentrations from re-centrifuged SSTs and PSTs on the Roche Cobas 8000, which may save time and costs associated with recollection and retesting.

## B-271

### Impact of Centrifuge Deployment at an Outpatient Phlebotomy Site on Blood Glucose Levels

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**Background:** In vitro glycolysis in unseparated whole blood specimens is an underappreciated significant preanalytical challenge. In vitro glycolysis falsely lowers blood glucose levels and contributes to the misdiagnosis of diabetes mellitus and false alerts by increasing the number of spuriously low critical glucose levels. Test tubes with rapidly acting glycolysis inhibitors are not widely available. Test tubes with only enolase inhibitors are not as effective. Immediate separation of plasma from blood cells (even after placing tubes on ice-water slurry) by centrifugation is recommended, but not without practical challenges, especially for specimens from an outpatient setting.

**Methods:** We compared the percent of low critical ( $\leq 55$  mg/dL) and median glucose levels from plasma specimens (Lithium Heparin, BD, Franklin Lakes, NJ) collected at two different outpatient phlebotomy sites (Sites A and B) before and after centrifuge deployment at Site B. Site A, but not Site B, is connected to the core laboratory via a pneumatic tube system (control group). Specimens from Site B are transported to the core laboratory via a courier pick-up service.

**Results:** Median (interquartile range) glucose levels from Site A (n=39 432) were 96 mg/dL (86-108) in 2018, 94 mg/dL (85-107) in 2019, 96 mg/dL (87-112) in 2020, and 98 mg/dL (88-115) in 2021. The glucose level from Site B (n=39 902) before the deployment of the centrifuge was 82 mg/dL (72-97) in 2018 and 2019, which, after centrifuge deployment, had improved to 93 mg/dL (85-108) and 95 mg/dL (86-108) in 2020 and 2021, respectively. The rate of critically low glucose level before the deployment of centrifuge was 2.6% and 2.7% in 2018 and 2019, respectively, vs 0.51% in 2020 and 0.33% in 2021. The rate of critically low glucose values from Site A relatively remained similar (0.33%, 0.4%, 0.27%, and 0.25% in 2018 through 2021, respectively). Glucose median turnaround time (collected to received) in 2018 through 2021 for Site A (44, 50, 45, 42 mins, respectively) and Site B (170, 210, 232, 225 mins, respectively) remained mostly similar (<30 mins difference).

**Conclusion:** We observed an eight-fold reduction in critically low and 13-16% increase in median glucose values after the deployment of a centrifuge at an outpatient phlebotomy site. The findings highlight the preanalytical challenges in glucose measurement.

## B-273

### Detecting Pre-Analytical Error in the Laboratory: An Experimental Approach to Derive Delta Checks for Sample Contamination.

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**Background:** Pre-analytical errors account for more than half of the total errors in laboratory testing. A common source of pre-analytical error can occur when blood samples are drawn from an IV-line that is being concurrently used for the delivery of crystalloid solutions. Clinical laboratories often implement rules in the laboratory information system (LIS) or middleware to alert testing staff to specimens that warrant further investigation and communications with the clinical team to ascertain whether contamination of this nature may have occurred. However, laboratorians are left unguided by a paucity of literature on how to configure these rules. This is particularly true if the desire is to develop a set of rules that would detect more subtle, rather than

gross contamination of clinical blood samples by crystalloid solutions. Accordingly, the primary objective of this study is to determine the *in vitro* effect of increasing blood sample contamination from commonly used crystalloid solutions, and how these observations can guide the derivation of multivariate delta checks to detect more subtle, but clinically meaningful forms of preanalytical error.

**Methods:** In this study, we spiked increasing volumes of major IV-fluids (normal saline (NS), lactated ringers (LR), and 5% dextrose (D5W)) into clinical blood samples that were collected from healthy donors. Crystalloid solutions were serially spiked into blood samples at 10% (solution:sample) increments for NS and LR from 10% up to 90%, and in 5% increments for D5W, up to 35%, and then to 50% for maximum dilution. Basic chemistry analytes were analyzed and compared between neat and contrived samples. All testing was performed on Roche Cobas 8000 chemistry auto-analyzers.

**Results:** Increasing concentration of NS showed significant changes in several basic metabolic panel analytes. The most significant changes relative to baseline were observed with potassium, calcium, and chloride that shifted by -0.8 mmol/L, -1.8 mg/dL, and +7 mmol/L, for every 10% of contamination, respectively. For LR, the most significant changes relative to baseline were observed with glucose and calcium that shifted by -12 mg/dL and -0.8 mg/dL, for every 10% of contamination, respectively. For D5W, the most significant changes observed were for glucose, calcium, chloride and sodium that shifted by 275 mg/dL, -0.6 mg/dL, -7 mmol/L and -9 mmol/L for every 5% of contamination, respectively. Finally, reviewing one month worth of quality assurance reports from our institution reporting IV-line contamination, we observed that samples identified via manual review by staff were found to have multivariate changes that were concordant with the observed differences in this study. However, in most cases, the magnitude of change was not significant enough to trigger our current, single-analyte, absolute value-based rules.

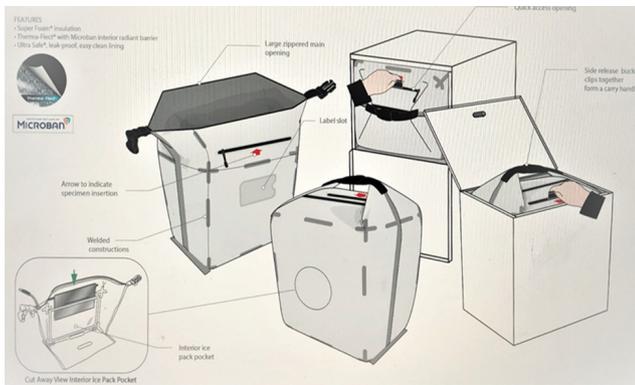
**Conclusion:** The results from this study support delta checks that consider multiple analytes, and fluid-specific bias profiles, as this may provide a more sensitive and specific approach to detecting contamination of clinical blood samples by crystalloid solutions. The *in vitro* experimental data collected in parallel to retrospective data analysis of institutional data will be used to derive multivariate delta checks in a data driven approach to detect pre-analytical error.

## B-274

### Insulated Courier Lockbox Insert Protects Specimens from High Temperatures

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**Background:** Traditional specimen lockboxes are metal with little or no insulation. Often these lockboxes sit outside the doctor's offices exposed to extreme hot and cold temperatures. Ambient seasonal temperatures are overlooked as a contributor to preanalytical variation and laboratory error, and to date, no guidelines or standards exist to improve this phase of the total testing process. In a recent study Dibbern and colleagues (Am J Clin Pathol 2021; 156:866-870), found significant negative effects of sample exposure to ambient temperature while stored in courier lockboxes. **Objectives:** We have designed and tested a prototype insulated insert that fits inside a common sized metal lockbox to offer better protection from extreme temperatures. **Methods:** Working with California Innovations we created a prototype insulated courier lockbox insert with approximate dimensions of 12" (L) x 9" (W) x 12 (H)". The lockbox had a Styrofoam lining but no other insulation. We compared different high-density foam materials such as high density expanded polyethylene (EPE), high density ethylene vinyl acetate (EVA), and a radiant barrier film laminate; EPE with radiant barrier film was chosen for testing. Air temperatures was monitored at the center of the lockbox at 23°C and 31°C and insulated lockbox insert was monitored at 29°C and 32°C ambient temperature. One 760 g ice pack was placed in the bottom of the lockbox; three 250 g ice packs were placed in the insert, on each side and bottom. **Results:** Air temperature in the lockbox exceeded 21°C at all timepoints; At 29°C and 32°C the insulated courier insert maintained temperatures of 5-11°C and 1-13°C, respectively, for five hours and 13°C or 15°C, respectively, after eight hours. **Conclusions:** The insulated courier lockbox insert protects specimens for considerably longer than the lockbox alone.



## B-275

### Verification of Sodium-Heparin Plasma Collection Tubes for Roche Cobas® Pro Analyzers

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**Background:** Many assays performed using the Roche cobas® analyzer series, accept the use of sodium-heparin plasma for various analytes as described in their accompanying product inserts. With the release of the next generation cobas analyzers, Roche eliminated sodium-heparin collection tubes from several of the updated assay product inserts. As a result, laboratories changing from older cobas analyzers to the newer cobas systems, are potentially faced with no longer accepting sodium-heparin plasma for patient testing of various analytes.

During our recent upgrade to the newer cobas pro systems, we evaluated the performance of sodium-heparin collection tubes for those assays that no longer indicated sodium-heparin plasma as acceptable. Because serum continues to be appropriate for these assays, comparison studies were performed utilizing the two specimen types. Here we report the results of those efforts.

**Methods:** Paired serum (serum separator tube) and plasma (sodium-heparin tube) specimens were drawn from volunteers. Specimens were collected according to Institutional Review Board approved protocols.

Paired specimens were tested (n=20 specimen pairs; 10 female, 10 male) using the cobas analyzers according to manufacturer's protocols. The analyzer and corresponding analytes tested were as follows: cobas pro c503 - alanine aminotransferase, amylase, beta-2 microglobulin, total bilirubin, complement component 4, haptoglobin, lipoprotein(a), low-density lipoprotein, phosphate, prealbumin, rheumatoid factor and soluble transferrin receptor; cobas e602 - free prostate-specific antigen and total prostate-specific antigen; cobas pro e801 - cancer antigen 15-3, cancer antigen 19-9, cancer antigen 125, carcinoembryonic antigen, creatinine kinase isoenzyme MB, dehydroepiandrosterone sulfate, follicle-stimulating hormone, myoglobin, parathyroid hormone, free prostate-specific antigen, total prostate-specific antigen, sex hormone-binding globulin, thyroxine, free thyroxine, thyroxinebinding capacity, thyroid-stimulating hormone and triiodothyronine.

For each specimen pair, the percent difference of results (plasma-serum) was calculated, and the mean absolute percent difference determined for each analyte. The primary criterion for sodium-heparin collection tube acceptability was a mean absolute percent difference of ≤10%. Additional assessment of sodium-heparin plasma acceptability for each analyte was completed by a paired t-test analysis and comparison with percent bias and within-subject CV data from biological variation databases (Westgard, European Federation of Clinical Chemistry and Laboratory Medicine).

**Results:** Sodium-heparin plasma was deemed acceptable for all but two analytes, rheumatoid factor and parathyroid hormone, based on the main criterion of ≤10% mean absolute difference. Eleven analytes, beta-2 microglobulin, haptoglobin, low-density lipoprotein, myoglobin, phosphate, prealbumin, free prostate-specific antigen (cobas e602), thyroxine, thyroxinebinding capacity, thyroid-stimulating hormone and triiodothyronine produced significant p-values (<0.05) upon paired t-test analysis. However, these analytes, except for thyroxinebinding capacity (not found in either database), were considered clinically acceptable based on data from one or both biological variation databases. Thyroxinebinding capacity, however, was judged acceptable based on precision and percent bias analyses.

**Conclusions:** Of the 31 analytes considered for sodium-heparin collection tube verification on Roche cobas analyzers, 29 were found acceptable considering overall, mean absolute difference, paired t-test analysis and biological variation. In an abundance of caution, sodium-heparin plasma was excluded as an acceptable specimen type for rheumatoid factor and parathyroid hormone.

## B-276

### Establishing new hemolysis Index for neonatal plasma glucose values measured using VITROS® XT 3400

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**Introduction** Hemolysis is still the most common reason for rejecting samples, especially in the neonatal population. Redrawing is very hard for neonates. Neonatal hypoglycemia, defined as a plasma glucose level of less than 30 mg/dL (1.65 mmol/L) in the first 24 hours of life and less than 45 mg/dL (2.5 mmol/L) after that, is the most common metabolic problem in newborns. This study aimed to investigate hemolysis's effect using different Hb concentrations on plasma glucose levels using Ortho Diagnostics VITROS® XT 3400 instrument. **Methodology** Four Plasma specimens with different glucose concentrations (49, 135, 201, and 397 mg/dl) were spiked with different hemolysate volumes prepared from re-suspending red cells in distal water to give a final 200, 300, 400, and 600 mg/dL concentrations of hemoglobin. Thermo Scientific Evolution 600 UV/VIS spectrophotometer was used to measure hemoglobin in the hemolysate. Each glucose value was run in triplicate, and the average for each sample was multiplied by the dilution factor. We used the CLIA'88 Total Allowable Error for glucose as target value ± 6 mg/dL or target value ±10%, whichever is greater as our acceptance limit, and the glucose values for the specimens without hemolysis as our target values. **Results** The VITROS® XT Hemolysis index correlates well with the spectrophotometer at low hemoglobin levels, and a negative bias increased directly proportional as hemoglobin values increased. The glucose results for each glucose concentration, and it's hemolyzed aliquots were compared. Samples with elevated glucose values (135, 201 and 397 mg/dl) were not significantly affected by all tested levels of hemolysis. However, samples with low glucose (49 mg/dl) had a significant (more than the CLIA specification) artificial elevation in samples when free hemoglobin exceeded 400 mg/dL. **Conclusion** The results suggest a hemoglobin interference cutoff of 400 mg/dL, higher than the Ortho package insert's limit of 200 mg/dl. 400 mg/dL of hemoglobin on the spectrophotometer correlates to a VITROS® XT heme index value of 320. Releasing glucose results with a hemolysis index of up to 320 would significantly improve the specimen's acceptance rate and allow fewer neonatal specimens rejection.

## B-277

### Evaluation of Serum Separator Collection Tubes for Serum Drugs Level Assays

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**Background:** Appropriate specimen collection and storage is essential to preserve sample integrity and ensure accurate test results. Default collection containers for blood drug levels are tubes without gel separators to avoid possible drug absorption. However, gel separator tubes are more convenient for collection, transport, and processing. As most routine chemistry tests are readily performed using gel separator tubes, collection of an additional gel-free tube is often required for drug level testing.

**Methods:** Citrated whole blood was pooled, spiked with drug, and transferred to three serum tubes (red, SST gold, RST orange) containing calcium chloride. Blood was allowed to clot before centrifugation and tubes were stored at ambient temperature (24 h) or in the refrigerator (7 days). At defined times, serum drug levels were measured (Roche cobas c502). Based on these results, specimen collection requirements were updated to allow serum separator tubes for 17 assays.

**Results:** Of the 21 drugs evaluated, 17 displayed acceptable stability in both gel-containing tubes (Figure 1). Three drugs displayed strong changes in measured levels after storage in one or both of the gel-containing tubes. Of the five drug tests most frequently ordered by the Emergency Department, ~94% were collected in serum separator tubes in the first 3 months following the update, with a total of 115 specimens collected in red top tubes and 1,867 collected in SST.

**Conclusion:** Evaluation of the stability of commonly monitored drugs revealed that a strong majority were not affected by prolonged exposure to gel separator material. Storage at room temperature for 24 hours or refrigerated for 7 days approximates conditions for outpatient clinic storage, courier transport and storage time during which

add-on testing may be requested. Expanding the collection requirements for appropriate drugs to include gel separator tubes decreases the number of specimens drawn and the complexity of laboratory workflows.

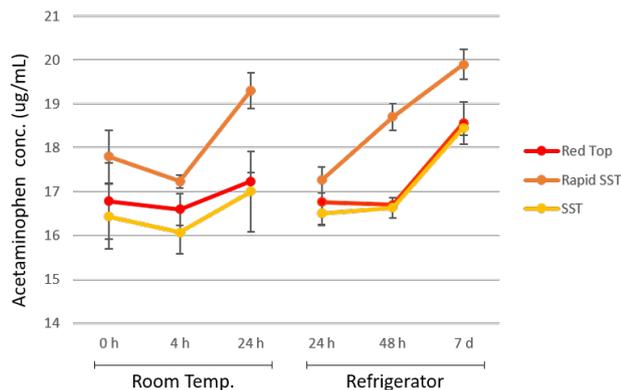


Figure 1. Average ethanol serum levels at defined time points for specimens collected in serum separator tubes stored at room temperature and refrigerated (mean±SD)

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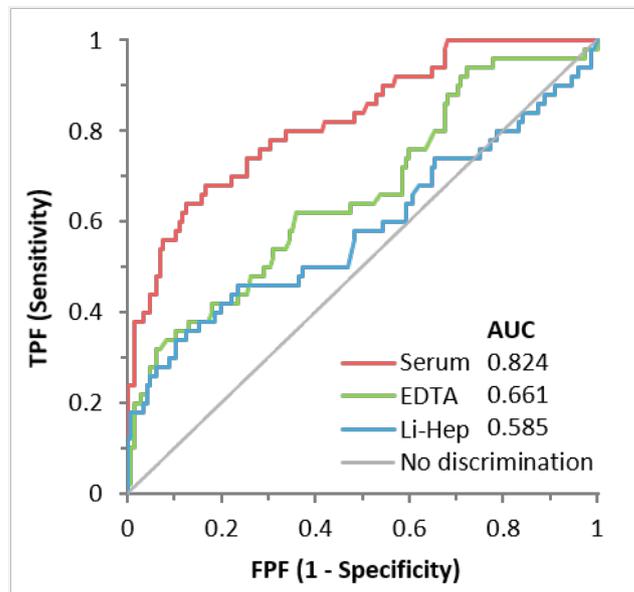
**Serum Calprotectin shows Superior Diagnostic Performance in Rheumatoid Arthritis compared to Calprotectin derived from Plasma**

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**Background:** Circulating calprotectin, a calcium-binding protein, is a promising marker with several potential applications, particularly in the diagnosis and monitoring of rheumatoid arthritis (RA) [1]. Several studies indicate that the measured concentration of circulating calprotectin strongly depends from preanalytical factors such as the sample matrix [2-4]. Therefore, the influence of the sample matrix on the diagnostic performance of calprotectin was evaluated.

**Methods:** Matched sets consisting of Serum, Li-Heparin- and EDTA-plasma obtained from 50 RA patients (DAS28 > 3.2) and 50 healthy controls. Calprotectin concentrations were measured using a newly developed particle-enhanced turbidimetric immunoassay (PETIA), BÜHLMANN sCAL® turbo, as well as with BÜHLMANN sCAL® ELISA. For rheumatologic patients, disease activity scores such as SDAI and DAS28 were available.

**Results:** Calprotectin concentrations were higher in sera from patients with RA than in controls for all matrices. Method comparison of the new BÜHLMANN sCAL® PETIA with BÜHLMANN sCAL® ELISA assay showed an excellent agreement with a Passing-Bablok regression of 0.983 for serum and 0.975 for EDTA-plasma samples. However, results were in poor analytical agreement for Li-Heparin plasma samples. Measured calprotectin concentration was lowest in EDTA-plasma and highest in serum for most matched serum/plasma sets. Calprotectin concentration was associated with disease activity score DAS28 in ROC analysis. The area under the curve (AUC) to discriminate RA from controls was 0.824 for serum, 0.661 for EDTA plasma and 0.585 for Li-Heparin plasma.



**Conclusion:** Our data confirm that calprotectin concentration strongly depends on the sample matrix. Serum calprotectin is superior to plasma calprotectin in discriminating RA patients from controls and seems to be the most suitable diagnostic matrix for the determination of calprotectin.

1. Ometto et al, *Exp.Biol.Med.*, 2017, 242(8), 859-873. 2. Van Hoovels et al, *Clin. Chem.Lab.Med.*, 2019, 58(1), 40-49. 3. Mylemans et al, *Clin.Chim.Acta*, 2021, 517, 149-155. 4. Nevejan et al, *Clin.Chem.Lab.Med.*, 2022, 60(2), E57-E60.

**B-279**

**Comparison of BD Vacutainer Barricor plasma with the Serum ST blood collection tubes for selected Routine Chemistry and Immunoassay Testing on Abbott Alinity ci-series**

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**Background** In parallel to serum-providing blood tubes with a barrier, plasma-providing blood tubes with a mechanical separator barrier are becoming more popular. Barricor Vacutainers are a non-gel separator blood collection tube with a surface coating of Lithium Heparin anticoagulant to inhibit blood coagulation and enabling samples to be processed much faster than serum collection vacutainers. A new tube with a mechanical separator has recently been launched (Barricor). According to the manufacturer may have these benefits to improve sample quality, centrifugation time, eliminate gel-related assay failures, such as instrument probe clogging and eliminate test interference due to gel. This study evaluated the stability of this tube to plasma gel tubes under clinically relevant conditions. For local clinical validation of this novel lithium heparin tube with a barrier, we compared selected routine chemistry and immunoassay analytes. These tubes enable for faster pre-analytic processing, significantly reducing turnaround time for routine patients. **Methods** Paired samples were taken from 60 outpatients at the KFAFH Hospital who were 18 years or older. Within two hours of collection, Barricor vacutainers were centrifuged for 3 minutes at 4000 g and SST vacutainers for 12 minutes at 1300 g at room temperature. On the Abbott Alinity ci system, all plasma samples were evaluated for chemical and immunoassay analytes on various days, and the bias of the data was determined between tubes. **Results:** All 56 analytes confirmed equivalent results across a broad concentration range between Barricor and SST vacutainers (Chemistry average percent bias -1.5% to 3.17%; Deming linear regression slopes 0.822 - 1.081; correlation coefficients ≥0.999) and (Immunoassay average percent bias -0.04% to 2.19%; Deming linear regression slopes 0.67 - 1.139; correlation coefficient 0.94 to 1.0). Platelet counts were statistically lower in Barricor™ compared to SST vacutainers. Alkaline phosphatase (ALP), carbon dioxide (CO<sub>2</sub>), total bilirubin (TBIL), lactate dehydrogenase (LD), and phosphate (PO<sub>4</sub>) had a significant bias in Barricor (-3.3%, 11.4%, 3.4%, 16.6%, 7.003%) relative to the reference tube, respectively. There was no statistical difference between different centrifugation duration or individual differences for K and LD in LiH and/or Barricor (p > 0.05). Human chronic gonadotropin (HCG) average (range) was 0.01 (0.00-0.14) mIU/MI, with the largest Error Index occurring at a concentration of 10.61 mIU/MI, while progesterone (PROG) was compared over a range of 1.50 to 27.10 nmol/l. The

average (range) was 0.03 (0.00-0.52) nmol/l. The largest Error Index occurred at a concentration of 27.10 nmol/l. **Conclusions** The BD vacutainer Barricor plasma blood collection tubes performed comparably to the more commonly used SST tubes. Using the Abbott Alinity-ci series, this study investigated the range, outlier, and linearity for the selected analytes.

**B-280**

**Specimen Contamination Rates With Residual Immunosuppressive Drugs Drawn From Central Venous Catheters**

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**Background:** Therapeutic drug monitoring (TDM) of immunosuppressive drugs is used to optimize therapy and minimize side effects. The use of central venous catheters (CVCs) is indicated for the administration of peripherally incompatible infusions and can also be used for blood specimen collection. However, blood collection from CVCs for the purpose of TDM needs to be properly performed to avoid contaminating the specimen. The aim of this project is to estimate the occurrence of blood specimen contamination with residual drugs in a bone marrow transplant service.

**Methods:** A retrospective review of critical results for cyclosporine (>600 ng/mL), tacrolimus (>25 ng/mL), and sirolimus (>25 ng/mL) in a pediatric population was performed for the period of 2020-2021. At our institution all TDM results are documented in the electronic health record with an interpretive consult written by a clinical pharmacist. We matched the critical value results with the interpretive consults to ascertain if the specimens were contaminated.

**Results:** Table 1 shows total tests performed, total number of critical results, and critical results due to specimen contamination. All critical results were from specimens collected via CVCs, and all but one was from bone marrow transplant recipients. Only six specimen contamination instances related to these three drugs were entered into our voluntary reporting system over the study period (6/20; 30%).

**Conclusion:** The observation of critical results for cyclosporine and tacrolimus being caused by specimen contamination are consistent with literature reports of drug adsorption to central venous catheters. Residual contamination of blood specimens with cyclosporine and tacrolimus has been reported to occur even after the lines are properly flushed and can persist for weeks after discontinuation of the infusion. Errors such as port misidentification or improper collection technique can lead to specimen contamination. Future efforts will use improvement science to reduce errors and the occurrence of contaminated specimens.

-Table 1-	Total Performed	Critical Results (%Total)	Critical due to contamination (%)
Cyclosporine	1269	19 (1.5%)	11 (58%)
Tacrolimus	1415	23 (1.6%)	9 (39%)
Sirolimus	1250	17 (1.4%)	0

**B-281**

**Improving Medical Students' Psychological Well-Being through Forgiveness Learning Program**

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**Background:** The psychological well-being of college students is of national concern given an increase in student reports of stress that negatively impacts their mental health. Previous research in forgiveness has shown forgiveness to be an important factor in the promotion of positive mental health. Dr. Robert Enright, a pioneer in forgiveness research, has decades of research on forgiveness and intervention programs. With his assistance, we have developed a 12-week online learning program utilizing a forgiveness and service love intervention for college students to investigate how the learning program impacts college students' well-being. The study also examines how students' spirituality and religiosity may affect the relationship between moral virtues and psychological well-being. **Methods:** 148 participants from business, law and medical schools at a Christian University were recruited for the study. Data from pre-and post-surveys were used to assess outcomes of the learning intervention. Participants were randomly assigned to an experimental group or a control group after being matched by college major, gender, service love score, and forgiveness score from the pre-survey data. The surveys consisted of demographic questionnaires and 12 measures, including service love, forgiveness, hope, self-esteem, empathy, anger, anxiety, depression, intrinsic spirituality, organizational religiosity, non-organization-

al religiosity, and intrinsic religiosity. Pearson correlation analysis was performed to investigate the association between moral virtues and psychological well-being. A multivariate analysis of variance (MANOVA) was used to examine differences between gender or schools in measures for the pre-survey. Then, the repeated measure analysis of variance (RM-ANOVA) was applied to investigate the effects of the learning program on moral virtues and psychological well-being. In addition to the quantitative analysis, interviews with selected participants from the experimental group were conducted. **Results:** The pre-survey data consisted of 52 males, 96 females, 95 professional students (medicine and law), and 53 business students. The pre-survey data presented statistically significant correlations between service love and empathy ( $r = .34$ ), hope ( $r = .24$ ), spirituality ( $r = .20$ ), organizational religiosity ( $r = .19$ ) and non-organizational religiosity ( $r = .18$ ), prejudice ( $r = -.24$ ), anger ( $r = -.20$ ), or depression ( $r = -.18$ ). A MANOVA test revealed that anxiety, empathy, depression, self-esteem, hope, intrinsic spirituality, and three religiosity measures demonstrated statistically significant differences between gender,  $F(13, 134) = 3.30, p < .01$ ; Wilk's  $\Lambda = .76$ . 88 participants completed both the pre-and post-surveys. A major finding of RM-ANOVA tests was a significant increase in forgiveness scores for the experimental group (12 points) after the learning program compared to the control group (2.25 points). More results will be presented in the poster. **Conclusions:** Pre-survey data showed strong relationships between service love and some psychological well-being and religiosity measures. Also, statistically significant gender differences in some measures were identified. The forgiveness learning program was revealed to be effective to improve participants' forgiveness. We expect that the learning program could improve not only students' forgiveness and well-being, but also their career readiness because professionals who deal with people having business problems, legal problems, or medical issues need to practice empathy and compassion.

**B-282**

**Evaluating the Feasibility of Using Preserved Urine for Testing Urine Chemistry Analytes to Reduce Pre-Analytical Variability**

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**Background:** Collection of 24hr urine can be challenging, especially if the urine needs to be collected using a splitter to collect two different sample types for collection. For samples collected at ambient condition and shipped ambient, we currently recommend collection with 10g of boric acid preservative for total protein and with no preservative for all other analytes including creatinine and albumin analysis. For this patient, we will have to use a splitter, collect urine into two different containers with and without preservative, then the sample needs to be transferred into respective tubes and sent it to the lab for analysis. This study was conducted to evaluate if one collection with boric acid would be suitable for analysis of urine chemistry analytes to eliminate the multiple pre-analytical steps and thus avoid any preanalytical errors. **Methods:** For this study both preserved (with 10g Boric Acid) and unpreserved urine were collected using a pee-splitter to split the 24hr urine sample into two separate jugs, from 21 presumed healthy volunteers. Upon receipt of the 24hr urine, the total volume was measured, specimens were thoroughly mixed and transferred into a 10.0 ml tubes for analysis. All chemistry analytes were analyzed on Roche Cobas 502 platform on both preserved and unpreserved sample in parallel. Data reduction was performed using the Alternate Method Comparison Module on Data Innovations EP Evaluator® Version 12.3.0 software. Preserved and unpreserved urines were considered combinable if they met our standard operating procedure acceptance thresholds for slope:  $1 \pm 0.05$ , y-intercept:  $< 5\%$  of Xmean, and correlation coefficient:  $> 0.95$  calculated from Deming regression. **Results:** Correlation outcome is demonstrated in the table below. **Conclusion:** Overall except Jaffe creatinine all other analytes showed acceptable correlation between preserved and unpreserved urine and does give us scope to use only one preserved urine collection to analyze all analytes when Enzymatic creatinine is chosen.

Method comparison data between preserved and unpreserved urine				
Analyte	Method	Deming regression	Correlation Coefficient (R)	Measurement Range
Creatinine Enz	Enzymatic Colorimetric	$y=0.972x + 0.125$	0.999	3.74 to 15.30 mmol/L
Creatinine Jaffe	Colorimetric	$y=0.940x + 0.274$	0.998	3.553 to 14.896 mmol/L
Microalbumin	Immuno-turbidimetric	$y=0.990x - 0.25$	0.995	3.4 to 61.9 mg/L
Magnesium	Colorimetric endpoint	$y=0.985x - 0.001$	0.999	0.91 to 5.28 mmol/L
Glucose	Enzymatic	$y=0.988x - 0.037$	1.000	0.11 to 373.62 mmol/L
Calcium	Colorimetric	$y=0.988x + 0.015$	0.999	0.64 to 6.50 mmol/L
Chloride	ISE Potentiometric	$y=0.982x + 0.10$	1.000	26.6 to 162.5 mmol/L
BUN	Enzymatic kinetic	$y=0.948x + 7.22$	0.991	112.1 to 312.8 mmol/L
Uric Acid	Enzymatic Colorimetric	$y=0.975x + 0.022$	0.999	0.67 to 3.35 mmol/L
NAG	Enzymatic Colorimetric	$y=0.974x - 0.05$	0.993	1.0 to 5.6 U/L
Phosphorous	Colorimetric	$y=0.973x + 0.285$	0.999	7.75 to 27.24 mmol/L
Potassium	ISE Potentiometric	$y=1.024x - 0.349$	0.997	11.60 to 65.20 mmol/L
Sodium	ISE Potentiometric	$y=0.987x - 0.76$	0.999	35.8 to 176.3 mmol/L

**B-283**

**Retrospective measurement of the effect of diurnal variation in specimens drawn weekly, either within 2 hr of previous week's draw time or outside of the 2 hr period**

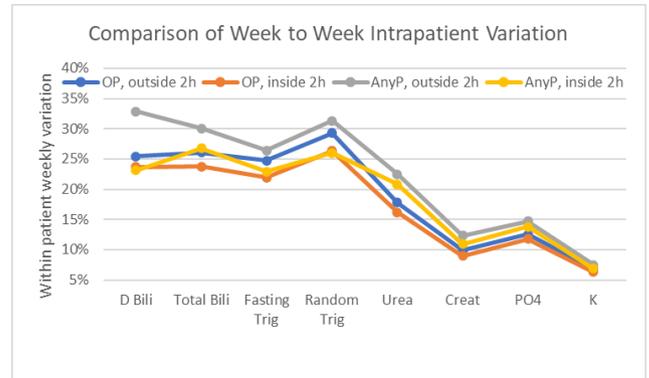
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**Background:** In 1989, Broughton observed that diurnal variations in bilirubin, phosphate, and triglycerides accounted for >5% of between subject variation. For these analytes, excess variation in blood draw times might falsely indicate significant analyte changes. We have developed a model for determining long term patient variation (PMID:35137000) and had access to 5 years of Siemens de-identified chemistry results from Ottawa Hospital. Based on the variability of the time of blood drawing, we determine the weekly inpatient variation of phosphate, direct and total bilirubin, fasting and random triglycerides as well as potassium, urea and creatinine.

**Methods:** For each analyte, if either drawn a week later and within 2 hr (or more than 2 hr) of its previous draw time, we tabulated consecutive pairs of inpatient truncated results by time intervals of separation: 0-1 weeks, 1-2 weeks, 2-3 weeks, up to 95-96w. We followed the procedure in PMID 35137000 and determined the long term variation of specimens that were drawn within 2 hr of the previous weeks specimen or more than 2 hours.

**Results:** The numbers of patients studied ranged from 14,400 for direct bilirubin to 261,000 for creatinine and 269,500 for potassium. Patients were classified as out-patient (OP) or outpatient and hospital (AnyP). The Figure shows that the between week variation is higher for AnyP compared to OP and noticeably higher for bilirubin and triglycerides with sequential blood draw time differences exceeding 2 hours. For AnyP direct and total bilirubin, the within subject variation is between 4 to 8% higher when drawn outside the 2 hour window. The differences are much less for a more highly controlled analyte like potassium.

**Conclusion:** As long as OP phlebotomy times are limited to 8 am to 5 pm, the diurnal variation expansion of Broughton's analytes' reference intervals is not excessive.



**B-284**

**Evaluation of the Stability of Fecal Immunochemical Test Samples at Ambient and Refrigerated Temperatures**

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**Background:**

Implementation of fecal occult blood test (FOBT) screening programs have decreased colorectal cancer mortality by 15-33%. The fecal immunochemical test (FIT) is the most common FOBT used in Canada. Currently at our institution, patients collect samples in a test kit at home and then return the kits to the lab within 7 days. A pilot study completed at our institution showed that 50, 60, 74, and 85% of positive samples near the clinical cut-off became negative after 3, 5, 7, and 14 days, respectively, when stored at room temperature (RT); refrigeration did not completely prevent this decrease over time. The objective of this study was to further evaluate the stability of FIT specimens after refrigeration or RT storage.

**Methods:**

A series of stability experiments were performed with the OC-Sensor DIANA iFOB Test system (Eiken/Polymedco) using a clinical cut-off of 75 ng/L hemoglobin (Hb) for a positive result. FIT samples were identified based on the initially measured concentration of Hb. Samples were analyzed at baseline, followed by consecutive measurements on days 3, 5, 7, and 14; samples were stored refrigerated or at RT throughout the experiment. Samples were categorized into three groups, based on their baseline measurement: Group A (n=40): 70-80 ng/ml, Group B (n=20): 90-110 ng/mL, and Group C (n=20): 190-210 ng/mL, with half stored at RT and half refrigerated. To further investigate the stability of RT storage for samples just above the cut-off within the first week of collection, an additional 10 samples (Group D) were collected with initial measurements between 75-85 ng/mL and measured at baseline, day 4, and day 7. Data from samples stored at RT with baseline Hb values of 75-100 ng/mL were combined with relevant pilot study data (n=24) to evaluate changes in positivity over time.

**Results:**

Group A showed a significant decrease in Hb on day three (p<0.05, paired sample t-test); this decrease was not observed until day 7 for refrigerated samples. Refrigerated and RT samples in Group B were significantly decreased on day 7 (p<0.05, paired sample t-test). Group C showed a significant decrease in Hb on day three (p<0.05, paired sample t-test), however, refrigerated samples did not significantly decrease throughout the study. Notably, all Group C samples remained positive over the course of the study, while Groups A and B saw decreases in the number of positive samples over time. Combined data with the pilot study for samples marginally above the cut-off (75-100 ng/mL) showed 100% positivity on days 0-1 (n=50), 62% on days 2/3 (n=37), 49% on days 4/5 (n=37), and 30% on days 6/7 (n=43).

**Conclusions:**

FIT samples showed decreased concentrations of Hb compared to baseline when stored at RT. Refrigeration appears to improve stability, but does not entirely prevent this decrease. FIT specimens with Hb concentrations near the clinical cut-off for positivity may be particularly susceptible to false negatives due to reduced stability. Shortening the acceptable stability time for samples with Hb levels just below (50-74 ng/L) the cut-off value could help prevent false negatives.

**B-285****Variables That Influence Bovine Serum Albumin Color and Their Importance to Diagnostic Applications**

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**Background:** Biologically derived proteins are commonly used in clinical diagnostics for standards, blockers, stability, and the capture and detection of analytes. The appearance of these proteins varies by lot, manufacturer, and origin. However, the reason for variations in color, particle size, and other properties are poorly understood and perceived to influence performance in final products. A better understanding of underlying causes for variation in appearance could provide insight on its impact on specific final applications. Bovine Serum Albumin (BSA) is a common blocker used in diagnostic applications whose appearance has been known to vary from manufacturers and is a good model protein to understand appearance variation in the clinical diagnostic space. BSA can be sourced in a range of colors and particle sizes from various manufacturers; color may range from colorless, yellow, green, or amber by visual inspection and particle size may vary from  $\mu\text{m}$  to mm dimensionality. The objective of this study is to quantify BSA color and determine the effect of particle size, residual chemical, such as bilirubin, on final BSA product color. **Methods:** Color measurements on lyophilized BSA powders ( $n=104$ ) were made using a Hunter Labs Miniscan XE Plus Colorimeter calibrated using white and black tiles on the daylight setting and a D65/10 degree reference. Data output in Hunter Labs was converted to CIE  $L^*a^*b^*$  values (EasyRGB, 2020) and D65/10 illuminant and observer values (refX 94.811, refY 100, refZ 107.304). Color analysis was performed using MATLAB and GraphPad Prism. BSA lots were sourced from Boone, IA or Feilding, New Zealand (NZ). To determine powder particle size distribution, three sieves with known mesh sizes were used to separate BSA samples. Bilirubin was quantified using the ABX Pentra Total Bilirubin Assay. Calibrations and testing on the ABX Pentra 400 were performed according to the manufacturer's recommendations. **Results:** CIE  $L^*$  is a measure of lightness, ranging from 0 (total black) to 100 (absolute white),  $a^*$  is a measure of red-green (positive to negative), and  $b^*$  is a measure of yellow-blue (positive to negative). When comparing BSA powders, NZ lots were slightly greener with an  $a^*$  range of 0.4 to -4.2 versus the US range of -1.6 to -6.2, but half of the US and NZ lots overlapped in  $a^*$  values. Decreasing the particle size led to significant increases in  $L^*$  ( $p=0.012$ ) and decreases in  $b^*$  ( $p=0.018$ ) but no significant change in  $a^*$  values. BSA samples with particle sizes  $\leq 10$  mesh were tested for bilirubin. As  $b^*$  values increased, bilirubin also increased in a linear manner ( $R^2=0.91$ ) explaining most of the yellow color variation. This is quite striking since bilirubin residuals were low, ranging from 4-10  $\mu\text{g-bilirubin/g-BSA}$  (ppm). **Conclusion:** CIE  $L^*a^*b^*$  values confirm plasma country of origin, bilirubin, and particle size influence color of BSA. When controlling for particle size, differences of 1 ppm bilirubin explain variations in yellow color. Based on the current analysis, it is unlikely color differences will cause issues in most diagnostic application unless there is sensitivity to particle size or ppm differences in bilirubin content.

**B-286****Assessment of Pre-Analytical Processing on Measurement of Calprotectin in Fecal Specimens**

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**Background:** Fecal calprotectin (fCal) is a biomarker of endoscopic inflammation approved for the diagnostic evaluation of patients with suspected inflammatory bowel disease; it is also used by some physicians for monitoring treatment response and mucosal healing. Testing for fCal requires extraction from stool, which can be accomplished by manual weighing or with graduated extraction devices. The pre-analytical processing and the analytical testing both contribute to the overall performance characteristics of the fCal assay.

**Methods:** Extractions of fCal from stool samples was performed using the Fecal Extraction Device (Werfen, San Diego, CA). Analytical testing for fCal was performed using the QUANTA Lite® Calprotectin ELISA or QUANTA Lite® Calprotectin Extended Range ELISA (Werfen) on the Dynex DS2 (Dynex, Chantilly, VA). Inter-assay reproducibility of the ELISA was evaluated using three extracted stool specimen pools tested in replicates ( $n=5$ ) over five analytical assays; acceptance criteria was set at  $\leq 20$  CV%. Inter-extraction reproducibility was evaluated using 6 stool specimens extracted in replicates ( $n=6$ ); acceptance criteria was set at  $\leq 20$  CV%. Continuous improvement program for the extraction process was performed on a quarterly basis and included extraction of stool specimens in replicate by five trained individuals with acceptance criteria of  $\leq 25$  CV%.

**Results:** Verification of the fCal ELISA using extract pools demonstrated inter-assay %CVs ranging from 6% to 16%. In contrast, %CVs obtained during precision studies in the verification of the Fecal Extraction Device ranged from 16% to 39%.

In response to the reproducibility in the extraction process observed during the verification, the laboratory initiated quarterly assessments of the extraction process as part of a continuous improvement program. The initial assessment included 9 samples which were extracted by 5 trained individuals; %CVs of the replicate extractions ranged from 11% to 36% (mean 25%). In the second assessment, %CVs ranged from 43% to 77% (mean 61%) for replicate extractions ( $n=5$ ) on 3 samples. Following the second assessment, several interventions were undertaken in an attempt to improve the reproducibility of the extraction process, including removing excess stool from tip of extraction device, mixing of stool before extraction, stirring of the stool till homogenous and after specimen has fully thawed, determining when a stool sample should be pipetted or stuck with the extraction device, and overall pipetting techniques. For the next 2 assessments following these interventions (3 samples/assessment and 5 extractions/sample), %CVs ranged from 5%-41% (mean 20%) and 5%-30% (mean 18%), respectively.

**Conclusion:** The majority of the variability observed with fCal measurements can be attributed to the pre-analytical processing. Our data suggest that monitoring of pre-analytical processing and a continuous improvement program is recommended to increase consistency and reliability of fCal analytical testing.

**B-288****Applying modified task centered strategies to rapid antigen self-testing instructions**

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**Background:** Lateral flow immunoassays have played a vital role in addressing testing needs during the pandemic for both screening and diagnosis. However, there is a lack of understanding regarding the widespread public use of self-testing and usability perceptions. The purpose of this study was to assess the overall user experience (UX) and general usability of self-testing kits to test for SARS-CoV-2 in relation to provided instructions.

**Methods:**

The study utilized two SARS-CoV-2 lateral flow antigen tests, and 60 participants over a 3-day study. Test (a) utilized the standard manufacturer format for patient instructions, test (b) utilized modified task-centered patient instructions. Test (a) consisted of a device requiring 8 steps to complete, with testing performed on day (1) and day (3). Test (b) consisted of a device requiring 12 steps to complete, with testing performed only on day (3). completed post-test questionnaires based on a 5-point Likert scale for each device, and after each day of testing to assess user experience. Test (a) demonstrated a UX score of 94.33% on day (1) and a 95.03% on day (3), for a total UX score of 94.68%. Test (b) performed only on day (3) demonstrated a UX score of 98.05%.

**Results:** While both tests demonstrated acceptable usability, and performance when compared to reference method RT-PCR testing. Test (b) utilizing modified task-centered patient instructions was ranked far higher by participants, despite participants having prior use experience with test (a) on day (1). Participant's age was determined not to be an influencing factor using a Wilcoxon rank sum test that demonstrated continuity across participant age ranges. Survey free text responses were analyzed using a latent Dirichlet allocation model, this analysis suggests the user experience for test (a) could be improved by modifying device instructions. This analysis also suggests that the instructional organization in relation to each performed task for test (b) was easier to follow.

**Conclusion:**

The study findings support that there are opportunities for improving self-testing instructions using an approach that better organizes task components to ensure user comprehension. This approach to user instruction development could improve user experience across other applications of self-testing as well as generalized point-of-care testing.

**B-289****Optimizing digital health application frameworks to support at-home testing**

L. B. Springer. *Brio Systems, Bellevue, OH*

**Background:** At-home testing has seen a large increase with the emergence of SARS-CoV-2. The primary contributor for this growth has been the need for the at-home

testing market to offset overburdened brick and mortar testing facilities. With this increase in at-home testing the need for digital health technology applications (DHT) supporting these tests has also increased. These applications play a key role in ensuring ease of use, test accuracy and facilitating public health reporting. Given the vital role that digital health applications play in public health testing it is imperative that they are optimized for patient engagement to ensure test validity. This is paramount for digital health technology to be considered a useful remote clinical tool.

**Methods:** A systematic analysis of digital health technology supporting at-home SARS-CoV-2 testing was performed to identify key areas of focus and to ensure optimal test outcomes. The focus of the study was to assess features of each application and its engagement in testing phases utilizing the mobile application rating scale (MARS). The study included 38 participants, and 7 digital health applications designed to complement SARS-CoV-2 at-home testing. To determine the correlating performance between testing sections of each application a nonparametric rank sum test was used. This allowed the mobile application rating scale to be aligned with each of the key testing phases of pre-analytical, analytical and post-analytical.

**Results:**

Higher ratings in the pre-analytical and post-analytical sections of the mobile application rating scale correlated to an overall high level of satisfaction and desired outcome. These correlations were highest in the functionality dimension exhibiting a mean score of 4.03 with an SD of 0.67, with the lowest correlations seen in information quality. This section exhibited a mean score of 3.04 with an SD of 0.21, indicating that result accessibility or report access was a key component of dissatisfaction. Additional correlations were seen between sections related to aesthetics and its perception to support pre-analytical processes effectively with a mean score of 3.90 and an SD of 0.44

**Conclusion:** Sections with higher ratings were deemed as being able to consistently produce better patient and result outcomes based upon the engagement in relation to test task. This provides a framework for test developers and manufacturers to focus on when developing digital health technology designed to support at-home clinical testing. Focusing on these areas can be a viable means to increase patient test comprehension, test compliance and optimize patient results. The optimizing of digital health technology in these areas is vital to support the widespread deployment of at-home testing to effectively address potential public health needs.

**B-290**

**Differentiating turbid versus truly lipemic samples with elevated lipemia indices**

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**Background** One common preanalytical error in the clinical biochemistry laboratory is sample turbidity without lipemia. This can be produced from suspended cells or debris in the specimen, potentially from transportation. Automated lipemia/turbidity indices (LI) and autoverification rules are utilized to assess lipemia or turbidity at a large academic health center. If the index is above the designated threshold, the specimen will be aliquoted and centrifuged to remove turbidity or, if the LI was not lowered below the threshold, centrifuged at high-speed to remove lipemia. This study aims to differentiate the number of turbid without lipemia from lipemic specimens using a shared LI rule to evaluate the operational impact of suboptimal turbid specimens.

**Methods** De-identified outpatient data from December 2021 was collected from the middleware repository (Data Innovations) for common analytes with relatively low LI thresholds: direct bilirubin (DBIL), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) with their corresponding LI (Roche Diagnostics). Samples with an initial LI greater than the threshold but dropped below the threshold after routine centrifugation were classified as false lipemia. Samples that required high-speed centrifugation were classified as true lipemia. The data were categorized into four groups: total samples, samples with an initial LI greater than the threshold, true lipemia, and false lipemia. It takes around 3 mins and 6 mins for a well-trained laboratory technologist to handle a false lipemic and a true lipemic sample. Total handling time was calculated as the sum of the true and false lipemia handling time. **Results**

	Total	Above Lipemia Index Threshold	True Lipemia	False Lipemia	% True Lipemia	% False Lipemia	Handling Time (min)
DBIL above the limit of quantitation (0.2 mg/dL)	2348	91	4	87	4%	96%	285
AST	33674	42	19	23	45%	55%	183
ALT	33674	15	9	6	60%	40%	72

**Conclusion** DBIL, AST, and ALT are high-volume tests with relatively low LI thresholds. Therefore, they are susceptible to turbidity from potentially improper transportation or centrifugation, confirmed by the high false lipemia rates (40%-96%). Of note, a total of 540 minutes for one month was dedicated to troubleshooting, which could be shortened if the preanalytical handling process is improved.

**B-291**

**Insignificant Differences in Hemolysis related parameters in Four-Way-Split Sample-Transfer from 22G-Needle-Syringe to Serum Tubes, Assayed for Potassium, LDH, AST, ALT & Free Hemoglobin**

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**Background:**

It has long been hypothesized that direct transfer of a blood sample from a syringe through its needle into a tube, especially if aided by the vacuum and/or additional force through the plunger, is likely to cause unacceptable hemolysis. Most western Phlebotomy Guidelines eg CLSI H3-A6 and textbooks recommend detaching the needle and attaching a "syringe transfer device" for transferring blood from a syringe into an evacuated tube. However, such maneuvers would add time for processing and involve a dedicated device/disposable which adds to cost and hospital waste and is rarely available in low or middle-income countries including India. These devices also puncture stopper with needle and currently cite safety and not hemolysis. To the best of our knowledge, there is not much concrete evidence on comparisons of hemolysis by different transfer techniques.

**Methods:** After taking informed consent phlebotomy was done on **36 volunteers** with syringes & **22G needles**. Immediately after drawing and mixing the samples were distributed to 4 identical **red-top BD** tubes in 4 different ways: **A: Closed Stopper: No Push**, only vacuum draw. **B. Closed Stopper: Extra push** on top of the vacuum. **C. Open stopper: Needle removed, D. Open stopper: Needle on** syringe. Parameters sensitive to hemolysis i.e. Potassium, LDH, AST, ALT and CK were measured with an ERBA fully automated analyzer. Free Hemoglobin was measured by Spectrophotometry at 415, 380 & 450 nm using **Allen Corrected Harboe** formula validated locally using serially diluted hemolysate.

**Results:**

Potassium, LDH, AST, and ALT were done on 144 (36x4) tubes, 100 (25x4) of which could be transferred and archived frozen in Eppendorf tubes for a Batch Spectrophotometry later. Four level Repeated Measure ANOVA did not show a significant difference between effects of the 4 transfer methods on Potassium, LDH, AST, ALT and free Hb)- all p values insignificant irrespective of sphericity assumption. Optical Free Hb correlated significantly but not so strongly with all the parameters, maximum with Potassium (r=0.293, p=0.003).

**Conclusion:** Transfer by these 4 different methods caused insignificant variation in hemolysis as measured by free hemoglobin, LDH, AST, ALT and Potassium. The transfers though were done without a transfer device (which is the norm in our country), the use of a rack instead of holding the tubes by hand made the transfers safe enough. Since there was no difference in hemolysis, time could be saved by simple direct transfer.

**Acknowledgment:**

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**B-292****Evaluating the Sample Transportation Time from Phlebotomy Center to Core Laboratory- Real World Evaluation of a Medical Center in Taiwan**

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**Background:**

Taipei Veterans General Hospital is one of the largest medical centers in Taiwan having 2,800 beds, 10,000 outpatient visits with around 3,000 blood drawings and 30,000 laboratory tests per day. Given the high demand on laboratory tests, the optimization of blood sample transportation procedures from the outpatient phlebotomy center to the core laboratory has become an important issue. Our phlebotomy center and core laboratory are located in different buildings having 500-meter straight distance. As the total laboratory automation (TLA) system in the core laboratory has been applied to clinical service, we implemented several automatic transportation systems to reduce the manual intervention in the sample transportation process, including the single-tube transport pneumatic tube systems (sPTS, 180 tubes/hour per sPTS) with a dispatch robotic arm for grasping and recognizing blood tubes (400 tubes/hr), the multi-tube PTSs, the carrier-based PTSs as well as some sorting machines connected to blood tube convey belts. All transportation processes were controlled and monitored by laboratory information system (LIS). In this study, we examined the different steps of sample transportation time including (1) T1a: the time from the blood collection counters to the sorting machines conducted by the convey belts; (2) T1b: the time from the sorting machines through PTSs to the sample Inlets of TLA system in the core laboratory, to see whether the performance have meet the laboratory goals in which the optimal thresholds for sample transportation were set as  $\geq 90\%$  within 20min for T1a and  $\geq 90\%$  within 30min for T1b.

**Methods:**

The laboratory data from 2020 January to 2021 November with a total of 2,894,501 blood tubes was collected for analysis. The mean, SD, 50, 90, 95 percentiles of T1a and T1b, and the percentages of T1a less than 20 min, T1b less than 30 min were calculated.

**Results:**

From 2020 January to 2021 November, the overall blood tubes from outpatient phlebotomy were 2,894,501 with an average of 125,848 (84,179-157,781) per month. The mean of T1a was 12.2 min ranging from 8.9 min to 18.9 min, and that of T1b was 27.5 min varied from 24.3 min to 30.7 min. The 50, 90, 95 percentiles of T1a were 6.6 min, 12.1 min, 21.2 min respectively, and were 23.2 min, 48.6 min, 61.5 min for T1b. There were 94.6% of tubes with T1a less than 20 min, and 68.3% with T1b less than 30 min. Compared with the previous process, we have saved 3 staff that originally handled with packing, singing up and delivering by manual.

**Conclusion:**

This study demonstrated that through implementing the automatic transportation systems from the phlebotomy centers to the core laboratory can not only improve blood tube transportation efficiency but also save labor costs (now we need only 1 staff for system monitoring and trouble shooting), which could be taken as a reference for clinical laboratories that have a large outpatient test volume.

**B-293****Falsely elevated urine total protein leads to the diagnosis of a rare metabolic disorder**

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**Background:** A 42-year-old patient was found to have positive urine protein by dipstick analysis. Follow up urinalysis was unremarkable, however, quantitative 24 h urine total protein (UTP) measured by main chemistry analyzer reported a concentration of 2.68 g/L (reference interval < 0.15 g/L) with minimal albumin (24 mg/L). UTP remained elevated for the next 4 months (2.12-3.10 g/L), while the underlying cause was investigated. The patient was non-diabetic, had a normal eGFR (> 100 mL/kg/1.73 m<sup>2</sup>) and other chemistries were normal. The patient's investigations for glomerulonephritis were negative - including serum and urine electrophoresis, autoimmune antibodies (anti-nuclear antibodies, rheumatoid factor), and complement C3/C4 levels. To patient was scheduled for a renal biopsy for further clarification. Days before the biopsy, the laboratory independently began investigating the patient's urine

specimen for spectrophotometric interference after the UTP was flagged by the instrument for having abnormally high absorbance. The flagged result was 3.10 g/L, which upon automatic dilution measured as 0.00 g/L. Repeat testing and manual serial dilution agreed with initial result prompting an investigation for analytical interferences.

**Methods:** Our laboratory measures UTP on the Roche Diagnostics Cobas® c701. The method includes two key reagents: NaOH to alkalize specimens and benzethonium chloride to produce turbidity. Change in specimen turbidity is assessed by measuring absorbance before and after the addition of benzethonium chloride. If absorbance exceeds the linear range of the instrument, results are flagged, and the specimen is automatically diluted 10-fold. Prior to reporting, dilutions are reviewed by technologists.

**Results:** The urine with suspected interference (specimen A) was clear-yellow. Upon agitation, no bubbles formed, which was inconsistent for urine containing elevated protein. The absorbance flag and the discrepancy in the measured UTP following dilution prompted a review of the reaction tracing. The tracing of Specimen A was compared with that produced by quality control (QC) material and another patient's urine (specimen B) with similar apparent protein levels (3.20 g/L). The reaction trace of specimen A was found to have a high background absorbance, which steadily increased 10-fold upon addition of NaOH without benzethonium chloride. No change in absorbance was observed with the QC material or specimen B with NaOH addition alone. NaOH was manually added to aliquots of the specimens A and B for visual inspection; specimen A changed to a red-brown color whereas no change in color was observed in specimen B. The ordering physician was contacted regarding the interference and the urine was sent off-site for testing by the pyrogallol red UTP method, which measured specimens under acidic conditions. Using this second method, the measured UTP was 0.13 g/L. A review of the literature suggested homogentisic acid (metabolite of Alkaptonuria) as a possible interferant. Notably, homogentisic acid does not interfere with the pyrogallol red UTP method. Urine organic acids were ordered, and the urine was found to be positive for homogentisic acid, resulting in a diagnosis of Alkaptonuria for the patient.

**Conclusion:** Homogentisic acid is a rare metabolite that can cause false elevated urine protein in methods that alkalize specimens.

**B-294****Effects of physicochemical properties on instillation of medications using dropper bottles**

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**Background:**

Dropper bottles are an alternative to pipetting devices that allow for consistent, accurate, repeated reagent dispensing in a dropwise fashion. They are used for point of care applications such as instillation devices for eye and ear drops, medication dosing, as well as in contact lens solutions and are used in self- or bedside- diagnostic kits (COVID-19 antigen test kits, pregnancy test, etc.).

Instillation of conventional aqueous solutions generally results in the delivery of drops that are accurate and precise to the denoted volume of the dropper bottles. However, in the pharmaceutical industry these are often used to instill solutions that contain viscolysers and in biotechnology applications to deliver lateral flow chromatographic buffers that often contain ionic and non-ionic surfactants such sodium dodecyl sulfate or Tween™ 20. These agents vary the density and viscosity of a solution, resulting in low accuracy or precision of the droplets delivered by the dropper bottle. This study investigates the effect of the solution's physicochemical properties has on the accuracy and precision of their instillation by dropper bottles.

**Methods:**

Similar LDPE dropper bottles from 5 separate manufacturers were tested for the accuracy and precision of volume dispensed, using liquids with different densities, viscosities, surface tension at both 4 °C and 25 °C. Dispensed volumes were measured gravimetrically using an analytical balance (0.001 g) and using the calculated density to convert mass to volume. A measurement of n=10 different droplets from n=3 different dropper bottles was performed from n=2 different technicians (total N=60 measurements per dropper bottle for each solution and temperature) and compared to the denoted droplet volume. Accuracy and precision data were analyzed with a student's t-test. A p-value of 0.05 or less was considered significant. Standard errors were calculated based on standard deviations of the droplets volumes and are reflected as error bars in all graphs of the study. To confirm the comparisons that were p<0.05 with the student's t-test, one-way ANOVA with Tukey's honestly significant difference (HSD) analysis was also performed for all pairwise comparisons of the mean values to separate groups. A value of q<0.05 was considered significant. Additionally, the dispense angle, volume in the bottle and user variation were considered.

**Results:**

Decreases in surface tension, resulting from the addition of surfactants and viscolysers to solutions, were observed to decrease the accuracy of the volume of the drops. Inversely, increases in the viscosity of the solutions decreased the volume of drops instilled. However, the decrease in accuracy observed under the above conditions could be counteracted to some extent by lowering the temperature of the solutions and maintaining this lower temperature during instilling the droplets.

**Conclusion:**

These results illustrate the effect of the solutions' physicochemical properties and temperature on the accuracy of the volume instilled by dropper bottles. Understanding these effects, manufacturers of pharmaceuticals or solutions for biotechnology applications can improve the accuracy of the volume of their solutions instilled using dropper bottles, ensuring the accuracy and precision of the amount of medication or reagent dispensed.

**B-295****“Specimen” is the Standard term for Collection, “Sample” is the Standard term for Statistics and Measurement**

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**Background:**

ISO 15189:2012 point 3.15 defines the terms “primary sample” and “specimen” as synonyms. ISO/TC 212 WG1 started the activity “13.3 Harmonization of terminology across ISO TC212”. ISO/TS 20658:2017 in the same definition modified “specimen” in “sample”. Besides “Term and definition” and “Bibliography” sections, ISO/TS 20658 has no recurrence of the term “primary sample” in the text, but the title of the is “Requirements for collection and transport of samples”. ISO 15189 terms are inherited by several ISO documents, such as ISO 5798, generating unnecessary ambiguity between material to be collected and material (sample or aliquot) to be examined.

**Methods:** We examined the documents of Clinical & Laboratory Standards Institute (CLSI), International Organization for Standardization (ISO), International Medical Device Regulators Forum (IMDRF), Global Harmonization Task Force (GHTF), World Health Organization (WHO), International Laboratory Accreditation Cooperation (ILAC), Centers for Disease Control and Prevention (CDC), European Committee for Standardization (CEN), and Health Level 7 (HL7) to find out the prevailing trend for the use of the words ‘specimen’, ‘sample’ and ‘primary sample’.

**Results:** CLSI changed all titles related to collection from “primary samples” to “specimen”. ISO 20916:2019 and ISO 6717:2021 deleted the term ‘primary sample’. The IMDRF uses the term specimen in its harmonized guidance documents to mean a sample of biological origin intended for examination by a medical laboratory. WHO, ILAC, HL7, CDC, CEN/TS 17742:2021 and CEN/TS 17747:2021 use the term “specimen” for collection activities. ISO/IWA 15:2015, ISO 20184-1:2018 and ISO 20166-1:2018 defines also the term “aliquot”. ISO 5492:2008 defines “sample” and “aliquot” as equivalent. ISO 15198:2004, ISO 3534-2:2006 and ISO/IEC/IEEE 24765:2017 define the statistical sample. CLSI GP33 distinguishes between the words ‘specimen’ and ‘sample’.

**Conclusion:**

The trend in standard documents, in guidelines and in many documents is clear. “Sample” is the harmonized term for the measurement and statistical activities. “Specimen” is the harmonized term for the collection activities. “Primary sample” is almost never used in the text of official documents and leads to potential ambiguity.

**B-297****Evaluation of the Automated pH Assay on the Roche cobas pro c503 for Urine Specimen Integrity Assessment**

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**Background:** Clinical testing of urine specimens can be used to aid in the diagnosis of a variety of diseases of the urinary tract, kidneys, and liver as well as metabolic disorders such as diabetes. Evaluating the integrity of urine specimens is important for producing reliable results and includes tests like pH, creatinine, and specific gravity. Many urine analytes require preservative addition to maintain sample integrity. For instance, some analytes are acidified avoid precipitation or bacterial growth, while other analytes are maintained alkaline to prevent degradation. The aim of the current study is to evaluate the use of an automated pH test on the Roche cobas c 503 platform. The

performance of the Roche Specimen Validity Test pH (SVTPH) was assessed using accuracy, linearity, imprecision, and reference interval verification. **Methods:** Method comparison testing was conducted on 30 residual urine specimens with pH ranging from 1-11. All urine specimens were tested on the Roche SVTPH assay and compared to a previously validated pH meter (Mettler Toledo, Columbus, OH) both quantitatively and qualitatively. Quantitative assessment included Deming regression analysis and %bias. Qualitative assessment compared the overall agreement in categorizing samples acidic (pH <4), neutral (pH 4-7), or basic (pH >8). Linearity was assessed using all 5 Roche SVT controls (ranging from 3.6 – 11.6 pH) testing each level in triplicate. Imprecision studies used same Roche SVT controls. Intra-assay precision was evaluated testing 10 replicates of 2 SVT controls (low/high) in a single day. Inter-assay precision was tested on all 5 SVT controls testing 4 replicates a day for 5 days for a total of 20 replicates. Acceptable imprecision criteria was coefficient of variation (CV) <10%. Lastly, reference interval was verified using 60 random urine samples (29 males and 31 females; age: 20-59 years) collected from apparently healthy volunteers. **Results:** Method comparison studies comparing Roche SVTPH assay to pH meter had acceptable correlation with a slope of 0.92 and a bias of 11.5%. Notable bias between the methods was observed at pH <2.5, corresponding to the lower measuring limit of the automated assay. Qualitative assessment showed good overall agreement (96.7%), having 1 discordant sample with a borderline neutral pH on meter and pH just above 8 on Roche. Linearity's had recoveries ranging from 99.1-107.8%. Imprecision and reference intervals met manufacturer's claims. **Conclusion:** The Roche SVTPH assay met all manufacturer claims. Comparison studies to pH meter demonstrated that the SVTPH assay was effective at categorizing samples as acidic, neutral, or basic. The performance was deemed acceptable to determine if samples submitted for basic chemistry urine analysis were adequately preserved, and thus suitable for clinical testing. Automated pH testing may provide a suitable and scalable alternative to pH specimen integrity testing by pH meter or pH strip evaluation for evaluating the integrity of urine specimens.

**B-298****Performance of the DiaSorin QSET Plus Device: Designed with Input from Laboratory Technologists**

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**Background:** Fecal calprotectin (FC) is a screening test for intestinal inflammation, which is helpful to distinguish between a non-inflammatory gastrointestinal issues, such as irritable bowel syndrome (IBS), versus an inflammatory one, like inflammatory bowel disease (IBD). Fecal Pancreatic Elastase (FPE) is a test used to screen for exocrine pancreatic insufficiency (EPI) caused by either pancreatic or intestinal issues. Given that the volumes of FC and FPE testing are steadily increasing, laboratories need to consider ways to increase the efficiency of testing. Our laboratory staff evaluated the original QSET extraction device and requested improvements to optimize workflow, resulting in the QSET plus device. We evaluated the performance of the QSET plus in comparison to manual weighing and the original device. **Methods:** Method comparison analyses were performed on the new QSET Plus extraction device versus the current QSET extraction device and manual weighing method using 49 stool samples for FC and 25 for FPE. Quantitative analyses between extraction methods were analyzed by Deming regressions and % biases. Qualitative agreements were assessed using reference interval cutoffs (FC: <50 µg/g = normal, 50-120 µg/g = borderline and >120 µg/g = elevated; FPE: >200 µg/g = normal, 100-199 µg/g = mild/moderate EPI, <100 µg/g = severe EPI). Imprecision evaluation of the QSET Plus extraction device was also performed using pooled stool QC material. Intra-assay precision was evaluated testing 10 replicates of pooled QC material in a single day. Inter-assay precision was tested over 5 days for a total of 20 replicates of pooled stool QC material. **Results:** Comparison of FC results based on the QSET Plus extraction device versus manually weighing and current QSET device had slopes of (0.964 and 1.020, Deming regression) and average biases of (-4.6% and -3.5%), respectively. Samples >800 µg/g were excluded in the quantitative analyses, since very elevated calprotectin measurements are known to have increased variability between extraction methods. For qualitative classifications of FC results, the QSET Plus device had comparable overall agreement with manual weighing and current QSET for all 49 samples tested (93.9% and 89.8%), respectively. Precision of the new QSET Plus device for FC was favorable with CV's <1%. Comparison of FPE results based on the QSET Plus extraction device method versus manually weighing and current device had slopes of (1.113 and 0.932, Deming regression) and average biases of (19.3% and -7.2%). For qualitative classification of FPE results, the QSET Plus device had comparable overall agreement with manual weighing and current QSET (88.0% and 100.0%), respectively. QSET Plus compared to manual weighing resulted in 3

discrepancies; (n=2 samples went from severe to moderate; n=1 went moderate to normal). **Conclusion:** Overall, the new QSET Plus device demonstrated good performance compared to manual weighing and the current QSET device for both FC and FPE for use with formed, homogenous stool samples. Improvements to the device include a longer wand, one-sided opening, and buffer added to the tube. These improvements were greatly appreciated by the lab staff to reduce contamination of gloves and repetitive movements while still maintaining high quality results.

## Precision Medicine

### B-300

#### Analytical Validation of $GFR_{NMR}$ : A Blood-Based Multiple Biomarker Assay for Accurate Estimation of Glomerular Filtration Rate

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Chronic kidney disease (CKD) is a major public health problem. Accurate and precise monitoring of kidney function is critical for a timely and reliable diagnosis of CKD. Determination of kidney function usually involves the estimation of the glomerular filtration rate (eGFR). We recently reported the clinical performance of a new eGFR equation ( $GFR_{NMR}$ ) based on the nuclear magnetic resonance (NMR) measurement of serum myo-inositol, valine and creatinine, in addition to the immunoturbidometric quantification of serum cystatin C, age and sex (Stämmler et al. *Diagnostics* 2021, 11, 2291). We now describe the analytical performance evaluation of  $GFR_{NMR}$  according to the Clinical and Laboratory Standards Institute guidelines. Single-site, within-laboratory coefficients of variation (CV) of the  $GFR_{NMR}$  equation, calculated from 4 different serum pools with  $GFR_{NMR}$  scores ranging from < 60 to > 60 ml/min/1.73m<sup>2</sup> and a total of 480 measurements, did not exceed 4.3%, with a maximum CV for repeatability of 3.7%. The inter-site reproducibility (between three sites), calculated from 4 serum pools and 360 measurements, demonstrated a CV of 5.9%.  $GFR_{NMR}$  stability was demonstrated for sera stored up to 8 days at 2-10°C and for NMR samples stored up to 10 days on board of the NMR device at 6 ±2°C. Over the storage time duration investigated, the linear regression slope p-values were not significant ( $p > 0.05$ ). The analytical performances of  $GFR_{NMR}$  combined with its previously reported clinical performance support the potential incorporation of this NMR method in clinical practice.

### B-301

#### Increased CA125 concentrations in Heart Failure with reduced ejection fraction: association with cardiac biomarkers and mortality.

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**Background:** Carbohydrate Antigen 125 (CA125) is the most widely used biomarker in ovarian cancer screening. In patients with heart failure (HF), increased levels of CA125 have been observed and related to disease severity. The aim of our study was to determine CA125 levels in HF patients with reduced ejection fraction (HFrEF) and its association of with cardiac biomarkers and adverse outcomes. **Methods:** Our retrospective study included 102 HFrEF patients and the outcome was cardiovascular mortality over a median follow-up of 3.7 years. CA125 circulating levels were determined with an electrochemiluminescent immunoassay. Concentrations of B-type natriuretic peptide (BNP), N-terminal proBNP (Nt-proBNP), Galectin-3 and C-terminal Fibroblast Growth Factor 23 (FGF23) were also measured by immunoassays. **Results:** CA125 levels were increased in HFrEF (mean concentration: 135 U/mL; range: 5 to 2587), were associated to disease severity according NYHA classes and were significantly and negatively correlated to left ventricular ejection fraction. Median CA125 concentration was also significantly related to cardiovascular mortality (log Rank =0.03). CA125 concentrations were positively and significantly associated to Galectin-3 ( $r=0.31$ ,  $p<0.001$ ) and FGF23 ( $r=0.38$ ,  $p<0.001$ ). CA125 was also significantly related to natriuretic peptides. In multiple regression analysis including age, EF, eGFR, BNP, NT-proBNP, Galectin-3 and FGF-23, the independent determinant of CA125 levels was BNP. **Conclusions:** Concentrations of CA125 are increased in patients with HFrEF, associated to disease severity and to adverse cardiovascular outcomes. CA125 levels are also correlated to several cardiac biomarkers in HFrEF patients and BNP appears as an important determinant of raise of CA125.

### B-302

#### Targeted metabolomic analysis suggests that Tacrolimus alters pipercolic acid and sarcosine metabolisms.

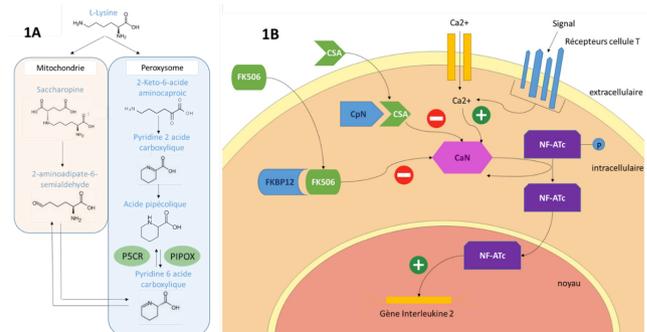
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**Background:** Tacrolimus (FK506), is an immunosuppressor that inhibit calcineurin. One of this side effects is nephrotoxicity. Pipercolic acid (PA) is a metabolite derived from lysine. Significant PA signals were routinely detected in patients treated with FK506. It is important to note that the structure of pipercolic acid is found in that of the molecule of FK506. Aims of this study are both to verify this possible analytical interference and to evaluate the link between use of FK506 and increase in plasma PA.

**Methods:** Analysis of amino acids (AA) was performed in plasma samples. To confirm the link between FK506 intake and the increase of plasma PA, AA measurements were performed in control patients without FK506, patients under FK506 (in vivo FK506) and control patients with FK506 added in vitro. To study the link between the metabolic impact of FK506 on PA and calcineurin inhibition, ciclosporin (CSA), was also investigated.

**Results:** Increased plasma PA was observed in patients under FK506 compared to control samples ( $p = 0.005$ ). In vitro additions of FK506 to control samples do not increase PA. Patients under CSA do not show increase in plasma PA compared to control samples ( $p = 0.18$ ), which does not support a metabolic link between the calcineurin and PA. However, in vivo FK506 was associated with higher plasma sarcosine and a decrease in the glycine/sarcosine ratio, as well as a tend to increased plasma lysine.

**Conclusion:** These results do not suggest interference between FK506 and AA assay, nor a metabolic impact of calcineurin inhibition on PA. However, the plasma AA changes in patients under FK506 highlight a possible link between FK506 and the action of an enzyme involved in both PA and sarcosine catabolism, the Peroxisomal sarcosine oxidase (PIPOX). This hypothesis needs to be confirmed by further mechanistic studies. Moreover, .



### B-303

#### Urine based PSA assay- A surrogate marker for invasive prostate cancer

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Serum PSA together with digital rectal examination and imaging of the prostate gland remained the gold standard in urological practices for managing and intervention of prostate cancer. Based on these adopted practices, the poor performance of serum PSA towards aggressive prostate cancer has led us to evaluate whether urinary PSA levels might have any clinical utility in prostate cancer diagnosis. **Results:** Utilizing the established diagnostic PSA assay (Beckman Coulter Access 2 immunoassay), we evaluated a total of n=437 urine specimens from prostate cancer patients. In our initially cohort, urinary PSA tests from a total of hundred and forty-six (n=146) urine specimens were obtained from aggressive (Gleason Score ≥8, n=76) and the non-aggressive (Gleason Score=6, n=70) prostate cancer patients, followed by a large set of n=291 urine samples from aggressive (GS≥7, n=168) and non-aggressive (GS=6, n=123) prostate cancer patients. Our data demonstrated that patients with aggressive disease had lower levels of urinary PSA compared to the non-aggressive patients while the serum PSA levels were higher in patient with aggressive prostate disease. The discordance among serum and urine PSA levels was further validated by immuno-histochemistry (IHC) assay in biopsied tumors and in metastatic lesions (n=65).

**Conclusions:** Our data demonstrated that aggressive prostate cancer was negatively correlated with the PSA in prostate cancer tissues and unlike serum PSA, urinary PSA might serve a better surrogate in capitulating tissue milieu in detecting aggressive prostate cancer. We further explored the utility of urine PSA as cancer biomarker alone or in combination with serum PSA and their ratio (serum to urine PSA) to predict the disease status. Comparing the AUCs for the urine and serum PSA alone we found that urinary PSA has a higher predictive power (AUC= 0.732) in detecting aggressive disease. Furthermore, combining the ratios between serum to urine PSA with urine and serum assay enhanced the performance (AUC= 0.811) in predicting aggressive prostate diseases. These studies strongly support the role of urinary PSA in combination with serum for detecting aggressive prostate cancer.

**Acknowledgement:** This work was supported by the NCI Early Detection Research Network (EDRN, U01CA152813) and the Patrick C Walsh (PCW) Prostate cancer research grant to Dr. Hui Zhang (Johns Hopkins University)

## B-304

### Multisite Imprecision for the Abbott, Beckman, Roche and Siemens High-Sensitivity Cardiac Troponin Assays at the Female 99<sup>th</sup>-Percentile: A Prospective Analytical Sub-Study From the CODE-MI Trial

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**Background:** One component that defines a high-sensitivity cardiac troponin (hs-cTn) assay is achieving a 10% coefficient of variation (CV) at the 99<sup>th</sup>-percentile concentration of a healthy population. Often, this imprecision is assessed at the overall population's 99<sup>th</sup>-percentile concentration (i.e., a cutoff derived from both healthy females and healthy males). However, as sex-specific 99<sup>th</sup>-percentiles are recommended by clinical and laboratory guidelines, it is imperative that the imprecision is also assessed at the lower female 99<sup>th</sup>-percentile concentration given this cutoff is used to identify myocardial injury in this population. In Canada, a stepped-wedge, cluster-randomized trial aims to assess whether use of the lower female 99<sup>th</sup>-percentile concentration cutoff improves the diagnosis, treatment, and outcomes of women presenting to the emergency department with possible myocardial ischemia (CODE-MI

Trial ClinicalTrials.gov Identifier: NCT03819894). Our objective in this multi-site sub-study of CODE-MI was to assess the hs-cTn assay imprecision at the female 99<sup>th</sup>-percentile concentration for different hs-cTn assays. **Methods:** Patient samples (EDTA plasma) with cTn concentrations near the upper analytical limit were identified and pooled together. Human serum was purchased from Sigma-Aldrich and used as the base material into which the high cTn pool was spiked to produce different quality control (QC) materials. For each QC material, the initial target concentration was derived from testing the spiked serum ten times, with the first and last aliquot tested prior to freezing the QC aliquots below -70°C. Two aliquots were thawed and retested (to confirm stability) for a total of 14 measurements prior to sending the QC aliquots on dry ice to laboratories across Canada. The analyzers used were the Abbott ARCHITECT i1000 (hs-cTnI), Beckman Access 2 (hs-cTnI), Roche Cobas 602 (hs-cTnT), and Siemens ADVIA Centaur (hs-cTnI) for the initial assignment of QC hs-cTn concentrations targeted slightly below the manufacturers' female 99<sup>th</sup>-percentile hs-cTn concentrations (average concentration of QC material | package insert outside United States 99<sup>th</sup>-percentile concentration [ng/L]: Abbott=14.1|16; Beckman=9.7|12; Roche=8.8|9; Siemens=36.6|39). Instructions on storing, handling, and testing the materials were provided, with the frequency of testing being once a month for one year. Data obtained from the first three months for assays that had at least ten data points was used to determine the pooled SDs and weighted mean used to calculate the overall CV for each company.

**Results:** Testing of 168 samples occurred on 57 different instruments from 35 sites across 8 provinces from January 2022 to March 2022. Instruments included the Abbott ARCHITECT i1000 (n=1), i2000 (n=5), Alinity (n=2); Beckman Access 2 (n=1), DxI 600 (n=3), DxI 800 (n=5); Roche Cobas e411 (n=9), e601 (n=4), e602 (n=12) e801 (n=11); and Siemens Atellica (n=4). The range of QC material concentrations were 10.7-15.6 ng/L for Abbott (n=24); 9.3-13.5 ng/L for Beckman (n=25); 3.7-11.5 ng/L for Roche (n=107); and 29.2-37.0 ng/L for Siemens (n=12). The overall average hs-cTn concentrations (CV) were: Abbott=12.7 ng/L (7%); Beckman=11.6 ng/L (7%); Roche=8.6 ng/L (10%); Siemens=33.4 ng/L (5%).

**Conclusion:** The initial estimates from this analytical sub-study of the CODE-MI trial indicate acceptable and comparable imprecision of hs-cTn assays at the female-specific 99<sup>th</sup>-percentile concentration cutoff.

## B-305

### Combined study of tumor tissue genotype and serum tumor circulating DNA provided additional diagnostic and prognostic value in pediatric rhabdomyosarcoma.

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#### Background:

Pediatric cancer evolution may be compromised by the presence of low represented mutations. In this way, bad evolution or relapse could be associated to the increase of minority clones with pathological mutations. Next generation sequencing (NGS) of tumor circulating DNA (ctDNA) may be an interesting alternative for genotypic evaluation of the tumor before treatment intervention. Thus, this approach may permit the detection of low prevalence mutations that could be monitored during the patients' treatment. Rhabdomyosarcomas are high aggressive tumors frequently located on skeletal muscle. It represents 3-4% of all pediatric cancers and is the most common mesenchymal cancer in children and adolescents. Two different subtypes are described, embryonic rhabdomyosarcoma (ERMS), more frequently observed, and alveolar rhabdomyosarcoma (ARMS).

#### Methods:

We present a clinical report analyzing the ctDNA by NGS of a child diagnosed of ERMS showing progression after treatment. The patient was re-diagnosed of ARMS after relapse analyzing tumor cells genotype from the biopsy after new surgery. At this time point serum circulating DNA was purified and sequenced by Agilent Technologies for Illumina.

**Results:** An eight-year-old patient presented a left nasal tumor. After surgery, the anatomopathological analysis was indicative of ERMS. The patient suffered a relapse one year after treatment, with a large but localized tumor mass that was removed by radical surgery, followed by treatment with maintenance chemotherapy. At this time point, patient was re-diagnosed of ARMS due to the presence of *PAX3-FOXO1* fusion, characteristic of ARMS, and the absence of 11p15.5 chromosomal deletion, a typical alteration of ERMS, in the genotypic analysis of tumor cells. Even though,

patient continued presenting a bad evolution, showing a submandibular nodal relapse and undergoing both, second-line chemotherapy and radiotherapy. Finally, the child started metronomic chemotherapy and palliative radiotherapy.

From serum ctDNA NGS analysis, two variants that might be associated with the presence of rhabdomyosarcoma were found (*CHEK2*: NM\_001005735.1: c.1549C>T, p.R517C; *HRAS*: NM\_176795.4: c.425T>A, p.I142N). Rhabdomyosarcoma progression has been associated with alteration on the Ras/PI3K pathway. Besides, although 11p15.5 chromosomal deletion was not observed, bad evolution of ERMS has been also associated with different *RAS* related point mutations, that might be the case of the *HRAS* mutation detected.

**Conclusion:** Due to the torpid evolution of the patient, we speculate that in some ARMS tumors, the *HRAS* variant found may have a role in the progression of the disease. Besides, the presence of this variant might be explained by presence of a mixture of both rhabdomyosarcoma subtypes in this particular patient.

## B-306

### An Automated Estimated Atherosclerotic Cardiovascular Disease Risk Score

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**Background:** Estimation of Atherosclerotic Cardiovascular Disease (ASCVD) risk is a key step in Cardiovascular Disease (CVD) prevention but typically requires healthcare workers or patients to collect additional risk factor information and entering it into a computer for the calculation. We developed a simplified ASCVD risk score that can be automatically calculated by the clinical laboratory information systems on all patients tested with a standard lipid panel. **Methods:** Equations for an estimated ASCVD (eASCVD) risk score were developed for four race/gender groups: Non-Hispanic White men (NHWM) or women (NHWW) and Black men (BM) or women (BW), using the following variables: TC, HDL-C, TG, and age. The eASCVD risk score was designed by multiple regression analysis to yield similar risk estimates as the standard ASCVD risk equations for 6,027 non-diabetic and lipid-lowering therapy-free individuals from the National Health and Nutrition Examination Survey (NHANES). Additionally, eASCVD risk score was compared to the standard ASCVD risk score for its association with ASCVD events in Atherosclerosis Risk In Communities (ARIC) population (N=14,742). **Results:** At a cut-point of 7.5%/10-year, the eASCVD risk score had an overall sensitivity of 69% and a specificity of 97.5% for identifying statin-eligible patients with at least intermediate risk based on the standard ASCVD risk score. By using the sum of other risk factors present (SBP>130 mmHg, blood pressure medication use, and cigarette use) to adjust the eASCVD score, its overall sensitivity increased to 94%, with a specificity of 92%. For each race/gender group, the eASCVD risk score performed similarly, but the positive predictive value (PPV) for men was better (88.6% for NHWM and 88.7% for BM) than for women (79% for NHWM and 78.7% for BW). Furthermore, eASCVD risk score showed 90% concordance with the standard risk score in predicting ASCVD events in the Atherosclerosis Risk In Communities (ARIC) study (N=14,742). **Conclusion:** The routine use of the automated eASCVD risk score for all patients tested with a standard lipid panel could improve CVD outcomes by being used as a primary prevention tool for screening patients at risk for ASCVD and as a decision aid for statin therapy.

## B-307

### Evaluating large NGS panels for RNA fusion detection in FFPE samples

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**Background:** Gene fusions have emerged as important biomarkers for precision oncology. It is a result of structural variants that can drive tumorigenesis by producing chimeric transcripts and abnormal proteins. The detection of these rearrangements can help cancer diagnosis and guide therapeutic decisions. Next-Generation sequencing (NGS) techniques are being widely used in routine patient care and oncology workflows by facilitating tumor molecular characterization. There are different platforms and NGS approaches, each one having its own advantages and disadvantages. Since gene fusions detection still presents more challenges than other gene variants, this

study aimed to validate and compare two large NGS panels for RNA fusion detection in clinical formalin-fixed paraffin-embedded (FFPE) samples. **Methods:** RNA was extracted from 18 FFPE samples. Samples' quantity, quality, and integrity were evaluated using Qubit 3.0 (Thermo Fisher Scientific) and 4200 TapeStation (Agilent Technologies). RNA fusion testing was performed by hybrid capture-based enrichment on Illumina's TruSight RNA Fusion assay (TSRNA), according to the manufacturer's instructions. Samples were also compared according to their previous results with amplicon-based enrichment on Thermo Fisher's OncoPrint Focus assay (OFA). **Results:** The average total RNA concentration was 69.2 ng/μl. The RNA Integrity Number (RIN) was low ( $1.1 \leq \text{RIN} \leq 2.6$ ) for all samples. The fraction of fragment sizes above 200 bases ( $\text{DV}_{200}$ ) was also measured. Samples with a  $\text{RIN} \leq 1.9$  exhibited a higher  $\text{DV}_{200}$  (> 50%) than samples with a  $\text{RIN} \geq 2$ . Thus,  $\text{DV}_{200}$  was superior for evaluating RNA in low-quality samples such as FFPE. The concordance rate between TSRNA and OFA was 88.8% (n=16/18) and all samples with a known fusion were accurately detected in both assays (n=5/5). Discordant results (n=2/18) were due to rare fusion events. OFA generates a 3'/5' imbalance score allowing the detection of fusions involving an unknown 5' partner. This analysis is absent in the TS5NA pipeline. **Conclusion:**  $\text{DV}_{200}$  proved to be a superior metric for RNA quality assessment and successful RNA-based sequencing results. Despite RNA quality and NGS enrichment methods, all known fusions were precisely identified. Further analysis with ALK-RET-ROSI FFPE RNA Fusion Reference Standard (Horizon Discovery, Cat. no. HD784) will be done to evaluate TSRNA performance and workflow from pre-analytical RNA extraction through to fusion detection.

## B-308

### Evaluation of next-generation sequencing (NGS) panels to detect genetic biomarkers related to PARP inhibitors sensitivity

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**Background:** Homologous Recombination Repair (HRR) is an important mechanism for the repair of double-stranded DNA. In some neoplasms such as prostate, ovarian, and breast cancer, alterations in HRR-related genes have been used as a biomarker to direct therapy with *PARP* (poly ADP ribose polymerase) inhibitor. Several studies have shown that patients with *BRCAl/2* mutations, key genes in the HRR mechanism, benefit from *PARP* inhibitor therapies, such as Olaparib, which represents a gain in treatment options. However, other genes and genomic loci, e.g. *ATM* and *CHEK2*, also play an important role in HRR. In this sense, the availability of genetic tests to evaluate several regions simultaneously, as well as NGS, is essential for a better clinical interpretation. Thus this work aims to evaluate two genetic NGS panels for *PARP* inhibitor biomarkers detection. **Methods:** Were evaluated twenty-seven FFPE samples from patients with ovarian or prostate cancer history and two commercial controls. All of them were previously tested for *BRCAl* and *BRCAl/2* variants search using the Ion platform (Thermo Fisher). The evaluated kits were AmoyDx® HRD Focus (HRD) and SOPHiA® Homologous Recombination Solution (HRS). The quality of samples and libraries were evaluated on the TapeStation 4200. Libraries were prepared following the recommendations of each manufacturer and sequenced using NextSeq500 (Illumina). Data analyses were performed using ANDAS AmoyDx® and SOPHiA® DDM softwares. **Results:** Sequencing demonstrates on target + flanked target coverages ~74% for HRS. In HRD 12 out of the samples failed in quality control due to the insufficient DNA library concentration according to its stringent analysis quality criteria. The poor initial quality of the FFPE DNA was directly related to the failure of the library to reach the quality cut-off. In approved HRD libraries, the on-target coverage average was ~95%. Therefrom, the 100x depth was greater than 80% and Q30 mapped reads close to 94% in both tests. From 132 variants surveyed in *BRCAl/2*, the positive percent agreement of SNV/Indel was 100% in both tests, and the consistency between *BRCAl/2* deficient and genomic scar score calculation (GSS) positive was 95.71% in HRD. In HRS it was possible to identify other 87 variants with probable clinical significance. From the 5% of allele frequency detection limit, the sensitivity, specificity, and accuracy were 100% in both tests. **Conclusions:** Both performance tests reached the expected results regarding the identification of variants in the *BRCAl/2* genes, with satisfactory intra- and inter-run reproducibility. HRD has the advantages of the GSS and a shorter library preparation time (<= 1 day) but requires a tumor percentage higher than 30% to avoid false negatives and DNA quality that is not always obtained from FFPE samples. In another hand, HRS presents a greater number of analyzed regions (16 genes) and superior control of the bioinformatics analysis steps but presents susceptibility to DNA degradation (deamination), which had to be mitigated by manually checking the variant calls in the IGV. This study highlighted the great usability and importance of both exams for expanding the access of patients with ovarian/prostate cancer to personalized medicine.

**B-309****Study of Mutations in Myeloid Neoplasms Using the Next Generation Multigene Sequencing Panel**

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**Background:** Myeloid neoplasms (MNs) are a heterogeneous group of hematologic malignancies that include acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), and myelodysplastic/myeloproliferative neoplasms (MDS/MPN). The World Health Organization (WHO) classification for MN has consolidated the importance of molecular changes in the diagnosis and prognosis of these neoplasms. With the increasing use of the genomic approach in the studies of MNs, new molecular biomarkers have been shown to be relevant for prognosis and therapy. Multigene Next Generation Sequencing (NGS) panels allow a more complete analysis of several genetic alterations, such as single nucleotide variants (SNV), small deletions and insertions, among others. However, the benefits of using an NGS panel in the investigation of MNs are not fully defined.

**Objective:** This study aims to investigate gene mutations in patients diagnosed with MN. **Methods:** Between November/2019 and July/2021, 30 samples of peripheral blood or bone marrow were collected from patients diagnosed with MN, at the Hematology Service of Hospital das Clínicas da UFMG. DNA and RNA samples from each patient were sequenced using the OncoPrint Myeloid Assay panel (Thermo Fisher Scientific), with the sequencing of 40 genes (23 hotspots and 17 full genes), gene fusions involving 29 driver genes. **Results:** Samples from 30 patients were sequenced: 11 MDS (36.7%), 10 (33.4%) AML, 6 MPN (20.0%), 2 MDS/MPN (6.7%) and an acute leukemia phenotype mixed (3.3%). The median age was 52 years (18 to 83 years). Nineteen patients (63.3%) were male and 11 (36.7%) were female. In 26 cases (86.7%), at least one mutation was detected, with the number per case varying from 1 to 5. A total of 57 mutations were detected in 22 genes, with the most frequent mutations in TET2, IDH2, FLT3 (ITD) and ASXL1. Mutations were detected in all cases of AML, also standing out for the greater number of mutations, with the most frequent mutation being the FLT3 -ITD. All of the FLT3-ITD mutations had mutant/wild allelic ratios greater than 0.5, confirmed by capillary electrophoresis. In MDS, mutations in TET2 and ASXL1 stand out. Mutations in TET2 were also the most frequent in NPM and SMD/NMP. In 12 cases (40%), at least one mutation was detected in genes associated with adverse prognoses, such as FLT3, ASXL1, TP53, RUNX1, U2AF1 and DNMT3A. We detected the fusions RUNX1-RUNX1T1, KMT2A-MLL2 and PICALM-MLL10 in the LMA; and BCR-ABL1 in CML. Mutations in TET2, SF3B1 and IDH2 were detected in three cases of triple-negative NPM. **Conclusions:** Our results demonstrate a high frequency and co-occurrence of mutations, some associated with an adverse prognosis. As multiple genes are related to the pathogenesis of MNs, with a significant overlap of mutations between groups, the use of a multigene myeloid NGS panel is relevant and feasible.

**B-310****Validation of biomarkers detection for Alzheimer's disease in Brazilian patients: a pilot study**

F. d. Marinho, M. A. Pereira, D. A. Zauli. *Pardini Group, Vespasiano, Brazil*

**Background:** In June 2021, the U.S. Food and Drug Administration (FDA) approved aducanumab (Aduhelm™) for the treatment of Alzheimer's Disease (AD). Aducanumab is a human immunoglobulin monoclonal antibody (IgG type) directed against aggregated soluble and insoluble forms of amyloid  $\beta$ . It is intended for the treatment of AD in the phase of mild cognitive impairment and mild dementia before the neurodegenerative process is established. To be approved by The Brazilian Health Regulatory Agency (Anvisa), tests should also be performed in the Brazilian population. Techniques providing AD earlier diagnosis, such as cerebrospinal fluid (CSF) biomarkers and amyloid-PET neuroimaging, can be used. **Objective:** This study aimed to validate the Euroimmun's kits for beta-amyloid 1-42, beta-amyloid 1-40, total tau (tTau) and phosphorylated tau (pTau) proteins measurement in CSF samples from Brazilian patients diagnosed with AD. **Methods:** This was an observational and analytical study to evaluate the performance of Euroimmun's kits "Beta-Amyloid (1-40) ELISA", "Beta-Amyloid (1-42) ELISA", "Total Tau ELISA" and "pTau (181) ELISA" for AD. 18 patients diagnosed with AD (n=6) and other dementias (n=12) were tested for all kits. Statistical analysis was performed using MedCalc for Windows (Ostend, Belgium). **Results:** As expected, patients with typical AD had a decreased 42/40 beta-amyloid ratio ( $\leq 0.1$ ) and an increased tTau ( $> 614$  pg/mL) and pTau ( $> 61$  pg/mL). Two AD patients failed for tTau and will be retested. The remain-

ing patients, presented 42/40 beta-amyloid ratio  $\geq 0.1$  and low pTau ( $< 61$  pg/mL). For tTau, only three patients were borderline (466 to 614 pg/mL). **Conclusions:** Several studies investigated the utility of AD biomarkers in CSF samples. Our results were concordant with literature findings and are a strong indicative that this method may be suitable for AD differentiation in Brazilian population. Future experiments will be performed with more samples.

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