2021 AACC Annual Scientific Meeting & Clinical Lab Expo
Poster Session Schedule

Posters of accepted abstracts were viewed in the Poster Hall of the Georgia World Congress Center, on Tuesday, September 28 and Wednesday, September 29.

Below are the topics and their scheduled times.

TUESDAY, SEPTEMBER 28, POSTER SESSIONS

9:30am – 5:00pm

Analytical Techniques and Applications . . . . . A-002 – A-130 . . . . . S2
General Clinical Chemistry . . . . . A-131 – A-220 . . . . . S41
Hematology/Coagulation . . . . . A-222 – A-242 . . . . . S64
Precision Medicine . . . . . A-243 – A-253 . . . . . S70

WEDNESDAY, SEPTEMBER 29, POSTER SESSIONS

9:30am – 5:00pm

Data Analytics and Informatics . . . . . B-001 – B-033 . . . . . S75
Laboratory Management and Leadership . . . . . B-034 – B-062 . . . . . S85
Laboratory Stewardship and Patient Safety . . . . . B-063 – B-073 . . . . . S94
Microbiology and Infectious Diseases . . . . . B-074 – B-167 . . . . . S98
Molecular Diagnostics. . . . . B-169 – B-211 . . . . . S126
Preanalytical and Postanalytical . . . . . B-212 – B-234 . . . . . S138
Special Patient Populations . . . . . B-235 – B-262 . . . . . S145
Toxicology and Therapeutic Drug Monitoring . . . . . B-263 – B-298 . . . . . S154

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Tuesday, September 28, 2021

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

Analytical Techniques and Applications

A-002

Longitudinal Evaluation of the Abbott Architect SARS-CoV-2 IgM and IgG Assays in a Pediatric Population

C. Interiano, V. Leung-Pineda. *Children's Healthcare of Atlanta, Atlanta, GA*

Background: Clinical laboratory testing has been an essential part of COVID-19 management. Serology can provide valuable information regarding a patient's exposure to virus, and may have a larger role to play as vaccines becomes available. Limited data is available on the serological response in pediatric patients. Here we investigate the use of one manufacturer's commercial assays for detecting IgM and IgG in an exclusively pediatric population.

Methods: Abbott SARS-CoV-2 IgM and IgG assays were performed on an Abbott Architect i1000. For specificity studies, we tested 78 patient specimens collected before the COVID-19 pandemic, and 66 specimens from patients who tested negative for SARS-CoV-2 nucleic acid amplification test (NAAT) during the COVID-19 pandemic. For sensitivity we tested 181 specimens from 41 patients with a positive NAAT result. Precision data was acquired for 20 days.

Results: For IgM, the highest qualitative positive agreement with molecular results was observed to be 15 to 30 days after a positive NAAT result or after symptom onset. For IgG, the highest positive agreement was 31-60 days after a positive NAAT result or 61-90 days after the start of symptoms. IgM started to decline 30 days after NAAT results and faded by 90 days. IgG started to decrease 60 days after a positive NAAT result.

Conclusion: The Abbott IgM and IgG assays have negative agreements of 98.7-100% relative to NAAT results. The IgM and IgG levels assayed by these methods start to decline months after positive molecular results and onset of symptoms in a pediatric population.

A-003

Effect of Hemolysis on HbA1c Measurement by the DCA Vantage Analyzer

K. Das, C. Tilghman. *Siemens Healthineers, Norwood, MA*

Background: An estimated 3.3% of venous sample rejection is reported due to pre-analytical hemolysis. The hemolysis effect is different for various assays. (Lippi *et. al.* Clin Chem Lab Med. 2008;46:764-72). Immunoassay-based DCA Vantage® analyzer, hemolyzes samples in the first step of the measurement process and can report HbA1c result for hemolyzed samples. In this poster, the pre-analytical hemolysis effect on HbA1c reported by the DCA Vantage analyzer is evaluated.

Methods: Four blood samples with various HbA1c levels, 5.3%, 6.6%, 7.1% and 7.6%, were tested. Part of each sample was hemolyzed by sonication. For each HbA1c level, a set of five test samples was prepared with various hemolysis levels by mixing hemolyzed and non-hemolyzed blood in different ratios. Respective non-hemolyzed sample was used as a control. Hemolysis indices were estimated using a CDC approved color chart. The HbA1c of the control and test samples was measured in 30 replicates using two DCA Vantage analyzers. Analysis included regression analysis for HbA1c vs. hemolysis indices, pared-bias analysis between the control and each hemolysis level and comparing mean HbA1c of each hemolysis with standard-deviation range of the respective control.

Results: Slopes for regression analyses for the four samples were 0.0007, 0.0031, 0.0029 and 0.0035 respectively. The slopes were statistically not different from zero (p value > 0.05) for clinically relevant hemolysis levels. The bias values were 1.88% for the completely hemolyzed first sample and 0.3%, 1.52%, 0.51% for the second, third and fourth samples respectively at 500-1000 mg/dL hemolysis level. The mean HbA1c of 30 replicates for all four samples at all hemolysis levels fell within the ±2SD range of the respective control sample.

Conclusion: The hemolysis impact on DCA Vantage analyzer's HbA1c measurement, is statistically minimal and clinically insignificant. Therefore, the DCA Vantage analyzer can reliably report HbA1c for hemolyzed samples.

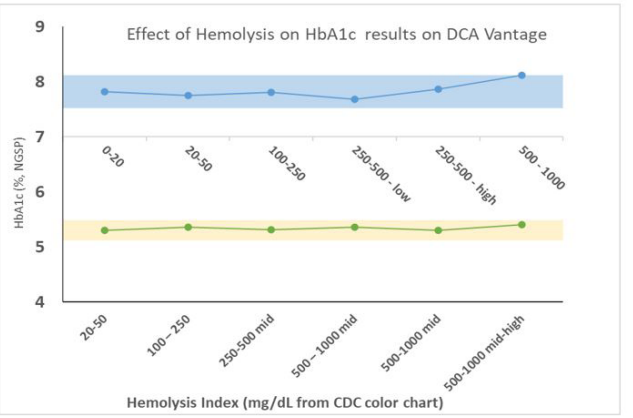

Figure 1: Mean HbA1c reported by the DCA Vantage analyzer at various hemolysis levels falls within ±2SD range of the non-hemolyzed control sample indicating minimal hemolysis effect. Data shown here is for a normal and a diabetic sample.

A-004

Performance Evaluation of the Atellica UAS 800 Urine Sediment Analyzer in Clinical Settings

K. Das, T. Briseno, M. Vallen-Thompson, Q. Mcmullen, B. Holmes, S. Kennedy, G. Mollino, C. Tilghman. *Siemens Healthineers, Norwood, MA*

Background: Urinalysis is an old but effective method to aid in diagnosis of many diseases. Automated analyzers can improve urinalysis precision and workflow. The Atellica® UAS 800 analyzer is an image- and neural-network-based urine sediment analyzer to report common urine sediment analytes. In this study, the accuracy and precision of the Atellica UAS 800 analyzer were evaluated in a clinical setting.

Methods: Accuracy was determined by testing 3136 urine samples on the Atellica UAS 800 analyzer (Siemens Healthineers) and the iQ®200 analyzer (Beckman Coulter) at three hospitals across the U.S. Precision was determined by running DIP&SPIN (Quantimetrix, U.S.A.) and qUAntify Plus (Bio-Rad, U.S.A.) quality control materials on the Atellica UAS 800 analyzer at three sites for 20 days, with two runs per day and two replicates by two operators.

Results: Slope, intercept, and coefficient of determination of Passing-Bablock regression analysis were 1.05, -0.809, and 0.799 for RBC and 1.034, 1.354, and 0.893 for WBC. Exact-block agreement values were 87.1%, 94%, 91.8%, and 83.5% and within-1 block agreement values were 99.1%, 99.3%, 99.7%, and 97% for BAC, CRY, EPI, and HYA respectively. Positive and negative percent agreement values were 86.1%, 90.9%, 93.1%, 92.8% and 90.4%, 98.1%, 95.3%, 85.8% for BAC, CRY, EPI, and HYA respectively. Standard deviation values were 3.23 p/µL for RBC (11.74 p/µL) and 2.64 p/µL for WBC (10.17 p/µL). Within-lab coefficient values were 8% for RBC (216 p/µL) and 8.3% for WBC (114.6 p/µL).

Conclusion: In this clinical performance study, the Atellica UAS 800 analyzer reported RBC and WBC analytes with slopes of ~1.0 compared to the iQ200 analyzer. When compared with iQ200 analyzer, the Atellica UAS 800 analyzer reported >90% within-1 block agreement for BAC, CRY, EPI, and HYA and reported >80% negative and positive percent agreements for BAC, CRY, EPI, and HYA.

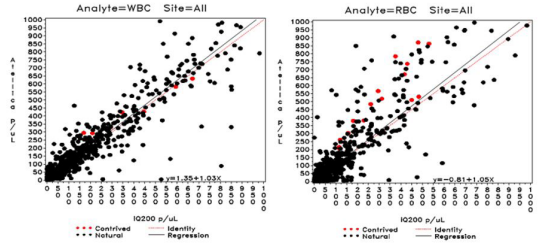

Figure 1: Passing Bablock regression plots between Atellica UAS 800 and iQ200 analyzers for RBC and WBC analytes. Red data points indicate contrived samples. (Atellica UAS 800 is not available for sale in the U.S.A. Product availability may vary from country to country and is subject to varying regulatory requirements.)

A-005

Free Light Chain Assays using Nephelometry and ELISA: a Reference Lab Experience for Multiple Myeloma Cases

B. T. Copet, M. J. Pido, J. M. Larkin, T. K. Tamire, Z. V. Som, K. T. Herbstritt, B. M. Hirner, D. M. Kuol, S. J. Kalass, M. A. Martinez, M. W. Nelson, M. J. Kurke, C. R. Poterucha, R. J. Bengal, C. S. Blixt, J. A. Dunker, D. L. Murray, M. R. Snyder, A. Dispenzieri, S. C. Bryant, M. V. Willrich. *Mayo Clinic, Rochester, MN*

Background: Serum kappa and lambda free light chains (FLC) are part of the diagnostic criteria for multiple myeloma requiring treatment. The most common FLC assays utilized are nephelometric or turbidimetric immunoassays (The Binding Site); however, a novel ELISA-based method (Sebia Inc.) is now being evaluated by laboratories. Both assays use polyclonal antibodies against FLC as reagents. The Sebia FLC assay was compared to the BNII nephelometric FLC assay to determine if the new assay is suitable for routine diagnostics and follow-up of patients with multiple myeloma (MM).**Methods:** 453 serum specimens from patients clinically characterized with MM were analyzed for kappa and lambda FLC using a Siemens BNII nephelometer with The Binding Site Freelite assay and manually performed Sebia's ELISA-based immunoassays. Reference intervals (RI) for Freelite were determined by the laboratory, while Sebia's were provided by the manufacturer. Method comparison was performed using Passing-Bablok (PB) regression and concordance analysis for all samples, diagnostic samples and follow-up samples. **Results:** The MM cohort had 125 unique subjects (62% males, ranging from 33 to 85 yo, median 62 yo). Samples were obtained at diagnosis (n=125) and follow-up (n=328). Kappa PB regression was y=0.362x+2.42, Spearman correlation r=0.925 and lambda y=0.819x+2.96, r_s=0.823. Using each method's RI, samples were classified as low/normal/high. All samples had an overall 68.1% concordance for kappa, 75.7% for lambda and 69.9% for the κ/λ FLC ratio. When only diagnostic samples were analyzed, the concordance was similar for kappa (79.0%) and lambda (76.0%), but greater for the FLC ratio (95.2%), likely due to higher elevations of FLC observed at diagnosis. Overall, the two assays have very similar diagnostic sensitivity based on the FLC ratio for diagnosing MM at 96.8% (95%CI 92.1-98.7%), Freelite and 96.8% (95%CI 92.0-98.7%), Sebia. The follow-up samples showed an overall concordance of 64.0%, 75.6%, and 60.4% for kappa, lambda, and FLC ratio, respectively. 17.7% of follow-up samples had normal FLC ratio with Sebia and elevated with the Freelite assay, which may be attributed in part to a Freelite reagent drift upwards previously reported (PMID 32829402). When the tests are reviewed in light of the clinical response criteria established by International Myeloma Working Group in the 328 follow-up samples, the assay drift and analytical concordance between the tests has smaller impact as the categorization of response criteria met at each stage of follow-up for patients and achieves 87.3% concordance.**Conclusion:** Nephelometry and ELISA are different platforms for the clinical laboratory. Nephelometry allows random access; ELISA allows for batched testing in smaller volumes using common laboratory instrumentation, has a wider measuring range, and eliminates polymerization over-quantitation. The assays have similar reference intervals, and equivalent diagnostic sensitivity, making both assays appropriate for diagnosis of MM. The variation in agreement between assays during follow-up stages should prompt laboratories to reconsider the use of FLC testing for monitoring MM as new assays enter the market, bearing in mind the high FLC biological variability, recent assay drifts and influence of renal function on appropriate RIs as potential confounding factors to the analysis.

A-006

Multiplex Bead Assay of Serum based Biomarkers as a Proposed Panel for Colorectal Cancer Diagnosis.

M. K. Eldeeb[1], M. Kandil[1], A. Farouk[1], N. Michael[2], N. E. E. Elbanna[3]. *[1]Medical Research Institute, Alexandria, Egypt, [2]Faculty of Medicine, Alexandria, Egypt, [3]Medical research institute Alexandria Egypt, Alexandria, Egypt*

Background: Colorectal cancer (CRC) is one of the most common causes of cancer-related morbidity and mortality worldwide. Early diagnosis improves CRC prognosis and treatment. However, the most widely used diagnostic tests include non-invasive tests limited by their insufficient performance and invasive ones limited by patient non-compliance. Profiling inflammatory mediators with carcinogenic roles could aid in CRC diagnosis. We aimed to use the Multiplex Bead assay in evaluating the utility of serum eotaxin-1, macrophage-inflammatory protein-1 beta (MIP-1 beta), granulocyte colony-stimulating factor (G-CSF), vascular endothelial growth factor A (VEGF-A), and Fas ligand (FasL) as potential biomarkers in CRC. **Method:** The study was conducted on 87 patients undergoing colonoscopy with history of gastrointestinal symptoms. Based on colonoscopy findings; patients were divided into 35 CRC and 52 non-malignant subjects. Non-malignant group was further subdivided into 9 with colon polyp, 24 with inflamed mucosa (colitis) and 19 with normal mucosa (control) patients. Serum samples were analyzed for eotaxin-1, MIP-1 beta, G-CSF, VEGF-A, and FasL using multiplex bead assay. **Results:** The median values of eotaxin-1, MIP-1β, G-CSF, and VEGF-A were significantly higher in CRC patients compared to non-malignant group. The area under the Receiver operating characteristics curve for a panel of studied markers (eotaxin-1, MIP-1β, G-CSF, and VEGF-A) was 0. 863. On the other hand, it was only 0.597 for routinely used occult blood in the stool. Also, there was a statistically significant increase in serum level of G-CSF and VEGF-A between CRC patients compared to those with precancerous colon polyp. **Conclusion:** Serum profiling of eotaxin-1, MIP-1β, G-CSF, and VEGF-A could be used as potential biomarkers in early CRC diagnosis with better discriminatory power than stool occult blood; and may increase occult blood performance thus reducing false positives rates and unneeded colonoscopy. Using multiplexing bead technology represents a promising approach for CRC screening and diagnosis.

TABLE 1: Sensitivity of SARS-CoV-2-Ab assays by days post-first positive SARS-CoV-2 RT-PCR - recommended limits of reactivity versus optimized limits of reactivity

Days POS	Roche Nucleocapsid		Roche Spike		Abbott IgG (nucleocapsid)		Snibe IgG (spike)		Abbott IgM (spike)	
	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)
Recommended limits of reactivity										
0 – 6	45/59	43.3 (33.6-53.3)	50/54	48.1 (38.2- 58.1)	2/52	3.6 (0.4 -12.3)	19/35	35.2 (22.7-49.4)	7/38	15.6 (6.5-29.5)
7 – 13	29/10	74.4 (57.9-87.0)	31/8	79.5 (63.5 -90.7)	23/8	74.2 (55.4-88.1)	25/6	80.6 (62.5-92.5)	17/7	70.8 (48.9-87.4)
≥ 14	39/2	95.1 (83.5-99.4)	39/2	95.1 (83.5-99.4)	33/1	97.1 (84.7-99.9)	34/0	100 (89.7-100)	23/6	79.3 (60.3-92.0)
Optimized limits of reactivity										
0 – 6	66/38	63.5 (53.4-72.7)	59/45	56.7 (46.7-66.4)	10/44	18.5 (9.3-31.4)	22/32	40.7 (27.6-55.0)	14/31	31.1 (18.2-46.6)
7 – 13	36/3	92.3 (79.1-98.4)	32/7	82.1 (66.5-92.5)	23/8	74.2 (55.4-88.1)	25/6	80.6 (62.5-92.5)	22/2	91.7 (73.0-99.0)

A-007

Automating System Diagnostics on Prototype Automated Immunoassay Analyzers*

T. Gregory, J. Mohr, C. Murray, S. Sidda, G. Mcdowall, C. Erickson, J. Wheatley, S. Maik, N. Sovde, T. Mizutani. *Beckman Coulter, Chaksa, MN*

Background: System diagnostic routines for automated immunoassay analyzers can be onerous to run and take valuable time away from other laboratory tasks. Many diagnostic routines are nonspecific, making diagnosis and repair of system issues slow and inefficient. To combat both scenarios, Beckman Coulter's next-generation automated immunoassay analyzer is being designed with diagnostic reagents stored onboard the analyzer paired with pre-programmed routines. Diagnostics can be scheduled to run automatically, avoiding conflict with running patient samples. Furthermore, new suites of diagnostics are being developed to assist in locating system issues to significantly reduce time to repair, and to identify discreet system issues that may impact the most sensitive assays-previously referred to as canary assays. **Method:** The automated system diagnostics (ASD) pack will have two components: a concentrated system check solution and a buffered solution of paramagnetic particles. Between the two of these components, all analyzer subsystems can be tested for functionality through different test methods. These test methods will be used for manufacturing release testing, field service return to service protocols, and to help predict if additional maintenance may be needed before a system failure occurs. **Results:** Wash efficiency is one diagnostic routine that measures how the vessels are washed; it is a critical process of the wash wheel subsystem. For this test, system check solution from the ASD pack is added to a vessel and subjected to the analyzer's normal washing process. Any remaining system check is measured, and results are automatically compared to established specifications. The sensitivity of the diagnostic was optimized to detect all failure modes of the wash subsystem. One such failure mode, reduced wash volumes, results in insufficient wash solution being delivered to the vessel. To test this, the wash efficiency diagnostic was compared to a historic canary immunoassay with sensitivity to the wash process. Results showed that a 40% reduction in wash volume yielded a "detected" result with the Wash Efficiency ASD, while not being detected by the canary assay. A 60% reduction in wash volume was detected by both the wash efficiency ASD and the canary assay. These results show that the diagnostic is more sensitive to this failure mode than the canary assay, demonstrating that reduced wash volumes can be detected before affecting patient results. **Conclusion:** The full automation of this new collection of diagnostic tests will significantly decrease

the time and effort needed to monitor the functionality of the prototype analyzer*. Tracking the trends of data will allow for proactive monitoring of each individual subsystem in order minimize analyzer downtime and significantly improve time to repair. This will allow for scheduling additional maintenance/service prior to impact of assay results. *† The prototype is not cleared nor approved for commercial use in any geography.2021-8747

A-008

Image analysis of particle concentration as system diagnostic tool

J. Mohr, T. Gregory, S. Chiang, T. Mizutani, A. Sawhney, M. Willette, N. Sovde, K. Seo. *Beckman Coulter, Chaska, MN*

Background: Beckman Coulter's next-generation, high-volume automated immunoassay diagnostic prototype analyzer* requires a complex set of system diagnostic tools to troubleshoot and maintain analyzer performance. Utilizing onboard camera systems and test methods designed to resuspend and retain known concentrations of particles, a fully automated diagnostic tool can be used to determine analyzer precision and potential subsystem failure, leading to faster analyzer service times and less laboratory downtime, as well as preventing failures that can cause erroneous patient test results. **Methods:** Samples of paramagnetic particles suspended in wash buffer were created in increments of 0.01 mg/mL, from 0 mg/mL to 0.16 mg/mL. Images of the samples were taken using the analyzer's onboard camera system, and the samples' average dark counts were determined. Using the average dark counts, particle concentration within the sample could be calculated from the images taken by the camera. Particle concentration in the samples were confirmed using a Hitachi spectrophotometer, and a statistical model was developed to predict sample concentration from the image dark count values, as well as determining sample creation precision. Multiple cameras are on-board the prototype analyzer* and can be used for this assessment; by employing the precision pipettor camera, the system diagnostic test can be run in a manner comparable to assay processing. This method lends confidence in the number of resuspended and retained particles aspirated from the system diagnostic pack, and therefore less variability in detecting particle failure modes. However, using this imaging method was not without challenges. Imaging with the precision pipettor camera requires analyzing particle dark counts through a disposable tip; as fluid is pulled through the tip, a gradient from light to dark occurs, thus providing inconsistent dark count values throughout the length of the tip. Multiple regions of analysis proved to be necessary to provide an accurate dark count of particles for concentration calculations. **Results:** Preliminary results from the wash wheel camera analysis have resulted in a statistical model with a prediction error of +/- 10% CV. Initial results using the precision pipettor camera show clear differences in image dark counts for select concentrations. Gradients are measured and defined as indicated for samples of 0, 0.005, 0.01, and 0.02 mg/mL, respectively. **Conclusion:**

Initial results using the precision pipettor camera to characterize sample particle concentration are promising. Use of this camera limits extensive software intervention. Additionally, a more accurate picture of particle resuspension for assays is obtained and it allows for targeted failure mode detection and troubleshooting. Using the onboard image processing software, diagnostic tests which utilize resuspended particles can have each replicate evaluated for particle concentration. Samples with particle concentrations that fall outside of the expected values can indicate analyzer hardware failures or need of additional maintenance in advance of a system failure, allowing for more targeted troubleshooting and decreased laboratory downtime. *The prototype is not cleared nor approved for commercial use in any geography. 2021-8746

A-009

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

C. Forsythe[1], S. Syed[1], B. Cummins[2], R. Thillen-Chennault[2], S. Brophy[1]. *[1]Abbott, Abbott Park, IL, [2]Abbott, Irving, IL*

Introduction: Lactic Acid or Lactate is a biproduct of glucose metabolism and is generated in red blood cells, muscle, brain and the gut. When the concentration of lactic acid increases in the blood the result is referred to acidosis. Accurate quantitation of the levels of lactic acid present in human plasma is key in identifying the presence of acidosis. An improved Research Use Only (RUO) lactic acid assay on Alinity c and ARCHITECT c Systems has been developed for the quantitative determination of lactate in human plasma.**Method**: The new REACH compliant lactic acid assay is now a two-reagent enzymatic assay for the quantitative determination of lactate in plasma which allows for greatly improved robustness to endogenous interferents. The enzymatic cascade includes the oxidation of lactate to pyruvate and hydrogen peroxide by lactate oxidase followed by peroxidase catalyzing the oxidation of a chromogen and 4-aminoantipyrine to a colored quinoneimine dye by hydrogen peroxide. Generation of the colored dye as measured at 604 nM correlates to lactic acid concentration in the sample. Multiple research reagent formulation components were evaluated to identify a best in class enzymatic Lactic Acid assay. Testing was performed on an automated clinical chemistry analyzers. **Results:** The reagent formulations with the best combination of assay performance and reagent stability were selected and resulted in a diagnostic assay that accurately and reproducibly measures the concentration of Lactic Acid. Total within laboratory imprecision was ≤ 0.8%CV on Alinity c and ≤ 0.6%CV on ARCHITECT c Systems. The assay has a demonstrated linearity up to 129 mg/dL. The limit of quantitation was 0.5 mg/dL on Alinity c and ARCHITECT c Systems. This two-bottle Lactic Acid assay demonstrated robustness to endogenous interferences (per CLSI EP 07, 3rd ed.) as follows: 60 mg/dL of unconjugated and conjugated bilirubin, 15 g/dL protein, 1500 mg/dL human triglycerides, and 1000 mg/dL hemoglobin. Calibration & Reagent onboard stability of at least 30 days was observed with a tighter TAE (Total Analytical Error allowance). Method comparison to a current on-market Lactic Acid assay on Alinity c showed a slope of 1.0 with correlation coefficient of 1.00 for the plasma concentration range of 3.1 - 118 mg/dL.**Conclusion:** A newly formulated REACH compliant RUO Lactic Acid assay was designed to be analytically robust with best in class performance to numerous endogenous interferents.

A-010

Multicenter Performance Evaluation of the New Elecsys Vitamin D Total III Assay

P. Findeisen[1], M. Leis[2], G. Bendig[3], J. Grimme[4], E. Moser[4], R. Christenson[5]. *[1]MVZ Labor Dr. Limbach & Kollegen, Heidelberg, Germany, [2]TRIGA-S, Habach, Germany, [3]Roche Diagnostics GmbH, Penzberg, Germany, [4]Roche Diagnostics Operations, Indianapolis, IN, [5]University of Maryland School of Medicine, Baltimore, MD*

Background: We characterized performance of the Elecsys® Vitamin D total III assay (Roche Diagnostics International Ltd), including intermediate precision and repeatability, by conducting method comparisons versus commercially available comparators, examining serum and plasma matrices, and calculating diagnostic accuracy versus reference LC/MS-MS values.

Methods: The Elecsys Vitamin D total III assay (cobas e 601 analyzer) was examined under routine conditions at three laboratories (Heidelberg, Germany; Habach, Germany; Baltimore, Maryland, USA). Intermediate precision and repeatability were calculated using five human serum pools (HSP1-5) and two PreciControl materials (PC1 and PC2; 5-day model, one reagent lot in accordance with CLSI-EP05-A3 criteria) and compared against prespecified acceptance criteria. Method comparisons were accomplished using a serum sample verification panel with predefined, reference LC/MS-MS values (Centers for Disease Control and Prevention; CDC). Characterization of the Elecsys Vitamin D total III assay was performed at all three sites. Comparator assay studies were performed at Heidelberg and Baltimore. Between-method differences were assessed using unweighted Deming regression. A separate serum/plasma comparison analysis with the Elecsys Vitamin D total III assay was conducted at a single site (Washington University, USA), using samples from apparently healthy adults and assessed using Passing-Bablok regression (PBR).

Results: CV% for repeatability and intermediate precision met the prespecified acceptance criteria across all three sites (**Table**). Good agreement was observed between the Elecsys Vitamin D total III assay and the comparator assays (Pearson's r, 0.958-0.982; **Table**), and also between the Elecsys Vitamin D total III assay and the CDC verification panel target values (Pearson's r, 0.960-0.986) across sites, and in serum and plasma samples (n=462; Pearson's r, 0.972; PBR, y=0.103+0.984x).

Conclusion: The Elecsys Vitamin D total III assay demonstrated good analytical performance and compared favorably with other commercially available assays, supporting its use as an aid in determining vitamin D sufficiency.

Table. Elecsys Vitamin D total III assay (cobas e 601 analyzer) analytical performance and method comparison

Imprecision across the three sites				
Specimen*	**Sample number (n)**	**Mean Vitamin D concentration (ng/mL)**	**Repeatability (within-run)§**	**Intermediate precision (within-lab)‖**
HSP1	75	16.8–18.4	SD, 0.870–1.07	SD, 1.14–1.77
HSP2	74†	32.1–34.6	CV%, 2.33–5.19	CV%, 3.22–7.83
HSP3	74†	61.7–64.3	CV%, 2.76–5.97	CV%, 3.16–8.37
HSP4	74†	80.2–82.8	CV%, 2.51–6.43	CV%, 3.10–7.66
HSP5	75	94.5–98.0	CV%, 1.58–2.76	CV%, 2.00–4.13
PC1	75	19.8–21.1	SD, 0.875; CV%, 3.91–6.13	SD, 1.05; CV%, 5.35–9.71
PC2	75	38.3–40.1	CV%, 2.50–5.61	CV%, 3.18–6.87
Method comparison‡				
Comparator assay	**Sample number (n)**	**Deming regression**	**Pearson's r**	**Bias at medical decision point (30 ng/mL), %**
ADVIA Centaur Vitamin D Total	117	y=–2.57+0.963x	0.958	–12.3
ARCHITECT 25-OH Vitamin D	117	y=0.907+0.921x	0.982	–4.88
Access 25(OH) Vitamin D Total	116	y=–1.92+0.966x	0.969	–9.77
LIAISON 25 OH Vitamin D TOTAL	117	y=–5.26+1.15x	0.963	–2.60

*Human serum pools and PreciControls were from Roche Diagnostics.
†n=1 sample excluded; 'without outlier analysis' performed according to predefined study parameters.
‡The serum sample verification panel used for method comparison analyses was obtained from the CDC.
§Prespecified acceptance criteria for repeatability were: lower end of measuring range (LEoMR) to 20 ng/mL, standard deviation (SD) ≤1.6 ng/mL; >20.0 ng/mL to higher end of measuring range (HEoMR), percentage coefficient of variation (CV%) ≤8.0%.
‖Prespecified acceptance criteria for intermediate precision were: LEoMR to 20 ng/mL, SD ≤2.2 ng/mL; >20.0 ng/mL to HEoMR, CV% ≤11.0%.

A-011

Guidance for the Analytical Characterization of Point-of-Care High Sensitivity Cardiac Troponin Assays intended for Plasma and Whole Blood Measurements

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Background. High-sensitivity cardiac troponin (hs-cTn) assays are more precise than contemporary assays and can be used in accelerated diagnostic protocols for rule-out/rule-in of acute myocardial infarction (AMI) at presentation or the first 1 to 2 hours. Point-of-care (POC) hs-cTnI measurement streamlines pre-analytical, analytical and post-analytical processes, and further improves the management of patients. We present a roadmap for regulatory validation of POC measurement using lithium heparin plasma (PL) and whole blood (WB) exemplified by validation of the Atellica® VTLi hs-TnI Assay. **Methods.** Characterization was based on Clinical Laboratory Standards Institute (CLSI) and best practice protocols. Characteristics were determined using several reagent lots. hs-cTnI measurements in PL and WB were compared using Bland-Altman regression and Passing-Bablok bias plots. The relationship of hematocrit and hs-cTnI dependence was determined. High-sensitivity performance of the Atellica® VTLi hs-cTnI Assay was assessed in accordance with AACC/IFCC collaborative recommendations using limit of quantitation (LoQ), precision data, limit of detection (LoD), and the AACC Universal Sample Bank measurements. **Results:** The table displays limit of blank (LoB), LoD, LoQ, precision, and linearity for PL and WB. The 10% CV for the POC hs-cTnI assay was 6.7 ng/L, well below the healthy women's 99th percentile URL of 18 ng/L (Table). Also, hs-cTnI values exceeded LoD for ≥50% of healthy >300-member cohorts of women and men, confirming high-sensitivity performance. Recovery was 90-110% for the 40 common medications and -0.007%-0.035% for cross-reacting substances. No high dose hook effect was detected. Sample matrix bias was minimal and correlation was excellent. No significant relationship between hs-cTnI and hematocrit was detected. **Conclusion**. The Atellica® VTLi hs-cTnI Assay POC demonstrated high-sensitivity performance and fit-for-purpose analytical characteristics across multiple reagent lots and excellent agreement between PL and WB with the robust validation and regulatory-required roadmap.

Characteristic	Method	Plasma (PL)	Whole blood (WB)
Limit of Blank	CLSI-EP17-A2	0.55 ng/L	
Limit of Detection	CLSI-EP17-A2	1.2 ng/L	1.6 ng/L
Limit of Quantitation	CLSI-EP17-A2, 20% CV	2.1 ng/L	3.7 ng/L
10% CV cTnI concentration	Lowest cTnI with 10% CV	6.7 ng/L	8.9 ng/L
Linearity	CLSI EP06-A; LoQ to upper limit	2.1 ng/L to 1250 ng/L	3.7 ng/L to 1250 ng/L
Precision	CLSI EP05-A3, intermediate precision	CV: 4.6% to 8.5%; cTnI: 13.1 to 262 ng/L	CV: 4.6% to 9.1%; cTnI 16.8 to 689 ng/L
Specificity: 40 substances	CLSI-EP07-3/EP37-3	hs-cTnI low:30 ng/L & high:850 ng/L; Overall recovery 90-109%	
Cross-reactivity: 4 substances	CLSI-EP07-3	Hs-cTnI low:30 ng/L & high:850 ng/L; Cross reactivity -0.007% – -0.035%	
High Dose Hook effect	Tested to >1,000,000 ng/L	No High Dose Hook effect	No High Dose Hook effect
Sample Matrix correlation, PL vs WB	CLSI EP09C; Passing-Bablok regression	WB = 1.02*PL +0.3 ng/L, r=0.996; n=152 matched samples	
cTnI Bias, PL and WB	CLSI-EP09C; Bland-Altman Bias plot	Bias=5.6%; 95%LoA: -28.7% to 45.2%	
99th percentile URLs	CLSI-EP28-A3C; AACC Universal Sample Bank	Non-parametric model: Women 18 ng/L, Men 27 ng/L; overall 23 ng/L	
Proportion >LoD	AACC/IFCC Expert Consensus	Women: 79.7%; Men: 87.3%; Overall: 83.7%	
Hematocrit dependence	hs-cTnI and hematocrit relationship	Bias=0.07-0.31*Hematocrit; R^2=0.003; not significant	

*Disclaimer: The products/features (mentioned herein) are not commercially available in all countries. Not available for sale in the U.S.A. Their future availability cannot be guaranteed.

A-012

The Development of an ICP-MS Method for Iron in Ovaprene® Vaginal Swabs

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Background: Ovaprene, an intravaginal contraceptive product developed by Daré Bioscience, is a novel non-hormonal monthly contraceptive option for women. The intravaginal silicone ring releases ferrous gluconate to impede sperm motility, while a knitted polymer barrier physically prevents sperm from penetrating the cervical canal. To demonstrate product efficacy, a method for assaying iron content in clinical vaginal samples was necessary. Previously-validated chromogenic assays failed due to the collection of mucosal samples on swabs – swabs that contained a high and variable amount of iron. Exacting assay development was necessary to maintain a low (undiluted) limit of quantitation while working with extremely small, variable sample quantities. An innovative method was developed to analyze vaginal swab samples via acid digestion and inductively-coupled plasma mass spectrometry (ICP-MS).**Methods:** ICP-MS was chosen as the analytical technique, affording increased accuracy with a lower limit of quantitation than would have been possible using a chromogenic or biological assay. Several swab brands were assessed for baseline iron content and variability. The digestion procedure was optimized to allow minimal dilution and complete digestion of samples with final acid concentration ≤10% (per instrument limitations). Standard solutions were prepared using ferrous gluconate in simulated vaginal fluid to accurately reflect sample chemical properties. Quality control ranges were established for blank samples, control swabs, and swabs spiked with iron (to be analyzed with each sample set), with acceptance criteria that the resulting group distributions did not overlap within ± 3*standard deviations. The final ICP-MS method was validated for specificity, accuracy, precision, linearity, limit of quantitation (LOQ), and system suitability.**Results:** The finalized method included pre-weighing individual, labeled swabs to obtain accurate final weights without compromising the sample. Swab and sample were digested at 100±10 °C for ≥12 hours with 1 mL of 20% nitric acid, employing a rinsate-recycling technique during the post-digestion dilution to maximize recovery and yield a final volume of 2 mL with 10% nitric acid concentration. Quality control ranges were established with >99.72% confidence in significant difference between blank, control, and spiked sample groups. The less common isotope of Fe-57 was used for ICP-MS analysis because it is subject to the least amount of polyatomic interferences. All validation parameters passed: accuracy (three recovery levels) 90-110%; precision <5% within-run, <10% total; correlation coefficient R = 0.9979; system suitability <20% drift (between bracketing standards in all sequences). The *undiluted* LOQ was validated at 4 parts-per-billion (ppb). All procedures were audited for GLP compliance.**Conclusion:** A highly sensitive ICP-MS method for analysis of iron in vaginal samples was developed with an undiluted LOQ of 4 ppb. The ability to analyze the swab and vaginal sample together is paramount when working with clinical samples of less than 10 mg. Initial swab brand optimization, coupled with the implementation of discrete quality check ranges, enabled the clinically-feasible collection of vaginal samples on swabs despite pre-existing iron content in the swabs themselves.

A-013

High-throughput Creatinine and Creatine Quantification by NMR in the Clinical Laboratory

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Background: Plasma/serum creatinine test is commonly used to assess renal function. Creatine, a precursor of creatinine, may be significantly increased in amyotrophic lateral sclerosis, dermatomyositis, myasthenia gravis, starvation, muscular dystrophies and trauma. Nuclear magnetic resonance (NMR) spectroscopy allows simultaneous detection of signals arising from multiple analytes in a complex mixture (e.g., plasma, serum) from the same spectrum. This avoids the need for separation techniques, use of multiple reagents and performing different analysis steps to quantify multiple analytes. The goals of this study were to: (1) develop and evaluate the analytical performance of a new NMR-based assay for quantification of creatinine and creatine from the same spectrum, and (2) apply the assay to determine circulating creatinine and creatine in apparently healthy adults.**Methods:** A high-throughput NMR-based method for quantifying serum/plasma creatinine and creatine was developed using a non-negative least squares fitting algorithm and its analytical performance was assessed in accordance with CLSI guidelines. To determine if the assay has adequate sensitivity to measure clinically relevant concentrations of creatinine and creatine, the assay was used to quantify creatinine and creatine in apparently healthy individuals.**Results:** The LOB, LOD and LOQ for the creatinine assay were 0.06, 0.12 and 0.15 mg/dL and for the creatine assay were 0.08, 0.13 and 0.14 mg/dL, respectively. Linearity was demonstrated over a wide range of creatinine (0.18 to 5.79 mg/dL) and creatine (0.15 to 5.23 mg/dL) concentrations. Coefficients of variation (%CV) for intra- and inter-assay precision ranged from 1.3 – 6.4% and 2.6 – 6.3%, respectively. Creatinine and creatine results as determined by the NMR assay were in good agreement with results obtained by the Kinetic Jaffé method (r = 0.990, slope = 0.997, y-intercept = -0.15, n = 1,172) and by enzymatic/creatinase/spectrophotometry (r = 0.998, slope = 0.999, y-intercept = 0.17, n = 50), respectively. Also, the creatinine values by NMR for the NIST SRM 967a material showed minimal bias (3%) with the certified values measured by ID-LC/MS. Specimen type comparison revealed <1% and -11.1% bias between serum and plasma samples for creatinine and creatine, respectively. The reference interval, in a cohort of fasting and non-fasting apparently healthy adult participants (n=559), was determined to be 0.49 to 1.36 mg/dL with a mean of 0.89 ± 0.23 mg/dL for creatinine, and 0.13 to 1.49 mg/dL with a mean of 0.62 ± 0.38 mg/dL for creatine.**Conclusions:** The newly developed NMR-based creatinine and creatine assay exhibits performance characteristics adequate for use in the clinical laboratory. The assay generates results comparable to the

commonly used clinical chemistry analyzers. The NMR assay offers an alternative method to quantify circulating creatinine and creatine.

A-014

An Analytical Throughput Challenge Between Nephelometry and Turbidimetry for the Free Light Chain Assay

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Background: Free light chain (FLC) testing was introduced in 2002 and has significantly improved the diagnosis, prognosis and monitoring of patients with monoclonal gammopathies. The Freelite assay (The Binding Site, UK) for measurement of kappa and lambda FLC is the most utilized in the United States and available on several automated platforms. Our laboratory utilizes the Freelite reagents on the Siemens BNII nephelometric systems and was considering a change to the Optilite, a turbidimetric platform by The Binding Site, to take advantage of several advertised testing efficiencies; an antigen excess flag incorporated in the software, and an improved dilution scheme of samples to use less reagent (1+9 for kappa and 1+7 for lambda). These options are not currently available for the BNII platform. Therefore, the laboratory decided to carry out a throughput challenge between the two platforms compatible with workload. **Methods:**100 residual waste serum specimens covering the analytical measuring range for kappa and lambda FLC were analyzed in parallel using The Binding Site Freelite reagents on the Siemens BNII platform and The Binding Site Optilite. Testing was carried out on the BNII per laboratory's standard operating procedure and for the Optilite according to manufacturer instructions. Timings were documented for each step of the respective instrument workflows and the amount of reagents (shots) utilized to obtain a final result recorded. Intra-assay precision was established at 3 levels with 10 measurements. Method comparison was performed using Passing-Bablok regression, and overall concordance between methods was assessed using semi-quantitative analyses for the FLC ratio. **Results:**Two technologists ran 100 samples for kappa and lambda in tandem on both platforms. For the BNII, it took 651 minutes to run all samples from instrument start-up to instrument shutdown, and the Optilite took 270 minutes to complete the same testing. The BNII system required 171 shots to report 100 kappa results and 135 shots to report 100 lambda results; the Optilite required 127 and 124 shots for the same reportable, respectively. The laboratory attributes these differences to the improved dilution scheme and antigen excess flag available for the Optilite. Passing-Bablok comparison between platforms (*x* axis=BNII) for kappa had a slope of 1.038 (95%CI 0.9585 to 1.1222), r=0.906 and for lambda slope of 1.138 (95%CI 1.089 to 1.193), r=0.991. The overall concordance for the FLC ratio was 94%; with 10% being low, 39% being within reference intervals and 45% elevated. The 6 discordant cases were near cut-off points, and no FLC ratio was >2.06 in either platform. Intra-assay precision was estimated as <6% CV for BNII, and <5% for Optilite. Out of the 100 samples, 6 samples were known for having antigen excess and required multiple manual dilutions to reach the final result on the BNII. The Optilite detected all 6 samples automatically with its antigen excess flag and identified one additional sample that the Freelite Kappa reagent had missed on the BNII. **Conclusion:**This initial analytical throughput challenge between the two platforms showed favorable results towards the Optilite workflow prompting the request for a full analytical validation for the platform

A-015

Evaluation of the Analytical and Clinical Performance of the Fujirebio Lumipulse G β-Amyloid (Aβ42/40) Ratio in Cerebrospinal Fluid

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Background: The Fujirebio β-amyloid 1-40 and 1-42 (Aβ40 and Aβ42) immunoassays are two-step chemiluminescent enzyme immunoassays for the determination of Aβ40 and Aβ42 in human cerebrospinal fluid (CSF). The β-amyloid ratio (Aβ42/40) is intended to be used in the assessment of patients presenting with cognitive impairment to aid in the differentiation of Alzheimer's Disease (AD) dementia and other causes of cognitive decline. A reduced Aβ42/40 ratio is associated with AD related pathology. The objective of this study was to evaluate the analytical and clinical performance of these assays.

Methods: The Fujirebio β-amyloid assays were performed on a Lumipulse G1200 per manufacturer instructions. The analytical performance characteristics were evaluated using CSF samples collected in Sarstedt low-bind, polypropylene tubes in order to avoid potential β-amyloid loss through tube adhesion. Studies were performed using at least two different reagent kit lots per analyte. Studies included stability, imprecision, accuracy, analytical measuring range (AMR), dilutions, limit of quantitation (LOQ), and interfering substances. For evaluation of clinical performance, the Aβ42/40 ratio was measured in a cohort of clinically characterized samples.

Results: Aβ40, Aβ42, and the Aβ42/40 ratio were stable for 48 hours ambient, 14 days at 4°C, 30 days at -20°C, and through 3 freeze/thaw cycles. Intra-assay imprecision (coefficient of variation; %CV) was <1.3% for Aβ40 concentrations ranging from 3952-19949pg/mL; <1.8% for Aβ42 concentrations ranging from 284-1002 pg/mL; and <1.6% for the Aβ42/40 ratio. Inter-assay imprecision (%CV) was <2.1% = for Aβ40 concentrations ranging from 3952-19949pg/mL; <2.5% for Aβ42 concentrations ranging from 284-1002 pg/mL; and <3.3% for the Aβ42/40 ratio. Aβ40 and Aβ42 accuracy was verified by measuring calibrators as unknowns. The mean recoveries were 102% (Aβ40) and 87% (Aβ42) of the expected concentrations. The AMRs were 5-30,000 pg/mL (Aβ40, slope: 1.00, R^2: 1.00) and 10-2335 pg/mL (Aβ42, slope: 0.94, R^2: 0.99). On-board dilutions exhibited a mean recovery of 94% (Aβ40,1:10). Manual dilutions were acceptable with mean recoveries of 115% (Aβ40,1:600) and 94% (Aβ42,1:4). LOQs were 7 pg/mL (Aβ40) and 10 pg/mL (Aβ42). Hemoglobin, bilirubin, biotin, and bovine serum albumin were acceptable up to 500 mg/dL, 27 mg/dL, 30 ng/mL, and 200 ng/mL, respectively. In a cohort of patients with mild cognitive impairment (MCI) (n=20) and AD dementia (n=16) the agreement of the Aβ42/40 ratio (normal cutoff of >0.058) with amyloid PET(normal cutoff of >1.48) were as follows: 88.9% overall percent agreement, 90.9% positive percent agreement and 85.7% negative percent agreement.

Conclusion: The observed analytical performance of the Fujirebio Lumipulse Aβ40, Aβ42, and Aβ42/40 ratio were acceptable for clinical laboratory utilization. Clinical performance using previously established cutoffs for Aβ42/40 was deemed acceptable.

A-016

Digital Proximity Ligation Assay for Highly Sensitive, No Wash Immunoassay

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Background: Although the technique of utilizing an antibody pair to target distinct epitopes on the target protein was first invented in 1971, many immunoassays employing this strategy have significant drawbacks. Other than lacking sensitivity, the workflows require multiple hands-on steps using ancillary and additional instruments for assay readout (~5 hours total assay length). Not to mention that standard curves are required for target quantification, significantly adding cost, and reducing consistency and accuracy. Proximity ligation assay (PLA) was introduced back in 2002, utilizing antibody pair conjugated with two non-complementary oligos for target protein detection. When the antibody pair binds to a target, a connector oligo acts as a bridge and allows the conjugated oligos to be ligated. This method takes advantage of PCR to both enhance specificity and sensitivity. However, standard curves are still required to correlate PCR cycle threshold with standard proteins. Researchers have attempted to run PLA using droplet digital PCR; however, the workflow remains complex and the sensitivity is poorer than conventional immunoassay, potentially due to the complex chemistry in droplet formulation. Here we reported absolute protein quantification by combining PLA with a novel microfluidic array partitioning digital PCR technology platform. It not only eliminates the standard curves, but also simplified the workflow by integrating all required steps into a single instrument and single consumable. The total assay time can be accomplished in less than 3 hours without any wash steps, with less than 10 minutes hands-on time. With ease of use, accurate and sensitive quantification and significantly reduced cost, the digital PLA platform has the potential to democratize accurate protein quantification to advance proteomics research and impact healthcare. **Methods:** Quantification of three protein targets were demonstrated: IL-6, troponin I and phosphorylated tau 181. The standard recombinant proteins were acquired from the commercially available kits. In each assay, only 1 uL of recombinant protein was incubated with the antibody-oligo conjugates for one hour and followed by 20 minutes of incubation with a solution of PCR master mix and ligase. Nine microliters of this mixture were loaded into a microfluidic array plate, which was then placed into the benchtop digital PCR instrument. Digitization, thermal cycling and fluorescence imaging were accomplished in 90 minutes. No template control (NTC) background subtraction was performed to further enhance sensitivity. **Results:** Troponin I was quantified from 5000pg/mL down to 1.6pg/mL with linear regression R-square value of 0.9984. Both phosphorylated tau 181 and IL-6 was quantified from 1000pg/mL down to 0.32pg/mL with linear regression R-square value above 0.9973. For all three proteins, the LLOD (Lower Limit of Detection) is 5x better than the reported qPCR results, without the need of standard curves. **Conclusion:** The digital PLA technology demonstrated here can detect ultra-low concentrations of protein samples down to 0.32pg/mL with a sample input of only 1µL (~320 attogram per reaction). The high sensitivity combined with simple workflow using only one instrument and one consumable significantly lowers the barrier to entry to performing studies of rare neurological, cancer or viral proteins in blood or other samples.

A-017

BioPlex 2200 SARS-CoV2 IgG Diagnostic Sensitivity and Specificity

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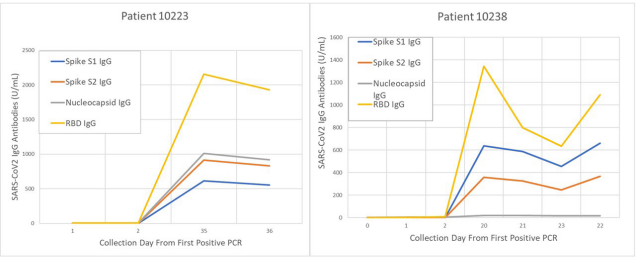
Background: Antibodies to SARS-CoV-2 proteins can be detected in patients with Coronavirus disease 19 after a few days of infection. The presence of antibodies against SARS-CoV-2 proteins is not diagnostic of infection but is a good tool for public health surveillance. In this study we investigated the presence of IgG antibodies against the nucleocapsid (NC), and the Spike protein S1, S2, and receptor-binding domain (RBD) in a patient population different than those used for assay development. **Methods:** Frozen remnant serum specimens from the following cohorts were tested using the BioPlex 2200 IgG panel: 241 CoV-2 PCR positive patients, 160 CoV-2 PCR-negative patients, and 10 longitudinally collected patients (2-10 days consecutive specimens). Specimens were obtained following Central IRB approval. Specimen were de-identified but information regarding age, gender, and PCR testing results were available. **Results:** The positive PCR cohort was composed of 98 females ages 19 to 96, and 139 males ages 21 to 96 (4 of the patients in this cohort did not provide gender information). The negative cohort was composed of 100 females ages 10 to 87, and 60 males ages 2 to 86. The longitudinal set were from 4 females ages 61 to 89, and 6 males ages 31 to 90. The percent positive agreement (sensitivity) and percent negative agreement (specificity) of the BioPlex 2200 IgG panel after 14 days of detection of SARS-CoV-2 virus by PCR was 100%. The seroconverted patients demonstrated antibodies to RBD, S1, S2 and NC to increase simultaneously, however, one of the patients NC antibodies was not detected as demonstrated in Figure 1 (cut-off is 10U/mL).

Conclusions: The percent positive and negative agreement of the BioPlex 2200 SARS-CoV2 IgG panel is consistent with the manufacturer's claim for performance.



A-018

Monoclonal Serum Amyloid A Antibodies Applicable to Human and Veterinary Diagnostic Immunoassays with Wide Detection Ranges

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Background: Acute-phase serum amyloid A (SAA), a family of apolipoproteins, are synthesized in response to inflammatory signals. SAA concentrations in blood increase within a few hours after the onset of inflammation. Normal levels vary from < 2-8 mg/L and increase 1000-fold during acute inflammation. Like CRP, SAA can therefore be used as an indicator for microbial infections or for various inflammatory conditions. Recently, SAA has been shown to have a prognostic value for patients with COVID-19. As SAA proteins are highly conserved in vertebrates, SAA measurements are also used in veterinary diagnostics.

Methods: We have developed eight mouse monoclonal antibodies against human serum amyloid A. The specificities of these antibodies, designated as Anti-h SAA 2201, 2203, 2205, 2208, 2209, 2211, 2212, or 2213 were studied in fluorescence-based immunoassays (FIA) with recombinant serum amyloid A proteins: human SAA1, SAA2, SAA4, equine, feline and canine SAA1, and native human SAA in direct coating immunoassay format. Selected antibody pairs were tested in *in vitro* diagnostic (IVD) applications: immunoturbidimetric assay (IT), lateral flow assay (LF) and fluorescent immunoassay (FIA), for differences in linear measuring range and ability to measure SAA in clinical samples. Overlapping peptide scan was applied to identify continuous epitopes for the antibodies.

Results: In IT, the antibody pair 2201 and 2211 showed the widest measuring range: Forty-three clinical samples in the range of 4 to 307 mg/L were measured with the selected pair, and the results show good correlation with the reference method (nephelometric assay from Siemens) with a coefficient of correlation of 0.95. In sandwich FIA, the widest linear measuring range of 4 µg/L to 1 mg/L was achieved with human SAA1 specific antibodies 2205 or 2208 paired with 2212. For lateral flow application, the pair with widest measuring range and excellent performance in clinical samples was 2201 with 2203. Linear epitopes were identified for three antibodies: 2205, 2208 and 2212. The epitopes were in loop structures of the SAA1 protein: 2212 in an N-terminal loop and both 2205 and 2208 in a C-terminal loop with overlapping epitopes in a region unique to human SAA1. Antibodies 2201, 2203, 2209, 2211 and 2213 did not bind to linear peptides, indicating binding to conformational epitopes. Their antigen recognition was more diverse, and they recognized also feline, canine and/or equine SAA1.

Conclusion: These results demonstrate that the monoclonal SAA antibodies can be used as tools to develop diagnostic assays with wide detection ranges for measuring SAA levels in human clinical samples. The results also emphasize that the best combination of antibodies in an IVD assay is dependent on the type of application. In addition to human diagnostics, these antibodies can also be applied in veterinary diagnostics for measuring equine, feline or canine SAA.

A-019

Evaluation of Point-of-Care Analyzer GEM Premier ChemSTAT versus Laboratory Analyzer Siemens VISTA 1500

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Background: A point-of-care (POC) chemistry test panel including whole blood (WB) creatinine determination could improve risk stratification and prioritization of high risk, acutely ill patients in the emergency department and expedite time to treatment. In our institution, the average turnaround time for a creatinine result from the laboratory is approximately 40 minutes. A new POC system, GEM Premier ChemSTAT (Instrumentation Laboratory/IL, Bedford, MA) provides results for electrolytes, metabolites, hematocrit, pH & total carbon dioxide (tCO_2) from a single WB sample in 70 seconds. The goal of this evaluation was to compare analytical performance and usability of the GEM Premier ChemSTAT system to the current laboratory chemistry assays, and other WB assays deployed at the University Hospital of Clermont-Ferrand.

Methods: More than 300 remnant WB samples from the intensive care unit and pre-dialysis patients were first evaluated on the GEM Premier ChemSTAT. As comparative methods, WB samples were then assayed on a GEM Premier 5000 (IL) and the plasma aliquots from the same samples were assayed on a Vista 1500 (Siemens Healthineers, France).

Results: WB results from the GEM Premier ChemSTAT correlated well with the Vista and GEM 5000 as summarized in Table 1. Biases were estimated at each medical decision level per analyte and were all within manufacture's claims except for tCO_2. The bias between comparative methods Vista and Roche cobas (GEM Premier ChemSTAT predicate) may be attributed to observed low slope and negative bias.

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

Table 1. Method Correlation Results for the GEM Premier ChemSTAT WB Assays *vs.* Comparative Methods (n/a - Analyte not available on the analyzer)

ChemSTAT Analyte	Siemens Vista 1500 (n=314)				GEM Premier 5000 (n=319)			
	Slope	Intercept	r	Sample Range	Slope	Intercept	r	Sample Range
BUN	0.990	0.4336	0.992	1.2 - 41.7 mmol/LL	n/a			
Crea	1.030	-4.286	0.996	24.4 - 1210 umol/L	n/a			
tCO_2	0.823	0.8458	0.933	17.0 - 36.3 mmol/L	n/a			
Na^+	1.076	-12.71	0.912	116.82 - 154.25 mmol/L	1.008	-1.642	0.942	113 - 151 mmol/L
K^+	1.111	-0.333	0.984	2.56 - 6.72 mmol/L	1.000	0.10	0.993	2.4 - 7.1 mmol/L
Cl^-	0.992	0.107	0.939	84.3 - 127.7 mmol/L	1.000	2.00	0.968	83 - 124 mmol/L
Glu	1.040	-0.121	0.996	2.87 - 19.56 mmol/L	1.000	0.0	0.997	3.1 - 20.5 mmol/L
iCa^{++}	n/a				1.000	0.01	0.943	0.82 - 1.51 mmol/L
Hct	n/a				1.028	-1.404	0.99	17 - 68 %
Lac	n/a				1.000	0.0	0.992	0.8 - 5.9 mmol/L
pH	n/a				1.065	-0.4782	0.976	7.190 - 7.610
pCO_2	n/a				1.000	1.00	0.989	27 - 79 mmHg

A-020

Evaluation of GEM Premier ChemSTAT versus Chemistry Analyzer Beckman Olympus AU5800

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Background: Coronavirus disease 2019 (COVID-19) has put extreme pressure on the health care system particularly the intensive care unit (ICU). GEM Premier ChemSTAT (Instrumentation Laboratory/IL, Bedford, MA, USA) is a point-of-care (POC) analyzer that can perform a chemistry panel consisting of sodium, potassium, ionized calcium, chloride, glucose, creatinine, blood urea nitrogen and total carbon dioxide plus pH, pCO_2 and lactate in one whole blood (WB) sample which could help patient assessment and monitoring. Evidence has demonstrated the relevance of electrolyte abnormality, acute kidney injury (AKI) and chronic kidney disease (CKD) to the severity and outcome of COVID-19 patients. The goal of this evaluation was to compare analytical performance and usability of the ChemSTAT to the current laboratory chemistry panel assays, and other WB assays deployed at the Vall d'Hebron Hospital Campus in Barcelona.

Methods: 128 Remnant WB samples mainly from oncology and nephrology departments were enrolled in the study. As comparative methods, WB samples were assayed on a GEM Premier 4000 (IL) and the plasma aliquots from the same samples were assayed on an Olympus AU5800 (Beckman Coulter Life Sciences, Brea, CA, US).

Results: WB results from the GEM Premier ChemSTAT correlated well with those from AU5800 and GEM 4000 as summarized in Table 1. Analyte biases were estimated at each medical decision level per analyte and were all within manufacture's claims except for K^+. The subtle bias observed in results vs. AU5800 were attributed to the different measurement methodology.

Conclusion: Strong correlations were observed between the POC GEM Premier ChemSTAT assays compared to the established laboratory and POC assays. With its operational simplicity, reliability and fast turnaround time, an ICU implementing the ChemSTAT system could find improved times to treatment and improved patient management.

Table 1. Correlation Results for the GEM Premier ChemSTAT WB Assays *vs.* Comparative Methods (n/a - Analyte not available on Lab or POC analyzer)

ChemSTAT Analyte	Beckman Coulter Olympus AU5800 (N=126)				GEM Primer 4000 (N=128)			
	Slope	Intercept	R	Sample Range	Slope	Intercept	R	Sample Range
BUN	0.957	0.695	0.995	2.73 - 42.37 mmol/L	n/a			
Crea	1.014	-0.009	0.993	0.37 - 10.15 mg/dL	n/a			
Na^+	0.985	1.86	0.960	124 - 176 mmol/L	0.9646	5.973	0.983	123 - 176 mmol/L
K^+	1.111	-0.333	0.984	2.8 - 6.6 mmol/L	1.000	0.10	0.993	2.6 - 6.7 mmol/L
Cl^-	0.992	0.107	0.939	78 - 135 mmol/L	1.000	2.00	0.968	80 - 135 mmol/L
Glu	1.159	-0.500	0.978	45 - 616 mg/dL	1.000	0.200	0.995	42 - 633 mmol/L
iCa^{++}	n/a				1.000	0.01	0.943	0.93 - 1.76 mmol/L
Hct	n/a				1.028	-1.404	0.990	25 - 67 %
Lac	n/a				1.000	1.00	0.974	1.0 - 5.5 mmol/L
pH	n/a				1.037	-0.6	0.999	7.08 - 7.49
pCO_2	n/a				1.056	-0.026	0.986	29 - 84 mmHg

A-021

Technical and interpretive aspects of measurement of hemoglobin %A1c in a titi monkey (*Callicebus donacophilus*) using Sebia Capillarys-2 capillary electrophoresis (CE)

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Background. An 8 year-old intact female Bolivian gray titi monkey (*Callicebus donacophilus*) exhibited persistent glucosuria, increased plasma glucose on samples since 2018 (coinciding with start of progestin contraception), and increased fructosamine. A blood sample was submitted for measurement of %A1c to evaluate glucose regulation following removal of progestin. We describe technical aspects of measurement and interpretation of %A1c for this specimen using Sebia Capillarys-2 CE. **Methods**. Whole blood was collected in a K-EDTA (purple top) microtainer tube for analysis by CE. CE was conducted according to manufacturer's procedures. Calculations were performed using Excel. **Results**. Non-hemoglobinopathy human CE chromatograms exhibit peaks for hemoglobins A1c, A0, other A, and A2. Automated determination of %A1c by CE relies on identification of A2 for validation of the chromatogram. For the monkey specimen, the apparent A2 peak had a prolonged relative retention time (RT=260) compared to normal human A2 (RT=240). For this reason, the monkey specimen chromatogram was identified as "atypical", whereby %A1c was not calculated automatically by the CE software. In such circumstances, %A1c can be calculated manually from the linear calibration curve for %A1c versus the ratio (α) of integrals A1c/(A1c+A0) from the chromatogram. Manual calculation has been shown previously to provide accurate %A1c results. The calibration equation was determined using chromatogram data from a subset of human specimens (n=18) on the same run, selected for a range of values for reported %A1c that were roughly evenly distributed between 4-16%. The calibration equation was found to be %A1c = 72.8 α + 1.9 (r2=0.999). Using this equation, and α=0.111 for the monkey specimen, %A1c for the monkey specimen was 10.0% (or 86 mmol/mol (IFCC units)). For human specimens, A1c >= 6.5% (48 mmol/mol) is diagnostic for diabetes. At our institution, A1c = 10% is approximately at the 85th percentile among patients having A1c >= 6.5%. The %A1c reference interval for this monkey species is unknown. Given that erythrocyte lifetime for this species is likely to be shorter than in humans, the upper limit of the reference interval for monkeys is likely to be less than that for humans. Correspondingly, the glucose level associated with %A1c = 10.0 is likely to be greater than the estimated average glucose (eAG) associated with this same result in humans (eAG = 240 mg/dL, or 13.4 mmol/L). Taking into account all of these considerations, the %A1c result for this monkey was consistent with a diagnosis of diabetes. **Conclusions**. By CE, hemoglobin A2 for the titi monkey was distinct from human A2. This circumstance prevented automated calculation and resulting of %A1c by Sebia Capillarys-2 CE for the monkey specimen. Manual calculation yielded a result of A1c = 10.0% for this case. Generally, interpretation of monkey %A1c results relative to the human reference interval for %A1c must consider a likely shortened erythrocyte lifetime in monkeys. In this case, %A1c was distinctly elevated and consistent with diabetes.

A-022

Assay for xylazine in urine by LC-MS/MS

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Background: Xylazine is an alpha-2 adrenergic receptor agonist approved for veterinary use as a sedative and analgesic. Xylazine exposure in humans has previously been reported to be associated with fentanyl use in our region. Based on animal studies, xylazine is expected in majority to be excreted intact in urine. Psychiatry services asked the laboratory if we could test for xylazine in fentanyl-positive urine as an aid to patient management. We developed an LC-MS/MS assay for this purpose. **Methods**: An LC-MS/MS assay using the AB Sciex API 3200MD was developed as a hybrid of methods from the literature for measurement of fentanyl or xylazine. Xylazine (Santa Cruz Biotechnology) was used to produce six standards (0, 1, 10, 50, 100, 250, 500 ng/mL) using mixed blank urine as a matrix. d5-norfentanyl (Cerilliant) was used as internal standard (IS). Controls were prepared from mixed xylazine-positive urine samples from patients. Standards, controls and patient urine samples were prepared for analysis as follows: 200 µl of MeOH containing IS was added to 100 µL of sample. After vortexing and centrifugation, 100 µL of supernatant was added to sample vials, with 20 µL injected for LC-MS/MS analysis. LC (Shimadzu UFL Prominence) was performed using a Phenomenex Kinetix C18 column (100 A, 5 µm, 50 × 4.6 mm) at 40 ºC. Binary mobile phases (A = H2O, 0.1% formic acid; B = MeOH, 0.1% formic acid) were used with fixed flow rate of 0.5 mL/min according to the following temporal profile (time relative to sample injection (min), %B): -1.0, 10%; 0.0, 10%; 2.0, 50%; 4.0, 100%; 4.1, 10%; 5.0, 10%. MS/MS was run using positive electrospray ionization. Multiple reaction monitoring utilized m/z transitions of 221>164 for xylazine (primary), 221>90 for xylazine (qualifier), and 238>84 for d5-norfentanyl (IS). Retention time was 3.9 min for both xylazine and IS. **Results**: The calibration curve was linear across all calibrators (up to 500 ng/mL; r>0.99). Intra-assay CVs (n=20) were 3.5% (12.2 ng/mL) and 2.4% (53 ng/mL). Inter-assay CVs (n=20) were 5.2% (18 ng/mL) and 6.6% (95 ng/mL). Accuracy of calibration was verified by 1:1 correspondence (±6%; r>0.99) between measured results and calculated spiked sample concentrations using xylazine from a second source (Sigma). Lower limit of quantitation (LLOQ) was set at 10 ng/mL (CV=15.5%); for practical purposes, lower limit of detection (LLOD) was also set at 10 ng/mL. Xylaxine recovery was 43% (400 ng/mL). No analytical interferences were observed for an array of therapeutic drugs, drugs of abuse, or presence of blood hemolysate. In a pilot study, 6 of 8 urine samples that were screen-positive for fentanyl (Roche Cobas immunoassay) were also positive for xylazine (xylazine > LLOD). This result suggests high prevalence of xylazine exposure among fentanyl-positive subjects. **Conclusions**: An LC-MS/MS assay for xylazine in urine was developed using the AB Sciex API 3200MD. Analytical performance characteristics were found to be suitable for routine use for detection of xylazine in urine. Psychiatry services has advised us that this information will be useful in treatment of fentanyl abuse patients.

A-023

Analytical Performance Assessment of a New Soluble Transferrin Receptor (sTfR) Assay on the Alinity c System

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Background: Soluble Transferrin Receptor (sTfR) along with other serum markers such as ferritin, TIBC and iron are used to assess iron status. In particular, sTfR has been used as an aid to diagnose and differentiate between two major forms of anemia: IDA (Iron Deficiency Anemia) and ACD (Anemia of Chronic Diseases). At times of iron deficiency, the transferrin receptor is cleaved from the cell surface and released as a soluble receptor into the blood resulting in an increase in serum sTfR whereas for anemia associated with chronic illness, the sTfR levels remain relatively unchanged. A new Research Use Only (RUO) REACH complaint sTfR assay has been developed for the quantitative determination of sTfR in human serum and plasma on the Alinity c system.

Methods: The newly designed sTfR assay (RUO) is a two reagent latex enhanced immunoturbidimetric assay. Agglutination occurs when the anti-sTfR antibody coated latex particles bind the sTfR antigen in the sample. This agglutination is detected as an absorbance change at 660 nm with the rate of change being proportional to the quantity of sTfR in the sample. The sTfR assay is a rate-up reaction type that uses a spline curve generated from a 6-point calibration.

Results: The new REACH complaint sTfR assay (RUO) has robust analytical performance on the Alinity c platform. The assay has a limit of blank, limit of detection and limit of Quantitation of ≤ 0.04 mg/L, ≤ 0.07 mg/L and ≤ 0.18 mg/L respectively. Total within laboratory imprecision was ≤ 2.8% for samples between 0.9 mg/L to 9.0 mg/L and SD of ≤ 0.03 for samples < 0.9 mg/L. The sTfR RUO assay demonstrated linearity up to at least 12 mg/L and has reagent onboard stability of at least 30 days with calibration curve stability of 30 days. The assay demonstrates significant robustness to HAMA and RF interference up to 480 reactivity titer and 1284 IU/mL respectively. The sTfR assay utilizes the latest CLSI guidance (EP07, 3rd ed.) and demonstrates exceptional robustness to common endogenous interferences; ≥ 60 mg/dL conjugated and unconjugated bilirubin; 1757 mg/dL hemoglobin, 1796 mg/dL human triglyceride, 1008 mg/dL glucose and 15.6 g/dL total protein when tested at 0.9 mg/L and 6.0 mg/L analyte concentrations. Method comparison to commercially available on market sTfR assays showed a slope of 0.98 and correlation coefficient of 0.99 for the serum samples spanning the assay range. The analytical measuring interval (AMI) for the newly formulated sTfR assay (RUO) was observed from 0.18 to at least 10 mg/L. The RUO sTfR assay has an auto-dilution feature for samples allowing for a reportable Interval (RI) of up to 80 mg/L.

Conclusion: An analytically robust and REACH compliant RUO sTfR assay was designed for use on the Alinity c system with excellent precision, exceptional modernized endogenous and exogenous interferences at lower and upper medical decision points and robust on-board stability without re-calibration.

A-024

A Mass Spectrometry Approach to Detect Beta-Lactamase Mediated Antibiotic Resistance In Clinical Specimens

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Background: Beta-lactam antibiotics account for the majority of antibiotics used worldwide. Resistance by beta-lactamase expression is a serious and growing threat. The typical workflow to identify beta-lactamase expression typically requires 2-3 days necessitating empiric treatment with potentially ineffective antibiotics until results are available. Our objective is to determine if beta-lactamase activity is directly detectable in urine specimens (without isolation and culture steps) enabling faster identification of resistance.

Methods: Urine samples from patients with Extended Spectrum Beta-Lactamase (ESBL) urinary infections were incubated with beta-lactam antibiotics. Beta-lactam hydrolysis was determined by mass-spectrometry based methods.

Results: Hydrolysis of ceftriaxone was demonstrated in 45 of 45 ESBL containing specimens from patients not treated with a beta-lactamase inhibitor before sample collection. Ceftriaxone hydrolysis was not demonstrable for 6 of 6 ESBL containing specimens derived from patients treated with a beta-lactamase inhibitor (either tazobactam or meropenem). Spiking studies demonstrate that ceftriaxone, cefazolin, and oxacillin hydrolysis can be observed within 30 minutes. The data also demonstrates that beta-lactam hydrolysis is evidenced by mass-spectrometry preceded by either liquid-chromatography or matrix-assisted laser desorption ionization sample processing methods.

Conclusion: Clinically significant beta-lactamase activity is robustly detectable in urine samples. Utilization of the described methods could enable detection of beta-lactam resistance 24-48 hours earlier than traditional culture-based methods and could be performed using mass spectrometers commonly found in clinical laboratories.

A-025

A Comparative Study between Immunoturbidimetry and Chemiluminescence Methods 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

A-026

Comparison of two quantitative rapid fecal calprotectin assays detecting active inflammatory bowel disease

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Background: Fecal calprotectin (FC) is widely used for the diagnosis and monitoring disease activity of inflammatory bowel disease (IBD). Quantitative rapid assay can be a reliable alternative to time consuming enzyme linked immunosorbent assay. This study aimed to evaluate and compare the two quantitative rapid FC assays (Ichroma calprotectin, and Buhlmann Quantum blue) for detection of active IBD. **Methods:** Total of 192 patients consisted of 84 patients with IBD (67 ulcerative colitis and 17 Crohn's disease) and 108 patients with non-IBD were included in this study. We compared quantitative levels of different disease status and evaluated the correlation between the two FC kits. Diagnostic performances predicting active IBD were analyzed according to various cut-off levels. **Results:** Forty-five patients with active IBD defined by endoscopic score had increased FC levels in both assays compared to the inactive IBD or other diseases ($P < 0.05$). Although two assays' results correlated ($r = 0.642$, $P < 0.001$), a significant deviation was present ($y = -45.2 + 8.9X$). Diagnostic performances predicting active IBD were comparable for iChroma (area under the curve (AUC) 0.812, cut-off 50, sensitivity 64.4%, specificity 85.0%) and for Buhlmann Quantum Blue (AUC 0.826, cut-off 100, sensitivity 84.4%, specificity 61.9%). FC levels using cut-off of > 250 μg/g confirmed 85.7% (iChroma) and 64.1% (Buhlmann) of active IBD patients.**Conclusion:** Two iChroma and Buhlmann rapid FC assays correlated, but the two test results were not interchangeable. It can be helpful predicting active IBD with optimized cut-off values.

A-027

Impact of Haemoglobin D Punjab Variant on Haemoglobin A1c Determination by High Performance Liquid Chromatography Method - A Case Report

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Background: Hemoglobin A1c (HbA1c) is routinely being used for diagnosing diabetes mellitus and to monitor long-term glycemic control. A number of techniques are available to measure HbA1c and many factors may interfere with its measurement causing falsely high or low results. Approximately 7% of the world's population have been reported to be carriers of a hemoglobin (Hb) variant. India with its multi-ethnic population has a plethora of haemoglobin variants with minimal to major clinical significance. Hb D hemoglobinopathies are widespread in northwestern India with the heterozygous form reported to have a prevalence of 1.1%. Hb D Punjab trait is usually clinically silent but coinheritance of Hb D with Hb S or beta-thalassemia produces clinically significant conditions of moderate severity while the homozygous state manifests as mild hemolytic anemia and mild to moderate splenomegaly. Some studies suggest a minimal impact of this Hb variant trait on HbA1c measurement, however, most evaluate only analytical interferences and there is lack of published data addressing potential limitations like altered RBC survival rate.Case report: Our patient is a 28 years old female, who came to our institution for a pre-employment check-up. On history taking she gave a positive history of diabetes mellitus for her father and paternal grandmother. Her fasting plasma glucose levels were 106 mg/dL and HbA1c concentration measured on D-10 (Bio-Rad Laboratories, Inc.) was 4.7% which is unlikely for a patient with impaired plasma glucose levels and a strong family history of diabetes mellitus. Chromatogram inspection revealed presence of an abnormal peak reported as Variant Window. The HbA1c concentration was cross-checked by capillary electrophoresis on Sebia Minicap Flex Piercing, which reported a concentration of 5.2% and an additional peak with a flag of Atypical Profile. Hb electrophoresis performed on Sebia Minicap Flex Piercing confirmed the presence of a Hb variant - Hb D Punjab. Conclusion: Clinical laboratories must be aware of the prevalent Hb variants and hemoglobinopathies in the population they cater to and their interference if any with the HbA1c testing method they are using. Also, HbA1c results should always be correlated to the clinical profile of the patient in case unexpected Hb variants are reported as many physicians have suggested that even a 0.5% HbA1c variation is clinically quite significant. Measurement of HbA1c levels via capillary electrophoresis is a relatively recent but well validated method that separates A1c and other Hb fractions via charge difference at high voltage using electro-osmotic flow and thus, is especially useful when Hb variants are present as capillary zone electrophoresis is very precise and accurate to estimate the Hb fractions.

A-028

Evaluation of the Siemens SARS-CoV-2 Total (COV2T) assay for the detection of anti-SARS-CoV-2 antibodies

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Introduction: There are already 12,748,747 infected with SARS-CoV-2 in Brazil with 321,515 deaths at the time of writing this report. One of the strategies to prevent the spread of this virus and consequently of COVID-19 is to identify whether the individual has been exposed to the virus. Serological tests, within the context of health control, are taking on more and more strength. Understanding the seroconversion curve for SARS-CoV-2 is essential for assays to be applied at the ideal time, ensuring that the diagnosis and identification of exposed patients are more assertive. Therefore, the objective of this study is to evaluate the performance of the Siemens SARS-CoV-2 Total assay for the detection of SARS-CoV-2 antibodies. **Methods**: The Atellica® IM SARS-CoV-2 Total (COV2T) assay is intended for use in *in vitro* diagnostics for the qualitative detection of total SARS-CoV-2 antibodies in human serum and plasma. Accuracy was assessed in the repeatability study with 12 replicates. Clinical sensitivity was determined by testing 61 samples from individuals with a clinical diagnosis of COVID-19 based on a positive polymerase chain reaction (PCR) result. Another 70 samples collected before November 2019 were selected from stored sera to assess the specificity of the assay. **Results**: Accuracy was 2.07% with an average index of 2.14. The sensitivity evaluated between 01 to 06, 07 to 13 and> 14 days was 60% (12/20), 76.67% (23/30) and 100% (11/11), respectively, and the specificity was 100%. It is interesting to note that the negative samples showed a very low average index of 0.06 with SD 0.08, showing little cross-reactivity of the assay. **Conclusion**: The Siemens Atellica® IM SARS-CoV-2 assay has high specificity (100%) and an excellent sensitivity over 14 days (100%) for immunocompetent adult patients with COVID-19. Performance is comparable to that reported in other studies that demonstrated similar sensitivity and high specificity.

A-029

Evaluation of the Analytical Performance of the Bone Health Laboratory Diagnostic Profile including Vitamin D

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Background: With population ageing, there is an increase in age-related diseases, such as cardiac and neurodegenerative diseases, among others, including bone diseases, which fractures lead to one of the main causes of death among the elderly. They are part of bone-metabolic diseases; osteoporosis, hypo or hypercalcemia, and osteomalaceae, for example, and use clinical analysis as a screening to signal bone problems. Osteoporosis, as the most common disease in this scenario, affects, according to the IOF (International Osteoporosis Foundation) 200 million women worldwide, and from 60 years, the prevalence by age practically doubles every 10 years. In Brazil, there are approximately 10 million cases, where 1/3 of women over 50 have the disease. Periodic monitoring through laboratory tests, such as Calcium, Potassium, Alkaline Phosphatase, PTH and recently Vitamin D guarantees greater resources for a more assertive diagnosis and effective treatment. In this context, this study aimed to evaluate the performance of the main biochemical parameters involved in bone health, using the Atellica Siemens Healthineers platform. **Methods**: The accuracy was verified through the repeatability study ($\%CV_R$) and within-laboratory precision ($\%CV_{WL}$) according to EP15-A3 and the comparison of methods according to EP09-A3. For the imprecision study, two concentrations were used; each level of QC materials was tested in one run per day, with five repetitions per run, for five days, resulting in a total of 25 replicates per sample for each assay. Method comparison studies were performed using at least 40 serum samples that covered the assay range. **Results**: The inaccuracy results are in accordance with the analytical quality specifications. The $\%CV_R$ was 0,3% to 8,5% and $\%CV_{WL}$ was 0,8% to 9,1% among all Atellica CH and Atellica IM Analyzer assays. The results of comparison of the Passing and Bablock methods ranged from (R^2) 0.9903 to 0.9999. The tests tested at Atellica CH and AU 5800 Beckman Coulter® demonstrated good agreement, as well as the tests tested at Atellica IM and Architect Abbott. **Conclusion**: All tests tested on the Atellica IM 1600 and Atellica CH 930 Analyzer demonstrated acceptable results of precision and method comparison with the Siemens Healthineers tests, essential in the detection and confidence in the results of liver changes. The precision results are consistent with the manufacturer's claims.

A-030

Validation of a Custom Multiplex Cytokine Assay for use in a Clinical Laboratory on Luminex® FLEXMAP 3D Platform

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Background: Cytokines are small molecules which are key mediators and regulators of immunity, inflammation and hematopoiesis. Evaluation of plasma cytokine levels can be used to investigate the etiology of infectious or chronic inflammatory diseases when used in conjunction with clinical information. **Objective:** Validate a custom magnetic microparticle-based multiplex immunoassay for quantitation of 14 cytokines in human plasma. The analytes evaluated were TNF, IL-6, IFN-beta, IL-10, MCP-1, VEGF, IL-6 receptor alpha soluble (IL-6Ra), IL-1 beta, IFN-gamma, MIP-1 alpha, GM-CSF, IL-2 receptor alpha soluble (IL-2Ra), IFN-alpha, and IL-18. **Method:** The custom 14-plex assay (R&D Systems) utilizes magnetic microspheres with unique color codes designated to specific analytes, allowing for simultaneous quantification of all analytes in a single sample. Samples or calibrators are incubated with capture antibodies coupled to the color-coded microspheres. A wash is followed by incubation with analyte specific detection antibody. A second wash is followed by a short incubation with a streptavidin-PE reporter, a final wash and a resuspension step. Finally, analyte detection occurs via a dual laser system within the Luminex® FLEXMAP 3D. Method validation included determination of imprecision, limits of detection and quantification, analytical measurement range (AMR), accuracy by spike recovery, and analytical specificity. The 95th percentile reference interval was determined for each analyte (n=119). **Results:** The table below summarizes key performance characteristics of the assay.

	TNF	IL-6	IFN-beta	IL-10	MCP-1	VEGF	IL-6Ra
AMR (pg/mL)	10.0-900	5.0-315	20.0-1200	7.0-750	40.0-9000	30.0-3462	246-35882
LOD (pg/mL)	4.5	2.9	8.5	3.7	30.8	11.9	47.6
LLOQ (pg/mL)	10.0	5.0	20.0	7.0	40.0	30.0	246
95% Reference Interval (pg/mL)	<10.0	<5.0	<20.0	<7.0	≤198	≤77.0	≤45431
% Recovery of spike	85%	74%	85%	104%	138%	94%	88%
	IL-1 beta	IFN-gamma	MIP-1 alpha	GM-CSF	IL-2Ra	IFN-alpha	IL-18
AMR (pg/mL)	20.0-3500	60.0-8500	220-5000	15.0-780	40.0-4000	20.0-1500	65.0-11000
LOD (pg/mL)	10.7	33.8	121.0	7.8	38.3	8.4	29.9
LLOQ (pg/mL)	20.0	60.0	220	15.0	40.0	20.0	60.0
95% Reference Interval (pg/mL)	<20.0	<60.0	<220	<15.0	≤959	<20.0	≤1015
% Recovery of spike	91%	126%	116%	77%	98%	100%	81%

Lipemia and hemolysis were acceptable up to 1000 mg/dL (triglycerides) and 500 mg/dL (hemoglobin). Tociluzumab significantly interfered with IL-6Ra at 50 ug/mL and 200 ug/mL. Significant cross-reactivity between analytes on the panel was not evident. **Conclusion:** The observed analytical performance of the multiplex cytokine panel on the Luminex® FLEXMAP 3D appeared robust and suitable for clinical laboratory utilization.

A-031

Validation of the Siemens Point of Care Atellica VTLi Cardiac Troponin I Immunoassay as High Sensitivity Including Sex-Specific 99th Percentiles

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Background: High sensitivity (hs) cardiac troponin (cTn) assays are defined per the IFCC Committee on Clinical Application of Cardiac Biomarker (C-CB) by the ability to measure ≥ 50% of concentrations greater than the limit of detection (LoD), with an impression (%CV) of ≤10% at sex-specific 99th percentile upper reference limits

(URLs). Our study determined the sex-specific 99th percentile URLs for males and females utilizing heparinized plasma from the AACC universal sample bank (USB) for the Siemens point of care (POC) Atellica® VTLi hs-cTnI immunoassay. Methods: Apparently healthy subjects were enrolled and included overall n =693, males n = 363, and females n = 330, following use of exclusionary surrogate biomarkers: hemoglobin A1c, NT-proBNP, eGFR, along with statin medication use. hs-cTnI was measured in a central laboratory, on multiple POC Atellica® VTLi immunoassay analyzers. Results: The LoD was 1.24 ng/L (CV 10%) and the limit of quantitation (LoQ) (CV 20%) was 6.7 ng/L. 99th percentile URLs were determined by the nonparametric (NP) method. Near Gaussian distributions of hs-cTnI concentrations for both males and females were observed. 99th percentile URLs were: overall 23 ng/L (90% CI 20-32 ng/L); male 27 ng/L (CI 21-37 ng/L); female 18 ng/L (CI 9-78 ng/L). The percentages of subjects having a measurable concentration > the LoD were: overall 83.7%, male 87.3 %, female 79.7%. Conclusions: Our findings are unique, showing the novel POC Atellica® VTLi hs-cTnI assay meets the designation of a 'high-sensitivity' assay using heparinized plasma specimens, demonstrating distinct male and female 99th percentiles.

A-032

A New Data Acquisition Strategy Using Orbitrap LCMS Platform for Fast Absolute Quantification of Neutralizing SARS-cov2 Antibodies and Other MAbs

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Background: Fast, direct and accurate analysis of anti-SARS-CoV-2 neutralizing antibodies, usually with unknown sequence, are the urgent needs to understand the direct mechanisms how their virus-neutralizing activities combat viral propagation at molecular level. It is vital for anitiviral mAb-based immunotherapies' efficacy and safety, and vaccine development. We aim to develop a novel integrated data acquisition and analysis method for the quantification of mAbs at subunits levels. The purpose is to address the unsolved challenges of routine peptide-centric MS detection of mAbs with unknown sequence. Bottom-up proteolytic digest methods tend to suffer from the common challenges, such as the introduction of artifacts from lengthy sample preparation, and loss of proteoform information in highly dynamic and complicated peptide pools. For mAbs quantification, bottom up ananlysis has some unique challenges: 1) It is incapable of selecting unique peptides from novel mAbs with a mutated or otherwise unknown protein sequence. 2) There are limited unique peptide selections available from the target IgG variable region. 3) The target region is might be difficult to be digested and the resulting fragments might show ionization efficiency issues. 4) Polyclonal immunoglobulin peptide increases the background signal. 5) Low sensitivity and specificity require costly antibody-based enrichment.

Methods: The intact mAb was digested by the IdeS protease and the disulfide bond breaker. IdeS cleaved heavy chains below the hinge region, producing F(ab')2 and Fc fragments. Following reduction of disulfide bonds, three antibody domains, about 25 kDa: light chain (LC) and VH-CH1 (Fd') and CH2-CH3 (Fc) domains of the heavy chain, can be further released. Two microliters of IdeS digest were injected, and chromatographic separation was performed on a Thermo Scientific Vanquish Flex UHPLC, followed by analyte detection on a Thermo Scientific Orbitrap Exploris 240 mass spectrometer. Total run time was 5 minutes. Thermo Scientific Chromeleon 7.3 chromatography data system software was used for instrument control and data acquisition. Quantification and data visualization were generated using both Thermo Scientific Chromeleon CDS 7.3, Xcalibur 4.3.73.11 and FreeStyle 1.7 software.

Results: We developed a novel integrated data acquisition method, leveraging ultra-high accurate, ultra-high resolution with narrow window middle down MS for the sensitivity, selectivity, accuracy, and proteforms resolving enhancement. An anti-SARS-CoV-2 neutralizing antibody was spiked to test the performance. The linearity of the assay was assessed by serial dilution and found to be 0.4 µg/mL to 25 µg/mL with R^2 > 0.9964. The method enables the sensitive enhancement of mAbs with high specificity and reproducibility, while simultaneously detecting its other proteoforms spinning around such as glycoforms or oxidations. **Conclusion:** The new method addressed the urgent needs of absolute mAbs quantification with unknown protein sequence. Its fast method development enables it to deploy to the measurement of novel antibodies for rapid evolution of viral pathogens.

A-033

Comparability of Selected Assays on cobas pure integrated solutions Under Routine-Like Conditions at Four Sites in Europe and Asia

H. Fleurkens[1], M. F. Rossier[2], F. Prevot[3], N. Vogt[3], H. Baum[3], J. Hong[4], H. Gu[4], S. Chun[4], P. Findeisen[5], J. Furrer[6]. *[1]Spital Wallis, Labor ICH-ZIS, Visp, Switzerland, [2]Spital Wallis, Labor ICH-ZIS, Sion, Switzerland, [3]Institut für Laboratoriumsmedizin und Transfusionsmedizin, Regionale Kliniken Holding RKH, Ludwigsburg, Germany, [4]Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of, [5]MVZ Limbach, Heidelberg, Germany, [6]Roche Diagnostics International Ltd, Rotkreuz, Switzerland*

Background: The novel cobas pure integrated solutions system (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) is a serum work area laboratory analyzer, comprising two analytical units: a clinical chemistry unit including ion selective electrodes (ISE) (cobas c 303) and an immunochemistry unit (cobas e 402). In this multicenter study, we assessed the comparability of the cobas pure integrated solutions system versus respective routine analyzers at four sites under routine-like conditions.
Methods: The study was conducted at five sites in Switzerland, Germany, and the Republic of Korea, from September to December 2020. At four sites, method comparison experiments using routine leftover samples evaluated comparability of the cobas pure integrated solutions system with respective routine analyzers: Beckman Coulter AU5822 Clinical Chemistry Analyzer, Abbott Alinity I, Siemens ADVIA Centaur, and Roche cobas INTEGRA 400 plus, cobas e 411, cobas pro and cobas 8000. In total, 47 selected analytes with 53 applications covering clinical chemistry (ALB, ALP, ALT, APO-A, AST, BIL-D, BIL-T, CA, CHE, CHOL, CK, CREA, CRP, FE, GGT, GLUC, HbA1c, HDL, LDL, LDH, LIP, MG, PHOS, TP, TRIGL, UA, UREA), ISE (Cl, K, Na), and immunochemistry (anti-TSHR, CA 15-3, CEA, E2, Ferr, FOL, FT3, FT4, HCG+Beta, LH, NT-proBNP, PROG, tPSA, TESTO, TnThs, TSH, Vit. B12) were assessed. Passing/Bablok regression analyses were carried out: slopes, intercepts, and correlations for method comparisons were calculated. Pre-defined acceptance criteria for each assay were determined before taking measurements for the method comparisons versus Roche methods.

Results: More than 35,000 result pairs were included in the analysis. All 53 applications showed good comparability between cobas pure integrated solutions and the initial results on the respective routine analyzers. A total of 218 method comparisons showed a median Passing/Bablok regression slope of 1.00 (67% were between 0.95 and 1.05), a median bias at the medical decision point of -0.1% (87% were ≤5%), and a median Pearson's r coefficient of 0.998.

Conclusion: The results of this study demonstrate that the cobas pure integrated solutions system delivers comparable and accurate results versus other commercially available analyzers across a selection of applications under routine-like conditions.

A-034

Result Precision and Accuracy on cobas pure integrated solutions Demonstrated at Four Sites in Europe and Asia

H. Fleurkens[1], M. F. Rossier[2], F. Prevot[3], N. Vogt[3], H. Baum[3], D. Radziszewski[4], J. Hong[5], H. Gu[5], S. Chun[5], C. Schneider-Thauern[6]. *[1]Spital Wallis, Labor ICH-ZIS, Visp, Switzerland, [2]Spital Wallis, Labor ICH-ZIS, Sion, Switzerland, [3]Institut für Laboratoriumsmedizin und Transfusionsmedizin, Regionale Kliniken Holding RKH, Ludwigsburg, Germany, [4]Diagnostyka Sp. z o.o., Wroclaw, Poland, [5]Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of, [6]Roche Diagnostics GmbH, Mannheim, Germany*

Background: The novel cobas pure integrated solutions system (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) is a serum work area laboratory analyzer, which comprises two analytical units: a clinical chemistry unit including ion selective electrodes (ISE) (cobas c 303) and an immunochemistry unit (cobas e 402). In this multicenter study, we evaluated the analytical performance and the overall system functionality of the cobas pure integrated solutions system. Here, we report on the result precision and accuracy.

Methods: The study was conducted between August and December 2020 at five sites in Europe and Asia (Germany, Switzerland, Poland, and the Republic of Korea). At all sites, quality control (QC) materials were measured for the applied assays (ISE, clinical chemistry [including enzyme, substrate, and specific protein], and immunochemistry) at two analyte concentration levels, and analyte recovery per QC was monitored. At four sites, precision studies based on the Clinical and Laboratory Standards Institute EP05-A3 protocol were conducted, over a 21-day period, for 34 selected applications (ISE: 3 analytes/6 applications [serum and urine]; clinical chemistry: 10

analytes/15 applications [serum and urine]; immunochemistry: 13 analytes/13 applications [serum only]) that represent a typical routine panel of assays. Coefficients of variation (CVs) for repeatability (within-run precision), intermediate precision (within-lab precision), and reproducibility (across-lab precision) were calculated and compared with the studies' pre-defined acceptance criteria. At four sites, result accuracy for 48 selected applications (ISE: 3 applications; clinical chemistry: 26 applications; immunochemistry: 19 applications) was validated in a ring trial. Ring trial samples included 16 commercial samples and two Roche QC samples (anti-hepatitis A virus immunoglobulin M), which were measured in triplicate over three consecutive days.

Results: For repeatability and intermediate precision, 152 CVs each were calculated. For repeatability, 34/48 CVs were ≤1% for ISE assays, 116/120 CVs were ≤2% for clinical chemistry assays, and 90/104 were ≤2.5% for immunochemistry assays. For intermediate precision, 47/48 CVs were ≤2% for ISE assays, 114/120 CVs were ≤2% for clinical chemistry assays, and 104/104 CVs were ≤3.5% for immunochemistry assays. For reproducibility, 11/12 CVs were ≤3% for ISE assays, 27/30 were ≤3% for clinical chemistry assays, and 20/26 were ≤3.5% for immunochemistry assays. For the 48 applications included in the analysis of result accuracy with the ring trial samples, 80% of median recoveries per assay and site were within 2% of the median per assay for all sites.

Conclusion: The findings of this study demonstrate that the novel cobas pure integrated solutions system provides precise and accurate results for a typical routine panel of assays for the measurement of ISE, clinical chemistry, and immunochemistry parameters, supporting implementation into routine clinical laboratory practice.

A-035

Serum Work Area System Validation Under Intended User Conditions

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Background: Testing a newly developed analyzer under routine-like conditions by the intended users is an established part of the Roche Diagnostics' system design validation concept. Therefore, external evaluations of the novel cobas pure integrated solutions system - a serum work area laboratory analyzer comprising a clinical chemistry unit including ion selective electrodes (ISE) (cobas c 303) and an immunochemistry unit (cobas e 402) - were performed at different phases of development. We evaluated the overall system functionality, reliability, and user satisfaction of the cobas pure integrated solutions system at various development phases.

Methods: We conducted a prototype study at one site in Switzerland, followed by a pilot study at two sites in Switzerland and Germany, and, finally, a comprehensive system performance evaluation study at five sites in Europe and Asia. Selected clinical chemistry, ISE, and immunochemistry applications were assessed during each study: prototype (n=22), pilot (n=17), and system performance evaluation (n=27). At the different development phases, routine simulation precision experiments based on the Clinical and Laboratory Standards Institute EP05-A3 protocol were conducted for the cobas pure integrated solutions system to evaluate all system components under routine-like intended user conditions, in order to identify deficiencies, and to analyze overall system functionality. Coefficients of variation (CVs) were calculated. The precision experiments allowed the identification of systematic and random errors for the selected assays (representing a typical routine panel of assays) included in the study. In addition to the precision experiments, system reliability (measured by the percentage of analysis runs completed without interruption) was compared at various development phases.

Results: The results from the cobas pure integrated solutions system prototype study led to the optimization of the fluid drain management; for the pilot study, the fluid drain system was redesigned and the installation procedure was adjusted. During the pilot study, sample pipetting was impaired after a sample with insufficient volume was tested; therefore, a software change was introduced during the performance evaluation study and regression testing demonstrated that the software change led to the cobas pure integrated solutions system exhibiting the correct behavior when pipetting samples in a repeat run after a sample short alarm occurred. In the performance evaluation study, 18 runs with 604 CV pairs for the 27 selected clinical chemistry, ISE, and immunochemistry applications were included in the data analysis; an overall system reliability of 99% was achieved, a good relative dispersion (most CVs <2%) was observed, and no systematic or random errors occurred.

Conclusion: The findings of this study demonstrate improvement in the functionality of the novel cobas pure integrated solutions system throughout the development process. The serum work area solution demonstrated good overall system functionality and reliability, which are essential for providing safe and accurate results to operators and patients.

A-036

Evaluation of β glucuronidases performance in LC-MS/MS testing of Opioid and Benzodiazepinesperformance in LC-MS/MS testing of Opioid and Benzodiazepines

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Background: β glucuronidase used in urine drug testing by LC-MS/MS to remove glucuronides and sulfates on drugs helps increase detection of compounds and their metabolized components. β glucuronidases from most vendors require a heated incubation step for 30-60 min around 50-70° C to achieve maximum enzyme efficiency and hydrolysis of drugs. Besides the routine sample preparation, this process requires additional staffing duties such as maintenance of oven, clock, and storage requirements. To reduce the upkeep and incubation efficiency, we sought to evaluate two b-glucuronidases types (β -Glucuronidase-1(BG1) and β - Glucuronidase Turbo (BGT)-from Kura Biotech) for efficiency in deconjugation compounds. The goals of this study were to find the glucuronidase that would give higher yield and shorter incubation time. **Methods:** Twenty patient samples with positive detection of opioids and benzodiazepines were used to evaluate BG1 and BGT performance over short and long incubation times using similar lot of calibrators and quality controls. Drug panels consist of 15 opioids, 9 benzodiazepines, and THC. Lab-developed LC-MS/MS (Vanquish LC and ThermoFisher Altis triple quad system) procedures were used to obtain the data. Concordance and comparison performance were done on results that were inside of analytical measuring range. **Results:** The results by both glucuronidases gave 100% concordance with current enzyme. 82% of the BZDs compounds were within the lab cut off and 72% of the Opioid compounds were within the lab cut off. A very high degree of correlation existed between the two enzymes (>95%) and between the two incubation periods (>98%). Enzyme BG1 with different incubations had 99.5% correlation for BZDs and 99.9% correlation for Opioids. Enzyme BGT with different incubations had 96.7% correlation for BZDs and 99.9% correlation for Opioids. With short incubation period, the two enzymes had a correlation of 88.8% for BZDs and 99.9% for Opioids. With Long incubation period, the two enzymes had a correlation of 96.5% for BZDs and 99.8% for Opioids. The agreement between the different enzymes and different incubation periods for both BZDs panel and Opioid panel was 100%. **Conclusion:** The Correlation analysis of drug concentrations in 20 patient urine samples treated with different enzymes and incubation periods reveals that Enzyme BG1 and BGT have similar performances. However, reduced incubation by BGT would reduce turnaround time of results and increase lab efficiency.

A-037

Multi-Site Verification of the Automated EXENT® MALDI-TOF-MS System and Immunoglobulin Isotypes Assay for the Identification and Quantification of Monoclonal Immunoglobulins

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Background. The advantages of MALDI-TOF-MS based methods for the identification and quantification of monoclonal immunoglobulins have been the subject of recent International Myeloma Working Group guidelines. Here we report on the first multi-site verification of this technology using the EXENT system. Standardisation of monoclonal peak quantification was achieved using intelligent peak picking software including integration of immunoglobulin assay data calibrated to DA470k. **Methods.** Samples were prepared using the EXENT system (The Binding Site Group Ltd, UK): modified polyclonal antibodies (anti-IgG, -IgA, -IgM, -total-κ and -total-λ) were covalently attached to paramagnetic microparticles. Patient samples were incubated with the microparticles, which were then washed, eluted (20mM TCEP in 5%(v/v) acetic acid) and spotted onto MALDI plates with HCCA matrix. Spectra were generated using the MALDI-TOF-MS system. Quantitative IgG, IgA and IgM values were obtained using the Optilite® analyser (The Binding Site Group Ltd, UK).Resulting light chain spectra from each reaction (G,A,M, κ & λ) were simultaneously interrogated using the EXENT software (employing proprietary peak modelling trained against clinical data), to yield respective immunoglobulin isotype, mass-to-charge

ratio (*m/z*) and quantity (g/L).Analytical performance of the system was based upon CLSI guidelines, including; LLMI (EP17-A2), reference interval verification (EP28-A3c; Hevylite reference range comparison), precision (EP05-A2), linearity (EP06-A), interference (EP07-A2), and stability (EP25-A). A comparison to 60 IFE positive clinical samples (31/60 >10g/L, 18/60 <10g/L and 11/60 unmeasurable by SPEP) taken from different patients throughout the course of their disease was performed. For quantitative comparisons with SPEP, Passing-Bablok regression analyses were performed on 49 samples and separately for those >10g/L and <10g/L.**Results.** LLMI was 0.015g/L for all specificities; dependent on the polyclonal background values of <0.005g/L were detectable. Published appropriate Hevylite reference intervals were verified. Acceptable 20-day total precision values for polyclonal samples (<7%) and for two monoclonal samples (~1g/L and 10g/L; <15% and <10%, respectively) were obtained, with between laboratory precision <8% for all samples. The assay was linear for all three major isotypes (IgG, IgA or IgM) across a 0.015 to 100g/L dynamic range (~1000 fold) using a 10% non-linearity acceptance limit. No significant assay interference was observed. On-board stability of reagents was 5-24h, and open-vial stability was 2.6 months. Qualitative comparison was performed on 60 IFE positive samples, obtained throughout the course of the individual patients' disease. There was 100% concordance with the monoclonal type. Additional monoclonal and immunoglobulin peaks were identified in 19/60 samples by the EXENT system; 3 of these had possible glycosylation modifications. There was acceptable quantitative agreement with SPEP for 49 samples (y=0.91x-1.14). However, there was a substantial discordance <10g/L (y=0.75x-0.87) compared to >10g/L (y=0.95x-2.59), suggesting over-estimation by SPEP at lower levels. **Conclusions.** This is the first verification data on the soon to be commercially available EXENT system. The data demonstrated acceptable performance in terms of precision, interference, and agreement with existing assays. The role of the EXENT software in the identification and integration of peaks will ensure reliable and reproducible results across different laboratories, an important step in standardising this exciting methodology.

A-038

Automated Analysis of a Panel of Fat-Soluble Vitamins A, E, D and K in Serum by Supported Liquid Extraction and LC-MS/MS

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Background: Fat-soluble vitamins are normally analyzed using multiple methods by HPLC or LC-MS/MS. We extracted and analyzed a panel of fat-soluble vitamins: alpha-tocopherol, retinol, beta-carotene, 25-OH-D2 and D3, phylloquinone and menaquinone-4 in a single method from serum employing supported liquid extraction (SLE) and LC-MS/MS.

Methods: Method development was performed using vitamin stripped serum (Golden West). Standards were purchased from Sigma-Aldrich. Samples were extracted using ISOLUTE® SLE+ 400 µL capacity 96-well plates (Biotage). Analysis was performed using a Waters Acquity Quattro Premiere equipped with a 100 mm x 2.1 mm, 2.7 µm Restek Raptor Biphenyl column and EXP holder and guard column with a gradient of 5 mM ammonium acetate with 0.1% formic acid in water and 3:1 methanol:2-propanol containing 5 mM ammonium acetate and 0.1% formic acid at a flow rate of 0.4 mL/min.

100 µL of serum was added to 10 µL of Internal standard solution and 10 µL of spiking solvent in methanol in a 2 mL collection plate which was capped, mixed and left to stand 1 hour in the dark. 400 µL of 2-propanol/heptane (1:3, v/v) with 1 mg/mL BHT was added to each sample, mixed and left to stand for 5 minutes. 500 µL of pretreated sample was mixed well and loaded as an emulsion onto the ISOLUTE® SLE+ and extracted with 2 x 500 µL of heptane following the standard SLE+ protocol. Samples were reconstituted in 150 µL of 2-propanol for LC-MS/MS analysis.

Results/Conclusions: Samples were extracted manually and compared to automated extraction using a Biotage® Extrahera™. Recovery, %RSD, lower and upper limits of quantitation (LLOQ and ULOQ) are listed in Table 1. This method provides high, reproducible recoveries of a range of fat-soluble vitamins in human serum in clinically appropriate concentration ranges using manual extraction or an automated process for increased throughput.

Table 1. Recovery, %RSD, LLOQ and ULOQ for Fat-Soluble Vitamins

Analyte	% Recovery Manual	% RSD Manual	% Recovery Extrahera	% RSD Extrahera	LLOQ (ng/mL)	ULOQ (ng/mL)
Retinol (A)	94.9	3.3	75.9	5.3	80	4000
Beta Carotene (A)	89	4.1	71.3	10.5	80	4000
25 OH Vitamin D2 (D)	101	5.1	79.2	5.4	4	200
25 H Vitamin D3 (D)	95.6	4.6	81.9	5.5	4	200
Alpha Tocopherol (E)	99.1	4.6	84	4.6	800	40000
Phylloquinone (K)	95.7	10.5	71	6.3	0.4	20
Menaquinone-4 (K)	95.7	9.6	73.4	4.5	0.4	20

A-039

Nitric Oxide Releasing Hydrogel Based on Inducible Nitric Oxide Synthase Embedded in Alginate-Polyethyleneimine as a Platform to study NO-driven Modulation of Carcinogenesis

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Background: Several biological processes including cell proliferation and programmed cell death (apoptosis) are affected by the multifaceted function of nitric oxide molecule. NO˙ is a ubiquitous free radical gas that exerts a wide range of biological effects and acts as a signaling molecule in the body. Over past decades, NO˙ has been suggested to modulate different cancer-related events including angiogenesis, apoptosis, cell cycle, invasion, and metastasis. On the other hand, it is also emerging as a potential anti-oncogenic agent under other conditions. Furthermore NO˙ also has the potential to enhance both radio- and chemotherapy. Nitric Oxide is synthesized by a complex family of nitric oxide synthase (NOS) enzymes. There is increasing interest in developing NO-releasing materials as potent tumoricidal agents in which high and localized concentrations of NO may be directly released in a sustained manner to the tumor site.

Methods: we develop Sodium alginate-Polyethyleneimine-iNOS Hydrogel discs via Cross linking using a layer-by-layer thin film building strategy to form layers of polyethyleneimine and iNOSoxy as NO-releasing coatings on alginate hydrogel. Here, the recombinant iNOSoxy enzyme protein was over expressed in E. coli BL21(DE3) strain using pCWori vector plasmid. The enzyme was purified by Ni^{+2}-NTA affinity column chromatography. The negatively charged iNOS oxygenase is adsorbed onto the positively charged matrix layer of polyethyleneimine which is built on a negatively charged sodium alginate hydrogel. The enzyme within resulting hydrogel produces nitric oxide when the hydrogel is exposed to a solution with all ingredients of the NOS reaction. We characterized the hydrogel discs by FT-IR and scanning electron microscopy. In Vitro cytotoxicity, protein adsorption and hemocompatibility tests were also performed.

Results: FT-IR shows the characteristic functional groups of sodium alginate and PEI. We also characterized the iNOSoxy enzyme on PEI using the amide-1 and amide-2 characteristic IR bands. After the structural characterization of the NOS-hydrogel films using spectroscopy, we examined their functional characteristics in terms of NO release profiles. We observed an initial "burst" of NO release during the first 4 hours of activity, followed by a decline and then stable NO release for up to 144 hours.

Conclusion: Successful NO release was achieved from our hydrogel discs. The measured fluxes are higher than what have been reported in the literature for other inorganic NO-releasing systems. This data will allow us to build NOS-alginate hydrogels with defined NO release profiles for application in cell biology to test the effect of sustained NO release on cell proliferation and cell death on specific cell lines. Specifically, we plan to test these films and their NO release capability on adenocarcinoma cells with and without a protein (WAVE3) that is important for actin reorganization and cell migration and where nitric oxide plays an important but mysterious role. The hydrogel/NOS platform that allow us to release defined profiles and fluxes of NO during cell culture and will give us the possibility to interrogate the role of NO on the balance of cell proliferation and cell death in these cell lines.

A-040

Clinical Performance and Method Comparison For NOVA Lite HEp-2 ANA Kit

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Background: The presence of anti-nuclear antibodies (ANA) in patients with systemic lupus erythematosus (SLE) and other connective tissues diseases can be recognized by indirect immunofluorescent assay (IIF). The term "anti-nuclear antibodies" describes a variety of autoantibodies directed at various cellular compartments including nuclear constituents, components of the nuclear envelope, mitotic spindle apparatus, cytosol, cytoplasmic organelles and cell membranes. This study aimed to assess the clinical sensitivity and specificity of the NOVA Lite HEp-2 ANA Kit and Kallestad HEp-2 Cell line substrate kit (Bio-Rad). **Methods:** A total of 421 clinical characterized samples (systemic lupus erythematosus (SLE, Hospital Clinic in Barcelona) (n=105), induced lupus (n=24), rheumatoid arthritis (n=40), scleroderma (n=24), dermatomyositis (n=14), Sjogren's syndrome (SjS) (n=14) and apparently healthy individuals (n=200)) were evaluated for this study. Samples were tested on NOVA Lite HEp-2 ANA Kit (INOVA Diagnostics) and Kallestad HEp-2 Cell line substrate kits (Bio-Rad). Resulting patterns and reactivity grades on respective slides using a manual microscope was captured by a trained technologist. **Results:** Between the NOVA Lite and Kallestad kits, a total agreement of 82.2% was found. The clinical performance of the NOVA Lite HEp-2 ANA Kit and the Kallestad HEp-2 Cell line substrate is included in the tables below.

Table 1 Clinical Performance for NOVA Lite Hep-2 ANA and Kallestad HEp-2 Cell line substrate Kit

	Sensitivity % (95% CI)		Specificity % (95% CI)
	SLE	SLE + Scleroderma + SjS	
Inova Diagnostics Inc.	82.9 (74.5-88.9)	79.9 (72.4-85.5)	77.6 (71.5-82.6)
BioRad	80.0 (71.4-86.5)	73.4 (65.6-80.0)	70.1 (63.6-75.8)

Table 2 Pos/Neg agreement between Inova and Biorad substrates

Method Comparison (N=421)		NOVA Lite HEp-2 ANA Kit			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate BioRad Kallestad Hep-2	Negative	192	40	232	NPA: 82.8 (77.4 – 87.1)
	Positive	35	154	189	PPA: 81.5 (75.3 – 86.4)
	Total	227	194	421	TPA:82.2 (78.19-85.84)

NPA=Negative Percent Agreement, PPA=Positive Percent Agreement, TPA=Total Percent Agreement

Conclusion: Data shows good clinical performance for Inova with the NOVA Lite HEp-2 ANA Kit showing higher sensitivity and specificity compared against BioRad Kallestad HEp-2 Cell line substrate Kit. The qualitative agreement, as well as pattern agreements between both methods was >80% and >90% grade agreement for positive samples.

A-041

Comparison of the automated EXENT® MALDI TOF MS system for the qualitative assessment of monoclonal immunoglobulins in urine

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Background: The advantages of MALDI TOF MS based methods for the identification and quantification of monoclonal immunoglobulins has been highlighted in recent international Myeloma Working Group Guidelines. To date methods have focused on using serum as the biological matrix for identifying monoclonal immunoglobulins. Here we report the use of the EXENT system for the identification of monoclonal immunoglobulins directly from aliquoted urine thus eliminating any pre-analytical manual sample handling. **Methods:** Samples were prepared using the EXENT system (The Binding Site Group Ltd, UK): Modified polyclonal antibodies (anti-IgG, -IgA, -IgM, -total-κ and -total-λ) were covalently attached to paramagnetic microparticles. Patient samples were incubated with the microparticles, which were then washed, and eluted (20mM TCEP in 5%(v/v) acetic acid) and spotted onto MALDI plates with HCCA matrix. Spectra were generated using the MALDI-TOF-MS system. All samples were run using total κ and total λ beads with a select number run using anti-IgG, -IgA, and -IgM beads.A comparison of urine EXENT to urine IFE results was performed on 102 clinical samples. **Results:**Analysts were blinded to all IFE results prior to reporting EXENT results. Agreement between EXENT and IFE was 95% for samples with a monoclonal light chain (97/102). The 5 samples out of the 102 that were not in agreement contained a monoclonal light chain peak identified by EXENT that was not observed by IFE. Furthermore, a total of 5 out of the 102 samples had possible glycosylation modifications that were detected by EXENT. Samples reflexed to anti-IgG, -IgA, and -IgM beads showed 100% agreement with IFE heavy chain isotype calls. Urine samples with high polyclonal background but no monoclonal immunoglobulin were also in 100% agreement between EXENT and IFE.

Conclusion: Here we describe expansion of the EXENT system for the detection of monoclonal immunoglobulin in urine, showing 95% concordance to existing IFE assays. The EXENT system was able to identify potential post-translational modifications that are not identifiable by IFE. The analytical workflow does not require any pre-concentration significantly improving laboratory handling of these samples.

A-042

Precision Study with a commercial urine control on AUTION EYE TM AI-4510 Urine Sediment Analyzer

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

Methodology: A precision study was performed per CLSI EP05-A3 *Evaluation of Precision of Quantitative Measurement Procedures*. Three different lots of Bio-Rad qUAntify Plus control level 2 were tested on three AI-4510 analyzers twice a day in triplicate over a 10-day period.

Validation: RBC at a cell concentration of 150 - 170 cells/μL and WBC at a concentration of 95 - 146 cells/μL had CV < 15 % for repeatability, between run, between day, between instrument and reproducibility.

Conclusion: Precision for RBC and WBC was consistent with a CV less than 15% with three lots of Bio-Rad qUAntify Plus control when analyzed on three different AI-4510 urine sediment analyzers over 10 days, twice a day in triplicate.

Figure 1:

RBC		Repeatability		Between Run		Between Day		Between Instrument		Reproducibility	
Lot	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	152.5	11.9	7.8%	7.6	5.0%	0.0	0.0%	7.6	5.0%	16.0	10.5%
2	168.9	13.5	8.0%	4.3	2.5%	7.1	4.2%	8.7	5.2%	18.0	10.7%
3	150.0	12.1	8.0%	1.4	0.9%	0.0	0.0%	6.1	4.1%	13.6	9.1%
WBC		**Repeatability**		**Between Run**		**Between Day**		**Between Instrument**		**Reproducibility**	
Lot	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	95.6	7.3	7.7%	4.3	4.5%	2.9	3.0%	9.4	9.9%	13.0	13.6%
2	145.7	9.5	6.5%	3.8	2.6%	3.4	2.3%	18.0	12.4%	21.0	14.4%
3	123.2	7.8	6.4%	0.0	0.0%	0.9	0.7%	9.9	8.0%	12.7	10.3%

A-043

Detection of CLIFT with NOVA View®Automated Fluorescence Microscope

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Background: Automated interpretation of indirect immunofluorescent (IIF) assays have the potential to improve reproducibility while improving workflow and efficiency. The purpose of this study was to compare the performance of NOVA View®, a computer-aided automated fluorescence microscope and interpretation system with the traditional manual method for *Crithidia luciliae* Immunofluorescence Test (CLIFT).

Methods: The study included 766 samples; clinically characterized SLE (n=391) and relevant disease controls (n=375). All samples were tested on the NOVA Lite® DAPI dsDNA (*Crithidia luciliae)* Kit, (Inova Diagnostics, USA). Correlation between endpoint titer by manual and digital reading versus single well titer (SWT), was evaluated using 7 anti-dsDNA clinical samples. Reacted slides were analyzed by both manual fluorescence microscope and NOVA View. Results obtained by NOVA View software and interpretation of digital images on the NOVA View computer monitor were compared to those obtained with manual microscopy.

Results:NOVA View digital image reading, manual reading, and software generated results showed high levels of agreement. Corresponding sensitivity/specificity values by each method for the disease and control samples were also captured (see tables).

Agreement	Manual vs. NOVA View	Manual vs. Digital	Digital vs. NOVA View
PPA (95% CI)	88.2 (83.3-91.8)	89.6 (84.9-93.0)	91.7 (87.3-94.7)
NPA (95% CI)	87.2 (84.1-89.7)	96.5 (94.6-97.8)	88.0 (85.0-90.4)
TPA (95% CI)	87.5 (84.9-89.6)	94.5 (92.7-95.9)	89.0 (86.6-91.1)
Cohen's *kappa* (95% CI)	0.71 (0.66-0.76)	0.87 (0.83 - 0.91)	0.75 (0.70 - 0.80)

PPA= Positive percent agreement; NPA= Negative percent agreement; TPA= Total percent agreement; CI=Confidence Interval

N=766	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Manual	48.1 (43.2-53.0)	91.2 (87.9-93.7)
Digital	48.1 (43.2-53.0)	92.3 (89.1-94.6)
NOVA View	57.0 (52.1-61.8)	88.8 (85.2-91.6)

Seven anti-dsDNA positive samples were evaluated at two CLIA laboratory testing sites. 100% (14/14) of SWT results at the two sites were within ± 1 dilution step of that of the manual titer, and 92.9% (13/14) were within ± 1 dilution step of that of the digital titer; 100% of SWT results were within ± 2 dilution steps of that of both the manual titer and digital titer.

Conclusion: This study demonstrates that the new CLIFT module on the NOVA View automated system generates results that are equivalent to CLIFT testing by manual microscopy. Based on these results, NOVA View is an efficient alternative for labs to automate and streamline the reading and interpretation compared to the traditional fluorescent microscopy.

A-044

A Comparison of Standard Electrophoretic Methods and the EXENT® Assays in a Longitudinal Follow Up of a Patient with Evolving Multi-Clonal MGUS

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Background: Patients with monoclonal gammopathy of undetermined significance (MGUS) require long term monitoring of their paraprotein due to the risk of progression to multiple myeloma. We present results from a patient with an IgA kappa paraprotein monitored >10 years, during which time they developed additional IgG kappa and IgA kappa paraproteins as determined by standard electrophoretic techniques. We retrospectively analysed sera from this patient using the EXENT assays to determine whether the evolving IgG and IgA kappa paraproteins were present at earlier timepoints using more sensitive technology. **Methods:** Capillary zone electrophoresis (CZE) and immunofixation (IFE) were performed on Capillarys2™ and Hydrasys2 Scan™ platforms, respectively (Sebia, France). Serum free light chain (sFLC) measurement was performed using Freelite™ reagents (The Binding Site, UK) on a Cobas c501 turbidimeter (Roche, Switzerland). Samples were also prepared using the EXENT reagents, by incubating diluted serum with antisera specific for IgG, IgA, IgM, total kappa, total lambda, free kappa and free lambda conjugated to paramagnetic beads. Purified immunoglobulins were reduced and eluted. Samples were mixed with matrix, spotted onto a MALDI target plate and acquired by MALDI-TOF MS. **Results:** At presentation and first follow up, CZE demonstrated a potential clonal abnormality and immunofixation identified the primary IgA kappa paraprotein. At second follow up, the primary IgA kappa and IgG kappa paraproteins were visible by CZE and IFE, and the second IgA kappa band was just visible by IFE. No other qualitative changes were observed through subsequent follow-up using electrophoresis, even though sFLC results were later reflective of worsening renal function as indicated by increasing serum creatinine. The kappa/lambda sFLC ratio remained within the Freelite renal reference range (0.3-3.1).

However, EXENT demonstrated a reportable IgA kappa paraprotein with a mass-to-charge ratio (*m/z*) of 11657 for the 2+ light chain, along with an additional IgG kappa abnormality at *m/z* 11765 and two further IgA kappa abnormalities at *m/z* 11931 and 12037 for the 2+ light chains, at presentation. These minor clonal abnormalities persisted, becoming more apparent as paraproteins at second follow up. All 4 paraproteins remained detectable by EXENT with consistent *m/z* values for the remainder of follow up. Furthermore, EXENT identified a clear clonal kappa sFLC with the same *m/z* as one of the IgA kappa monoclonal proteins from baseline when sFLC levels and sFLC ratio were normal and this persisted throughout the seven follow-up samples.

Conclusion: In this case the EXENT assays detected developing paraproteins earlier than standard electrophoretic techniques and was able to identify additional abnormalities which had not been visible during routine follow-up. The ability to monitor the monoclonal FLC through their unique *m/z* enabled detection and monitoring across this sample series of monoclonal kappa sFLC in the context of an increasing polyclonal sFLC background due to deteriorating renal function. The sensitivity of EXENT allows more careful and accurate monitoring of developing paraproteins, and, has potential to give increased confidence around reporting of patients on long term monitoring.

A-045

A RUO monoclonal antibody assay for IgG4 Subclass detection on ARCHITECT c8000 clinical chemistry analyzer

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Introduction: IgG4 is one of the four IgG subclasses with the lowest concentration in normal human serum. Testing of IgG subclasses helps to determine treatment options if IgG concentration is outside the normal range either in recurrent infections or inflammatory disease states. Due to the low analyte levels of IgG4 in human serum, a latex enhanced microparticle based immunoturbidimetric assay is the preferred clinical chemistry (CC) methodology. Selection of the antibodies for an IgG4 assay is very challenging, because the amino acid sequence homology between the four subclasses is 95%. Monoclonal anti-IgG4 antibody coating is preferred over polyclonal antibody coating as monoclonals provide controlled production minimizing reagent-lot-to-lot performance differences and would not need repeated animal inoculations or terminal bleeds.

Methods: Anti-human IgG4 monoclonal Abs were screened for cross-reactivity to other IgG subclasses proteins using ELISA. Monoclonal Abs with at least a 100-fold difference in ELISA cross-reactivity were chosen for EDAC coupling based coatings on submicron latex microparticles. The antibody coated microparticles were assessed for viability and analytical performance on ARCHITECT c8000 clinical chemistry analyzer.

Results: Five out of eleven monoclonal Abs showed selective cross-reactivity performance. One out of the five antibodies, when coated on particles and tested on the ARCHITECT c8000, yielded IgG4 concentration dependent absorbance responses and an active calibration curve. A method comparison slope of 0.96 and correlation coefficient of 0.97 were obtained for the monoclonal antibody assay relative to an on-market predicate polyclonal antibody IgG4 assay. The selected monoclonal antibody assay showed <3.0% CV across the assay measuring interval of 4 – 1500 mg/L. Endogenous interference assessment met desired CLSI guideline targets for clinical chemistry assays.

Conclusions: A selective IgG4 monoclonal antibody was identified that had a sufficient binding titer for human IgG4 with minimal to no cross-reactivity with the other IgG subclass molecules. As a result, a RUO monoclonal antibody IgG4 subclass assay was designed for use on ARCHITECT c8000 clinical chemistry analyzer demonstrating key performance attributes.

A-046

Technology Review and Performance Evaluation of Truvian's Automated Blood Analyzer

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Background: Truvian has developed a point of care automated blood testing analyzer capable of running clinical chemistry, immunoassay and hematology assays. The goal of creating this system was to enable point of care testing of routine analytes to be run in a single panel thereby allowing for fast actionable results to be provided to the clinician and patient. The first test panel for the Truvian's system is designed to perform a comprehensive metabolic panel, TSH and complete blood count from a low volume of blood. Here, we describe the design of the Truvian's system and present a preliminary performance evaluation for the absorbance module and cell imager module for clinical chemistry and hematology respectively.

Methods: Truvian's automated blood analyzer consists of three modules: absorbance reader, bead scanner and cell imager. The assay reagents are filled into wells within a plastic disc consumable and dried on the disc. Two plasma separation chambers are housed on the disc. An additional consumable known as the support pack holds pipette tips, the monolayer, diluents, sample dilution wells and the whole blood sample. A preliminary performance evaluation for the absorbance module and hematology module was performed. For the absorbance module testing, two dyes were used: crystal violet (475 nm) and pNP (405 nm). A 5-point dilution series was performed on each of the dyes. Linearity and precision were evaluated for the absorbance module. The hematology module was tested to confirm the system's capability of capturing in focus

images using the system autofocus algorithm and confirmed that the system is capable of producing images of white blood cells (WBCs) that are able to be classified via a 3 part differential.

Results: Results from this study demonstrate that the systems tested performed consistently in terms of linearity and precision for both crystal violet and pNP dyes. Dilution series of both crystal violet and pNP had excellent linearity with an R^2 of >0.9999. Precision of the dilution series of both crystal violet and pNP had a standard deviation of <0.01 for nearly all concentrations tested. All systems tested were able to produce >90% of RBC images that were in focus. All systems were able to produce >95% of WBC images that were in focus and able to be differentiated.

Conclusion: Results from this study demonstrate successful development of the chemistry module and hematology modules of Truvian's system. Preliminary performance evaluation of the system demonstrates excellent chemistry module and hematology module performance with consistent operation across systems.

A-047

Addition of cN-1A as a Target for Detection of Autoantibodies Associated with Inclusion Body Myositis to a Multiparameter Lineblot Immunoassay for Autoimmune Inflammatory Myopathies

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Background: Autoantibodies against cN-1A (Mup44) are primarily used as a biomarker for inclusion body myositis (IBM), they also occur with lower prevalence in systemic lupus erythematosus (SLE) and Sjögren's Syndrome (SjS). cN-1A has been included as a parameter on the multiparameter lineblot Autoimmune Inflammatory Myopathies 16 Ag et cN-1A (EUROIMMUN), allowing the detection of 17 myositis-specific and myositis-associated autoantibodies. The prevalences of anti-cN-1A autoantibodies were determined in different patient collectives and healthy blood donors

Methods: A lineblot immunoassay containing a highly purified, recombinant cN-1A expressed in *E.coli* was incubated according to the instructions. Sera of 197 patients with IBM, 48 with collagenosis (systemic sclerosis, undifferentiated collagenosis and mixed tissue collagenosis), 52 with polymyositis/dermatomyositis and 75 with SLE/SjS were screened for cN-1A autoantibodies. Additionally, 151 apparently healthy blood donors as well as a disease control collective comprising sera of 20 patients with thyroiditis, 26 with neuromuscular disorders, 24 with primary biliary cholangitis (PBC), 19 with Wegener's Disease, 17 with Coeliac disease, 2 with autoimmune liver disease (ALD) were analyzed. Signal intensities were automatically evaluated using the EUROLineScan software (EUROIMMUN) **Results:** Prevalence of cN-1A autoantibodies were 34.5% in IBM, 10.4% in collagenosis, 7.7% in polymyositis/dermatomyositis, 13.3% in SLE/SjS, 2% in the blood donor cohort and 4.6% in the disease control collective

Conclusion: The addition of cN-1A to a multiparametric lineblot immunoassay now reduces the risk of underdiagnosing IBM in patients with autoimmune inflammatory myopathies and increases the overall diagnostic sensitivity

A-048

Autoimmune Gait Disturbance Accompanying Adaptor Protein-3B2-IgG

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Background: Autoimmunity targeting the neuronal (B2) form of adaptor protein-3 (AP3), a synaptic vesicle coat protein, is accompanied by gait ataxia with a protracted course, sometimes stabilized by immunotherapy. Herein, we describe phenotypes, treatment response, and outcomes of AP3B2 autoimmunity

Methods: Archived serums (ten) and CSF (six) specimens from ten patients harboring unclassified synaptic antibodies mimicking amphiphysin-IgG on tissue-based indirect immunofluorescence assay (IFA) were re-evaluated for novel IgG staining patterns. Autoantigens were identified by Western blot and mass spectrometry. Recombinant western blots and cell-based assay (CBA) were used to confirm antigen specificity

Results: All specimens produced identical IFA staining patterns throughout mouse neural tissues, most prominently in cerebellum (Purkinje neuronal perikarya, granular layer synapses and dentate regions) spinal cord grey matter, dorsal root ganglia, and sympathetic ganglia. The antigen revealed by mass spectrometry analysis and confirmed by recombinant protein western blot and CBA was AP3B2 in all patients. The median symptom-onset age was 42 years (range: 24-58). Clinical information was available for nine patients, all with subacute onset and rapidly progressive gait ataxia. Neurological manifestations were: myeloneuropathy (three); peripheral sensory neuropathy (two); cerebellar ataxia (two); spinocerebellar ataxia (two). Five patients received immunotherapy: none improved, but three stabilized over the follow-up period (median: 36 months).Two patients suffering from cancer died

Conclusion: All patients with AP3B2-reactive IgG had a neurologic phenotype of gait dysfunction secondary to ataxia but differed in treatment response and outcome. Ideally, testing for AP3B2 IgG would be incorporated in laboratory testing in case of suspected autoimmune ataxia

A-049

Cell-based Immunofluorescence Test Applying Recombinant Laminin 332 for the Serological Diagnosis of Pemphigoid

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Background: Anti-laminin 332 mucous membrane pemphigoid (MMP) is an autoimmune subepidermal blistering disease with predominant mucosal involvement. A subgroup of patients with MMP exhibits autoantibodies against laminin 332 (Lam332), a glycoprotein important for structural integrity of the dermal-epidermal junction in the skin. Lam332 is a heterotrimeric protein consisting of three laminin chains, α3, β3, and γ2. Diagnosis of anti-Lam332 MMP is important since nearly 30% of these patients develop solid cancers. Yet, no standardized detection system for anti-Lam332 serum antibodies is widely available. Here, a cell-based indirect immunofluorescence test (IIFT) was validated

Methods: The Anti-Laminin 332 (Lam332) IIFT (EUROIMMUN) is based on the eukaryotic expression of recombinant Lam332 (heterotrimer and single chains) in HEK293 cells. The test was probed with anti-laminin 332 MMP patient sera (n=93; five with follow-up samples; 13 from patients with concomitant malignancy), as well as sera from patients with anti-laminin 332-negative MMP (n=153), bullous pemphigoid (n=20), pemphigus vulgaris (n=20), noninflammatory dermatoses (n=22) and from healthy blood donors (n=100) **Results:** The sensitivities with the laminin 332 heterotrimer and the individual α3, β3, and γ2 chains were 77%, 43%, 41% and 13%, respectively, with specificities of 100% for each substrate.When the anti-IgG4 secondary antibody was supplemented with an anti-IgG+IgG4 conjugate (EUROIMMUN), the sensitivity for detection of autoantibodies to the heterotrimer increased to 84%. Anti-laminin 332 reactivity paralleled disease activity. All thirteen patients with malignancy were anti-laminin 332 positive

Conclusion: The Anti-Laminin 332 IIFT will facilitate the serological diagnosis of anti-Lam332 MMP and may help to identify patients at risk of a malignancy

A-050

Validation of Respiratory Gases Measurements to Assist with Ex-Vivo Lung Perfusion in Support of Expanding the Lung Transplant Program

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Introduction: Ex-Vivo lung perfusion helps salvage would be rejected lungs in order to expand donor pool for lung transplantation. Perfusion of the lung helps rescue the lungs and maintain them in a perfused state until transplanted. Serial measurements of blood gases are essential for pulmonary physiological assessment of the

lung. This project describes the validation of a point of care devise in support of the Ex-vivo lung perfusion and transplant program at a tertiary care university hospital. **Methods:**Perfusion solution (STEEN) and perfusion samples (n=21) obtained at different times from both left arterial outflow (LA) and pulmonary arterial inflow (PA) were used to assess validity for accuracy, imprecision (four replicates assessed at two different levels for both sample types), linearity, sensitivity, specificity (STEEN solution samples collected following administration of Heparin, Solumedrol, and Zosyn, as per surgical intervention protocol, as well as correlation studies. Blood gases and pH were measured on all study samples using i-STAT point of care instrument (Abbott Point of Care, NJ, USA) and the comparator laboratory-based blood gases instrument (ABL-90 Radiometer, ABL Diagnostics, GA, USA). STEEN perfusion solution was obtained and used to test linearity and to assess for matrix effects. Serial samples are collected at one hour intervals up to 12 hours were used to establish measurement range. Experimental lungs perfusion experiments were conducted. Retrospective review of blood gases following transplant (and success of the transplant lung was assessed to validated performance of the POCT testing validation of lungs vitality and recovery.**Results:**Imprecision was less than 10% for all measured parameters and sample types. Limits of measurements were 6.6 for pH, 11 for pCO_2 and 108 for pO_2. Acceptable performance within allowable errors were <10% for a range of 6.6 to 7.54 for pH, 11 to 25 for pCO_2, and 108 to 418 for pO_2. No interferences were observed for Heparin and Solumedrol, however Zoysn exhibited interference. Serial pulmonary physiological assessment (oxygenation) PA and LA PaO_2, PaO_2/FiO_2 ratio were consistent with clinical outcomes.**Discussion:**The performance if the point of acre in the assessment of Ex-Vivo lung perfusion was found to be adequate. Technical validation study were within acceptable limits. Retrospective clinical correlation for transplanted lung performance was assessed. Decisions made based on the point of care biochemical data were appropriate given the successful outcome of the transplant procedure. In conclusion, use of point of care testing aids in assessment of Ex-Vivo lung perfusion.

A-051

Performance Evaluation of LumiraDx SARS-CoV-2 Ag Test for Rapid Point of Care Testing

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Background: The LumiraDx SARS-CoV-2 Ag test has FDA Emergency Use Authorization (EUA) for the identification of SARS-CoV-2 infection in individuals within 12 days post symptom onset. The test methodology utilizes a microfluidic immunofluorescence approach for the direct and qualitative detection of the nucleocapsid protein (NP) antigen in a nasal swab specimen of a patient suspected of having COVID-19. The assay is rapid, providing a result in ~12 minutes. Variation in performance is commonly observed among rapid point-of-care tests (POCTs) for the detection of SARS-CoV-2 antigens; therefore, there is a need for evaluating the performance of these new assays. Here, we report the performance of the LumiraDx SARS-CoV-2 Ag test.

Methods: Performance evaluation of the LumiraDx 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=71) inclusive of both symptomatic and asymptomatic patients. Of these, 16 samples from patients confirmed to be PCR positive for SARS-CoV-2 infection, were used to assess the sensitivity of the assay. Fifty-five 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 LumiraDx antigen assay observed was of 88% (95% CI: 62%-98%) and 100% (95% CI: 93%-100%), respectively. Additionally, the assay exhibited a 97% agreement with the PCR-based test, with an average PCR Ct count of ~22 for the SARS-CoV-2 positive samples. Only two specimens of 71 rendered discordant results and both results were in asymptomatic patients. The resulting PPV and NPV were 100% (95% CI: 77-100%) and 96% (95% CI: 88%-100%).

Conclusion: Antigen detection by the LumiraDx test was less sensitive in a mixed symptomatic and asymptomatic cohort than the PCR-based test, but offers a 100% specificity with the possibility of rapid results in a more inexpensive manner.

A-052

Increased Sensitivity for PR3 - ANCA Using a Novel Chemiluminescence Immunoassay

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IntroductionANCA-associated vasculitides (AAV) encompass granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA). In GPA, anti-neutrophil cytoplasmic antibodies (ANCA) are mainly directed against proteinase 3 (PR3), while anti-myeloperoxidase (MPO) antibodies are primarily associated with MPA and EGPA. Sensitive and accurate PR3- and MPO-ANCA detection would aid early diagnosis and improve monitoring of disease activity in patients with AAV. Here, we investigated the performance of the first commercial chemiluminescence immunoassays (ChLIA) that uses human recombinant PR3 as antigen.

MethodsPerformance of the Anti-PR3 ChLIA, processed on the RA Analyzer 10 (EUROIMMUN), was assessed using sera from 205 AAV patients (101 GPA, 104 MPA) and 162 disease control patients. Results were compared to that obtained by the established Anti-PR3-hn-hr ELISA and Anti-MPO ELISA (EUROIMMUN). Borderline results were considered negative.

ResultsSensitivities for PR3-ANCA using the Anti-PR3-hn-hr ELISA, the Anti-PR3 ChLIA and the Anti-MPO ELISA were 86%, 90% and 8%, respectively in GPA patients and 0%, 6% and 97%, respectively, in MPA patients. Although PR3-ANCA have the highest prevalence in GPA, they are also described in MPA. The Anti-PR3 ChLIA showed a higher specificity for PR3-ANCA than the Anti-PR3-hn-hr ELISA in AAV samples (99% vs. 98%)

ConclusionThe novel Anti-PR3 ChLIA outperformed the ELISA in sensitivity and specificity, thus providing a valuable tool for AAV diagnosis and ANCA target antigen differentiation. Future studies with larger cohorts and samples from AAV patients under treatment are necessary to further analyse assay performance and the relevance of monitoring PR3-ANCA levels in follow-ups.

A-053

Novel Line Blot Differentiates Between IgG Antibodies Against Three SARS-CoV-2 Antigens & Against Further Seasonal Coronaviruses

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Background: The determination of specific antibodies enables confirmation of ongoing or past SARS-CoV-2 infection and differentiation from infections with seasonal coronaviruses (HCoV). We evaluated a novel immunoblot (EUROIMMUN) for the parallel determination of IgG antibodies against the SARS-CoV-2 antigens S1, S2 and nucleocapsid protein (NP) and against four HCoV antigens (HCoV-HKU1-NP, HCoV-OC43-NP, HCoV-NL63-NP, HCoV-229E-NP) **Methods:** Prevalences of antibodies against SARS-CoV-2 and HCoV were determined by investigating 369 sera.

Diagnostic sensitivity and specificity were determined using a COVID-19 validation panel. 78 sera from 24 PCR-confirmed COVID-19 patients were used to investigate the prevalence of anti-SARS-CoV-2 antibodies relative to the infection phase. Cross-reactivity was evaluated by analysing samples from 199 patients with different infections. Incubated line blots were automatically evaluated using the EUROLineScan software (EUROIMMUN). The results for SARS-CoV-2 and HCoV were evaluated as positive if ≥2 SARS-CoV-2 antigen bands and ≥1 HCoV band showed a positive intensity value, respectively

Results: The prevalence for antibodies against SARS-CoV-2 and HCoV antigens were 0.3% and 74.8%, respectively. Sensitivity and specificity for the detection of anti-SARS-CoV-2 antibodies were 100% each. The prevalence of anti-SARS-CoV-2 antibodies in patients increased from the early (80%) to the intermediate infection phase (96.3%). The majority of patients developed antibodies against both S1 and NP antigens of SARS-CoV-2. Cross-reactivities were not observed

Conclusion: The novel line blot enables parallel investigation of the immune response against different SARS-CoV-2 antigens with high sensitivity and specificity. Combined detection of antibodies against different coronaviruses in one reaction can be performed with fully automated incubation and evaluation

A-054

Simple and fast assay for apolipoprotein E phenotyping and glycotyping: discovering isoform-specific glycosylation in plasma and cerebrospinal fluid

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Background: The mechanisms of how the *APOE* Ɛ4 allele (*APOE4)* increases the risk of Alzheimer's disease (AD) pathology have not been fully elucidated. In cerebrospinal fluid (CSF), apoE is heavily glycosylated. Our objective was to determine the impact of APOE genotype on the relative abundance of apoE protein isoforms and their specific glycosylation patterns in CSF and plasma, via a newly developed mass spectrometric immunoassay (MSIA) assay. **Methods:** Total glycosylation and isoform-specific glycosylation were analyzed in plasma and CSF from a group of non-demented older individuals (n=22), consisting of homozygous Ɛ3 and Ɛ4 or heterozygous Ɛ3/Ɛ4, Ɛ2/Ɛ3, or Ɛ2/Ɛ4 carriers. The glycan structures were further confirmed after treatment with sialidase. **Results:** In heterozygous individuals, the apo E3/E2, E4/E2 and E4/E3 isoform ratios were all significantly lower in plasma compared to CSF. For all individuals, a single O-linked glycan was observed in plasma, while two glycans (of the same type) per apoE were observed in CSF. The ratio of glycosylated to total apoE was greater in CSF compared to plasma for all apoE isoforms. In plasma and CSF, a trend of decreasing glycosylation was observed from apoE2>apoE3>apoE4. The difference in the percentage of secondary glycosylation in CSF was significantly greater in apoE4 compared to the other isoforms. **Conclusion:** The new MSIA apoE assay robustly distinguishes among apoE isoforms and glycoforms in plasma and CSF. ApoE4 is the predominant isoform and least glycosylated in CSF. Assessing apoE isoform-specific glycosylation by MSIA may help clarify brain apoE metabolism and AD risk.

A-055

Identification of DAGLA as an Autoantibody Target in Cerebellar or Hippocampal Degeneration

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Background: Presence of autoantibodies against Purkinje cell antigens τσ φρεθυεντλψ χοννεχτεδ with cerebellar ataxia. Diacylglycerol lipase alpha (DAGLA) is a newly identified cell membrane protein predominantly expressed in Purkinje and hippocampal pyramidal cells, which influences synaptic signalling

Methods: Sera of five patients suffering from neurological aberration (P1-P5) were subjected to comprehensive autoantibody screening by indirect immunofluorescence assay (IFA). Immunoprecipitation with lysate of cerebellum followed by mass spectrometry was used to identify the autoantigen. Antigen identification was verified by Western blot, IFA and in several immunoassays. 115 sera with a similar staining pattern as P1-P5 without known autoantibody reactivity and 51 negative control sera without a specific reaction in IFA with neuronal tissues were screened for anti-DAGLA antibodies.

Results: In IFA, P1-P5 showed IgG reactivity with the molecular layer of the cerebellum. Patient sera immunoprecipitated DAGLA, as detected by Coomassie-stained SDS-PAGE followed by mass spectrometry or Western blot. Immunolabelling of cerebellar sections with a monospecific rabbit anti-DAGLA antibody revealed the same staining pattern as with the patients' sera. Anti-DAGLA reactivity of sera from P1-P5 and seven additional sera was confirmed by IFA with the recombinant protein. Control sera were negative. In a neutralization experiment, recombinant DAGLA abolished the tissue reactivity of the samples, thus verifying the correct antigen identification. **Conclusion:**We identified DAGLA as novel Purkinje cell antigen targeted by patient sera with similar IgG reactivity on cerebellar tissue. Antibodies against DAGLA represent novel biomarkers for the diagnosis of autoimmune diseases associated with cerebellar or hippocampal degeneration.

A-056

Novel Anti-ATP4B ELISA to Aid the Diagnosis of Autoimmune Gastritis

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Background: Antibodies against parietal cell antigens (APCA) are the established serological marker for diagnosis of autoimmune gastritis (AIG). Also, APCA are present in 80 - 90% of patients with pernicious anaemia (PA), which is often associated with end-stage AIG. However, with respect to specificity, it must be taken into account that APCA are also prevalent in patients with endocrinopathies, such as Diabetes mellitus type I, Hashimoto's thyroiditis and Graves' disease, and to 10-20% in healthy persons. In this study, we investigated the performance of a newly developed ELISA (EUROIMMUN) based on a modified parietal cell antigen derived from the beta-subunit of the gastric H+/K+-ATPase (ATP4B). **Methods:**Sensitivity and speci ficity of the novel Anti-ATP4B ELISA (IgG) were examined by analysing serum samples from 29 AIG patients and 100 healthy blood donors. The results were compared to those obtained using an established standard Anti-PCA ELISA based on H+/K+-ATPase. **Results:**The novel ELISA revealed a higher specificity (99%) compared to the conventional ELISA (93%). In the AIG cohort, both assays demonstrated a high concordance rate, thus a similarly high level of sensitivity (96.6% versus 100%). **Conclusion:**We have demonstrated here that further customisation of the established antigen improves the performance of ELISA-based AIG diagnostics, resulting in increased specificity. Patients with symptoms leading to suspicion of AIG could profit from this enhanced diagnostic precision. Therefore, the performance of the new assay will be examined in a broader clinical setting to evaluate its diagnostic accuracy in the routine detection of autoantibodies in AIG and PA.

A-057

Increased Sensitivity for Detection of IgA Autoantibodies Against Tissue Transglutaminase Using a New Chemiluminescence Immunoassay

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Background: Coeliac disease (CD, also named gluten-sensitive enteropathy) is triggered by consumption of gluten in genetically predisposed individuals. CD is a systemic autoimmune disease, which may affect different organ systems. Its manifestation as a recurrent skin disease characterised by supepidermal blisters is named Duhring's dermatitis herpetiformis (DDH). The detection of autoantibodies against tissue transglutaminase (endomysium, tTG) confirms a diagnosis of symptomatic CD, indicates latent CD and potential CD in patients at risk. We compared the performance of two test systems from EUROIMMUN for detection of autoantibodies against tTG. **Methods:**Performance of the established Anti-Tissue Transglutaminase ELISA (IgA) and the new chemiluminescence immunoassay Anti-tTG-ChLIA (IgA), processed on the RA Analyzer 10 (EUROIMMUN), was assessed using sera from 102 patients with CD and from 43 patients with DDH. Specificity was evaluated in a panel consisting of 166 sera from patients with rheumatoid arthritis (n=30), Sjögren's syndrome (n=21), systemic lupus erythematosus (n=30), systemic sclerosis (n=15), Crohn's disease (n=10), ulcerative colitis (n=10), diabetes mellitus type 1 (n=30) and autoimmune thyroid diseases (n=20). **Results:**Both tests yielded a high sensitivity of 99% in sera from CD patients. In samples from DDH patients, the Anti-tTG-ChLIA (IgA) showed a higher sensitivity compared to the ELISA (88.4% vs. 81.4%). The specificity was 98.2% for the ChLIA and 97.6% for the ELISA. **Conclusion:**By yielding a performance that was comparable to the established ELISA in CD patients, and even more sensitive in sera from DDH patients, the Anti-tTG-ChLIA (IgA) provides a valuable tool supporting diagnosis of GSE.

A-058

Novel Antigen Assay for Acute Diagnostics of COVID-19 in the Laboratory

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Background: COVID-19 is a pandemic that has led to millions of infections and hundreds of thousands of deaths worldwide. It is caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which can result in severe symptoms and fatal outcome. During viraemia, COVID-19 diagnostics is based on the detection of SARS-CoV-2 RNA by reverse transcription polymerase chain reaction (RT-PCR) or of viral protein by various immunological methods. Here, we present evaluation data for a novel, fully automatable SARS-CoV-2 antigen ELISA for use in diagnostic laboratories **Methods:** The SARS-CoV-2 Antigen ELISA (EUROIMMUN) was developed for the semiquantitative determination of SARS-CoV-2 nucleocapsid (N) protein in human samples. The assay´s clinical performance was evaluated in 94 nasopharyngeal swabs, of which 59 originated from symptomatic COVID-19 patients and 35 from influenza patients, all precharacterised by PCR. Correlation of results obtained by ELISA and PCR (EURORealTime SARS-CoV-2, EUROIMMUN) was analysed by testing 101 naso- and oropharyngeal swab samples by both techniques in parallel, followed by comparison of PCR cycle threshold (Ct) values and ELISA ratios. To exclude cross-reactivity and interference, the assay was run against recombinant N protein of different human pathogenic coronaviruses as well as other potentially interfering viruses, bacteria and reagents

Results: Analysis of clinical samples revealed 93.2% (95% CI: 83.5-98.1%) positive agreement and 100% (95% CI: 90.0-100%) negative agreement of the SARS-CoV-2 Antigen ELISA with molecular diagnostic testing. In the correlation panel, the results obtained by SARS-CoV-2 Antigen ELISA showed high positive agreement (84-100%) with PCR Ct values up to 34. Spearman rank-order analysis revealed a significant negative correlation between both methods ($rs = -0.875$, $p<0.001$). Except for SARS-CoV-1, we did not find evidence of cross-reactivity or interference **Conclusion:** Based on high correlation with PCR test results and excellent performance characteristics, the novel SARS-CoV-2 Antigen ELISA can support acute diagnostics of SARS-CoV-2 infections, particularly during COVID-19 outbreaks. Infectious patients with a high viral load can be detected reliably using this ELISA.

A-059

Quantification and Characterization of the SARS-CoV-2 Antibody Response by ELISA and Mass-Spectrometry in Naturally Infected and Vaccinated Healthcare Workers

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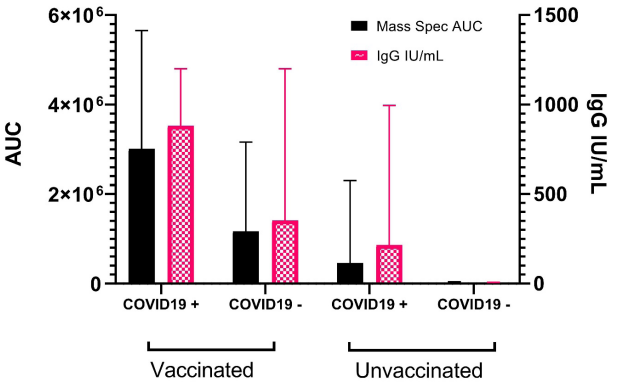
Background: Understanding the magnitude, quality and longevity of the serological response to SARS-CoV-2 spike glycoprotein arising from natural infection and vaccination is critical to pandemic control. Mass spectrometry offers novel insight into the nature of these serological responses. **Methods:** Relationships between a positive SARS-CoV-2 immunoassay serology result and mass spectrometry was determined in 43 UK healthcare workers, stratified by vaccination status and prior COVID19 illness: COVID19 (n=12, PCR positive), COVID19 followed by vaccination (n=10, Pfizer-BioNTech single dose), vaccination only (n=10) or neither COVID19 nor vaccination (n=11). Serological responses were measured using qualitative anti-IgG/A/M SARS-CoV-2 and quantitative anti-IgG ELISA assays (The Binding Site Group Ltd.). Both assays targeted the S-glycoprotein; the latter calibrated to the WHO 1st international standard (20/136). Immunocapture of serum immunoglobulins was performed using S-glycoprotein conjugated paramagnetic beads. Eluents were reduced and acquired by MALDI-TOF-MS (Bruker). Positive spectra were analysed focusing on Immunoglobulin light chains (10900-12500m/z). **Results:**The serological responses of each of the four groups was clearly differentiated by either method (Fig.1). The unvaccinated and COVID19 -ve patients had minimal response, while those who had natural infection followed by vaccination had the greatest response. A single dose of Pfizer-BioNTech generated antibody levels similar to those who have recovered from COVID19. MALDI-TOF characterisation of the antibody response identified a predictable kappa/lambda usage, with the presence of multiple dominant oligoclonal peaks. This pattern was the same regardless of whether seropositivity arose from vaccination or natural infection. Within the vaccinated groups two peaks were consistently present in addition to the expected light chain spectra; we hypothesise these to be C1q complement fragments based on their molecular weight. **Conclusion:** ELISAs and mass spectrometry assays using the SARS-CoV-2 S-glycoprotein can discriminate between naive and infected / vaccinated individuals. Furthermore, mass spectrometric analysis illustrates the diversity of the antibody responses in clinically relevant circumstances.

Quantification of the SARS-CoV-2 Antibody Response by ELISA and Mass-Spectrometry in Naturally Infected and Vaccinated Healthcare Workers



A-060

A New Approach to Discovering Surface Epitopes with Potential Antigenic Properties Using Molecular Imprinting

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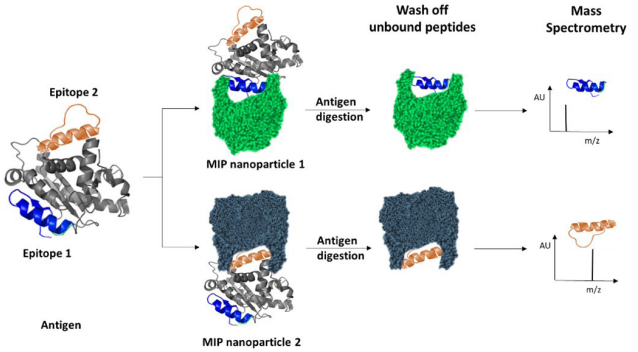
Background: The surface epitopes of a biomarker are key to the development of effective in-vitro diagnostic assays, since this is where antibody binding occurs. Mapping these surface epitopes using traditional techniques such as x-ray crystallography and oligopeptide scanning can be time consuming and expensive, plus success is not guaranteed. These techniques are more often utilized for the analysis of therapeutic antibodies as opposed to diagnostic antibodies, which are typically developed against a whole protein, or a peptide sequence already identified within the literature. A new rapid and cost effective method for identifying surface epitopes with potential antigenic properties prior to binder synthesis has been developed. In this method, molecular imprinting is used to identify multiple surface epitopes, and the peptides are then sequenced using mass spectrometry.

Methods: The target protein was brought into contact with a selection of monomers, which were polymerized to create a protein-polymer complex. The protein was then digested to leave a peptide-polymer complex in which the identified epitope(s) are 'captured' within the polymer pocket. The peptide-polymer complexes were then isolated, the peptide was removed, and later sequenced using mass spectrometry. The peptide sequence(s) identified correspond to potential binding sites for a binding partner such as an antibody, aptamer, affimer or molecularly imprinted polymer. The imprinting process was completed in 1 week.



Results: Several surface epitope peptide sequences were identified. They were compared with those already reported within the literature, and a handful were shown to be previously unknow peptide sequences.

Conclusion: This new method for epitope discovery had been shown to identify previously unknown surface epitopes on the target biomarker, which can then be utilized to create an anti-peptide antibody, apatamer, affimer of molecularly imprinted polymer for diagnostic applications. Other potential applications include the preliminary screening of therapeutic antibodies and to support vaccine design.

A-061

Investigation of Macro-Troponin in Patients Receiving Immune Checkpoint Inhibitor Therapy

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Background: Myocarditis is a rare, but potentially fatal complication of immune checkpoint inhibitor (ICI) therapy. Due to the severity of ICI-induced cardiotoxicity, it is important to identify patients at risk of cardiotoxicity early during immunotherapy to help guide treatment decisions. Cardiac biomarkers, especially troponin, play a key role in myocarditis diagnosis and management. Macro-troponin has been reported to cause falsely increased or decreased troponin levels. We investigated macro-troponin interference in ICI-patients using the Alinity STAT hs-TnI and Elecsys TnT-hs immunoassay following treatment with PEG and protein G. **Methods:** Cardiac troponin (cTn) was measured following PEG (2.5% and 12.5%) and protein G treatment. Deidentified residual serum samples from 10 ICI-patients, 10 control patients with cTnI values > 99th percentile, and 2 patients with cTnI values < 2 ng/L were analyzed using the Alinity STAT hs-TnI and Elecsys TnT-hs immunoassay. Samples from control patients with cTnI values > 99th percentile were used to determine the range of troponin recovery post PEG or protein G treatment in samples not suspected of falsely elevated troponin. cTnI and cTnT recovery was calculated by comparing cTn levels before and after immunoglobulin depletion with protein G and after volume correction using TSH (Alinity TSH immunoassay) as an internal standard. Recovery of cTnI and cTnT (as percentage) was calculated as follows: (cTn after immunoglobulin depletion / cTnI before immunoglobulin depletion) x (TSH before immunoglobulin depletion / TSH after immunoglobulin depletion). Recovery of cTnI and cTnT (as percentage) was also calculated for 2.5% PEG and 12.5% PEG treatment. **Results:** In control patients with cTnI values > 99th percentile, protein G showed a median cTnI and cTnT recovery of 75.20% (2.5th percentile: 39.98%, 97.5th percentile: 84.82%) and 79.16% (2.5th: 69.52%, 97.5th: 97.62%), respectively. Treatment with 2.5% PEG showed a median cTnI and cTnT recovery of 104.8% (2.5th: 96.71%, 97.5th: 125.2%) and 125.8% (2.5th: 104.6%, 97.5th: 155.3%), respectively. In addition, treatment with 12.5% PEG showed a median cTnI and cTnT recovery of 34.24% (2.5th: 24.07%, 97.5th: 53.11%) and 98.05% (2.5th: 39.74%, 97.5th: 144.1%), respectively. We considered a cTnI of < 40% following protein G treatment and a cTnI of < 20% following 12.5% PEG treatment to define the presence of macro-troponin. In ICI-patients, the range of troponin (cTnI) was 2.9 – 1650.5 ng/L. The recovery ranged from 9.3 – 100%, 15.7 – 158.6%, and 7.9 – 137.9% following treatment with protein G, 2.5% PEG, and 12.5% PEG, respectively. Linear regression analysis showed poor agreement between protein G and 2.5% PEG or 12.5% PEG treatment with an R^2 value of 0.035 and 0.36, respectively. Overall, in patients with cTnI > 99th percentile 1/5 showed macro-troponin based on a cut-off of < 40% recovery for protein G. In addition, 2/5 ICI-patients showed macro-troponin based on a cut-off of < 20% recovery for 12.5% PEG treatment. **Conclusion:** Treatment with protein G, 2.5% PEG, and 12.5% PEG were not equivalent. Macro-troponin was identified in ICI-patients following treatment with protein G and 12.5% PEG. Further studies are needed on the presence of macro-troponin in ICI patients.

A-062

Development and Optimization of Equilibrium Dialysis ID-LC/MS/MS Procedure for High-throughput Routine Measurement of Free Thyroxine in Serum

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Background: Free thyroxine (FT4) measurements are critical to evaluate thyroid function and diagnose thyroid diseases. Approximately 18 million FT4 tests are performed in the USA every year with the majority of measurements performed by using immunoassays (IAs). However, concerns about the accuracy and reliability of FT4 assays used in patient care exist. An internationally recognized, highly accurate and precise reference measurement procedure (RMP) for FT4, which is based on a well-defined equilibrium dialysis (ED) procedure, is now available. However, this RMP is not designed and intended for use with patient or study samples. The aim of the present study is to develop and optimize a high-throughput routine FT4 assay that is based on ED LC-MS/MS and traceable to the RMP. **Methods:** FT4 in serum was separated from protein-bound T4 at 37.0 °C in 96-well micro-ED plate. Several types of micro-ED plates, including Harvard Apparatus and HTDialysis were tested. The ED conditions were followed according to the CLSI C45-A guideline. An aliquot of 150 uL dialysate samples with FT4 was collected after the ED. FT4 in the dialysate was isolated from the sample with ethyl acetate liquid-liquid extraction before LC-MS/MS analysis. Chromatographic separation of T4 from the sample matrix was achieved on a C18 UPLC column eluted with a gradient mobile phase of methanol and water with 0.1% formic acid. FT4 in the samples was quantified by using selective reaction monitoring in positive electrospray ionization mode. The IRMM-468 certified primary reference material (JRC, Belgium) of T4 was used to construct calibration curves. **Results:** FT4 levels reached equilibrium after 18 hours and 4 hours dialysis for micro-ED plates from Harvard Apparatus and HTDialysis respectively using the same dialysis conditions. The developed routine FT4 assay with 96-well ED system was compared with CDC FT4 reference measurement procedure (RMP) using the human pooled serum. The bias between the routine assay and RMP was within 5%, and the CV of the routine assay was within 10%. A runtime of 8 min was sufficient to separate T4 from Triiodothyronine (T3), reverse triiodothyronine (rT3), and other interferences in the dialysate samples with specific LC conditions. The sample volume required for the routine assay was reduced to 150 uL from 1 mL, which was the required volume for the RMP. The sensitivity of the routine FT4 assay allowed quantification of samples from normal and hypothyroid patients. The use of 96-well-plates allowed for semi-automated sample preparation. **Conclusion:** The described routine FT4 assay based on ED LC-MS/MS significantly improves throughput and uses low sample volume, which is suitable for application to large biomonitoring and epidemiological studies.

A-063

To Skim or Splice? Comparing the Quantification of M Proteins Using Two Peak-Integration Methods Across Multiple Platforms

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Background: M-protein quantification by peak integration in serum protein electrophoresis plays a central role in diagnosing and monitoring monoclonal gammopathies. The conventional integration approach integrates M-spikes from baseline (thus known as the perpendicular drop (PD) or the "splice" method), which performs acceptably when the M-protein concentration is relatively high compared to the amount of polyclonal immunoglobulins present. The more recent peak-integration protocol by tangential skim (TS), however, allows for more accurate M-protein estimations by excluding background proteins. Despite guideline recommendations, TS has been poorly adopted, making an understanding of the differences between the two protocols and their potential impacts paramount. **Objectives:** To investigate the % differences in M-protein quantification over a large concentration range upon changing from PD to TS in 3 of the most popular electrophoresis platforms, and to assess the effect of this change on patient classification using two commonly used prognosis and diagnosis cut-offs at 15 and 30 g/L respectively. **Methods:** PD and TS were performed on patients with M-proteins migrating in the gamma region using the Helena gel, Sebia gel,

and Sebia capillary electrophoresis platforms. % differences were analyzed across the whole concentration range, and changes in patient classification were estimated using cut-offs at 15 and 30 g/L respectively. Regression analysis was performed for each platform to predict the expected change in M protein concentration when switching between the integration methods. **Results:** A total of 822 gamma-migrating M-proteins were integrated using both PD and TS protocols (Helena N = 113; Sebia Gel N = 458; Sebia capillary N = 251). On average, TS gave results that are 70% (Helena gel), 50% (Sebia gel) and 54% (Sebia CE) lower than PD. The differences increased tremendously and became more sporadic as M-protein concentrations dropped below 15 g/L in all 3 platforms. At < 15 g/L, the average % difference ranged from 80% to 94%, while at ≥15 g/L, the average % difference was only 13-31%. Interestingly, the change from PD to TS had varying impacts on patient classification with respect to prognosis (Helena gel = 55%; Sebia gel = 23%; Sebia CE = 6%) and myeloma diagnosis (Helena gel = 36%; Sebia gel = 34%; Sebia CE = 18%) on different electrophoresis platforms. **Conclusion:** Integration of M-proteins by the conventional PD protocol led to overestimation and possible confusion when significant polyclonal background is present, which is more pronounced at low M-protein concentrations. While the magnitude of change from PD to TS is consistently higher at low M-protein concentrations, it does vary among different electrophoresis platforms and so are the resulting patient classifications. Careful consideration of these concentration changes and the potential impacts on patient classification should be made when switching to TS.

A-064

High sensitivity COVID-19 detection using a molecularly imprinted polymer-based sensor

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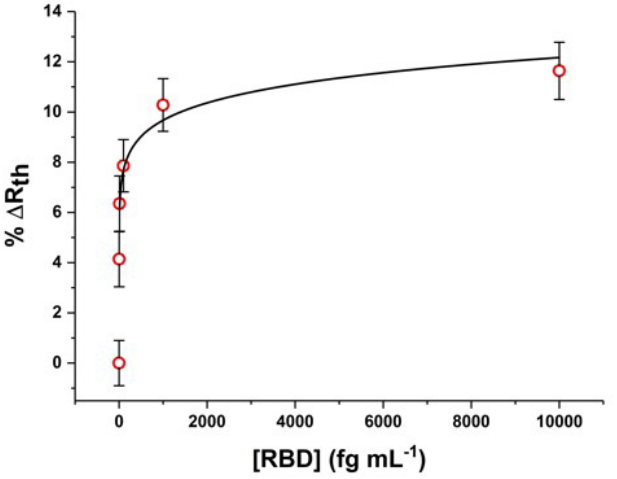
Background: Point-of-care rapid testing for COVID-19 is expected to continue for some time to support the 're-opening' of society and to continually reduce the spread of the virus. However, the first wave of antigen detecting COIVD-19 rapid tests have come under fire, suggesting they are too inaccurate for screening programs in hospitals, schools and other high traffic locations. Viral loads below the limit of detection have led to false negatives, and it is therefore essential that the next generation of COVD-19 rapid tests come with a low limit of detection. A SARS-COV-2 nanoscale molecularly imprinted polymer (nanoMIP) has been developed and integrated into a sensor device to enable high sensitivity COVID-19 detection.

Methods: SARS-COV-2 nanoMIPs were synthesized by a process in which a peptide of the receptor binding domain (RBD) region of the spike glycoprotein was immobilised on a solid phase, monomers and cross-linker were added, controlled polymerisation was initiated and high affinity nanoMIPs were eluted. Specificity of the nanoMIPs were tested in dot blot against other coronaviruses 299E, HKU1 and OC43. The nanoMIPs were covalently coupled to an electrode surface and integrated into sensor platform that relies on monitoring the thermal resistance (R_{th}) at the solid-liquid interface. Sensor sensitivity was tested in PBS buffer spiked with Sars-COV-2 receptor binding domain protein.

Results: The nanoMIP has shown to selectively bind to SARS-COV-2 spike protein and not bind other coronaviruses 299E, HKU1 and OC43. Sensitivity at the fg/ml level has been demonstrated in a sensor format in RBD-spiked PBS buffer.



Conclusion: nanoMIPs specific to SARS-COV-2 have demonstrated an extremely low limit of detection in a sensor-based platform. This level of sensitivity should facilitate a new wave of antigen detecting COVID-19 rapid tests with reduced false negatives.

A-065

Development of A Simple and Sensitive Method for Accurate and Reproducible Whole Blood Selenium Measurement in the Presence of Gadolinium on A Triple Quad Inductively Coupled Mass Spectrometer

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Background Selenium (Se) is an ultra-trace element with essential roles in many biological activities such as antioxidant defense and thyroid hormone regulation. Clinical manifestations of Se toxicity include nausea, emesis, diarrhea, hair loss, nail changes, mental status alteration, visual loss, and peripheral neuropathy. In contrast, Se deficiency, such as Keshan disease, is associated with skeletal muscle dysfunction and cardiomyopathy. Se is measured in whole blood, and the gold standard is inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS methods using kinetic energy discrimination (KED) mode are subjected to interference by gadolinium (Gd) widely used in contrast for magnetic resonance imaging (MRI). Gd interferes with the major isotopes of Se due to doubly charged Gd species. Although rare, we were unable to report 7 samples (2,971 total Se tests) in a 15-month period due to suspected Gd interference. The objective of this study was to develop and validate a TQ ICP-MS assay for reliable quantification of Se in the presence of Gd, via an O_2 mass shift approach. **Methods** We used the iCAP TQ ICP-MS (Thermo Fisher Scientific, Waltham, MA, USA) in O_2 mode to shift the mass of Se and a 4DX FAST autosampler (Elemental Scientific Inc., Omaha, NE, USA). The method was calibrated using a certified reference material (CRM) traceable to NIST SRM 3149 (VHG Labs, Manchester, NH, USA) and CRM internal standard traceable to NIST SRM 3120a. Succinctly, 0.5 mL of whole blood was added to 9.5 mL of 0.1% HNO_3, vortex mixed and centrifuged for 5 minutes at 4,000 rpm. The total run time is approximately one minute. Sensitivity, precision, analytical measurement range (AMR), matrix effect, method comparison, carryover, reference interval (RI) verification, and interference were evaluated. **Result and Conclusion** The limit of blank, limit of detection/limit of quantitation of the assay were 0.0 ug/L and 15.0 ug/L, respectively. The inter- and intra-assay coefficients of variation at 3 concentrations were <3.3% and <1.7%, respectively. The AMR of the assay was 15.0-500.0 ug/L with analytical recoveries of CRM ranging from 97.0-104.1 %. A mixing study performed using ten different patient samples to determine matrix equivalency with 0.1% HNO_3 was acceptable with a mean %bias of 3.1. Method comparison to a previously validated in-house ICP-MS/KED method (n=40) resulted a slope, intercept, and correlation coefficient of the linear regression of 0.99, -5.0, and 0.9983, respectively. No carryover was observed up to 500.0 ug/L. The RI of 58-234 ug/L was verified with 20 healthy individulas. Common endogenous interferents and MRI contrast agents were tested, and no interference was found up to 10,000 ug/dL. Samples from 2 patients who recently had MRI were tested in the old and new assays. In both cases, Se measured by the new assay was within the RI

but was falsely elevated with the old assay using KED mode (>5000 ug/L). In summary, the validated TQ ICP-MS method offers a simple, sensitive, reproducible, and accurate quantification of Se in the presence of Gd.

A-066

Accuracy between two anti-nuclear antibody platforms: multiplex bead-based technology vs. immunofluorescence assay

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Background: BioPlex 2200 uses multiplex flow immunoassay screens anti-nuclear antibody (ANA) is rapid and automated that permits simultaneous detection and identification of multiple antibodies. Historically, the most common test for ANA has been Immunofluorescence Assay (IFA) uses cell lines that may contain 120 different antigens, at times detect non-clinically relevant autoantibodies in patient samples that may result in a false-positive test results. Herein, we retrospectively examine the outcome of the ANA screen by comparing the overall performance of BioPlex 2200 and immunofluorescence assay. Methods: We reviewed ANA performance (Feb 2020 to Jan 2021) on BioPlex 2200 ANA using multiplex fluorescent beads (antigens dsDNA, chromatin, SSA 60, SSA 52, SSB, Sm, SmRNP, RNP A, RNP 68, Scl-70, Jo-1, ribosomal P, and centromere B) and immunofluorescence assay (using HEp-2 cell line). Results: Total number of cases tested on both multiplex flow and IFA platform were 133 (111 females and 22 males). The prevalence in our population divided by age groups 18 to 44 years (36%); 45 to 64 years (42%); 65 years or older, (22%) has reported lupus. The mean age of the lupus patients at diagnosis is clustering around 53 years. Out of 133 multiplex tests performed, 58 tests were negative and 75 were positive. 47% of the tests performed with a negative result (58 tests) on the multiplex, flow turned to positive on IFA assay. The breakdown of titers for these tests were: 30% (1:320); 30% (1:160); 15% (1:80); and 26% (1:20). 55% of the tests performed with a positive result (75 tests) on multiplex flow turned to negative on the IFA assay. The breakdown of titers for these tests were: 12% (1:1280), 18% (1:320); 6% (1:640); 15% (1:160); 15% (1:40); and 35% (1:80). Upon scrutinizing further, data shows a significant disparity in the dsDNA (27%) and RNP antibodies (51%) of the multiplex positive tests turned to negative on IFA assay. Conclusion: Our data suggest a disparity between the results obtained from both platforms. The common notion of IFA being a gold standard is flawed therefore manufacturers need to invest in optimizing assays that are less prone to errors. Clinicians should interpret the test results in a clinical context that includes history, assessment of signs & symptoms.

A-067

Assessing Suitability of use of Point of Care Testing (POCT) Devices for Blood Lipids Measurements in Low- and Middle-Income Countries (LMICs)

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Background: Cardiovascular diseases (CVDs) are the leading cause of death worldwide, causing over 17 million deaths each year. According to the World Health Organization (WHO), 85% of CVD deaths are due to heart disease and strokes, and over 75% occur in low- and middle-income countries (LMICs). Early detection and treatment are key to preventing the consequences of CVD. Many high-income countries have advanced infrastructure to transport blood samples from the point-of-collection to clinical laboratories for analysis. However, such infrastructure is often unavailable or only partially available in LMICs. Therefore, many people cannot get screened for CVD in these countries, creating major public health and economic problems. Point-of-care testing (POCT) devices, which are used at the point of blood collection and require minimal laboratory infrastructure, may overcome this challenge. POCT can be a cost-effective way to screen and identify individuals potentially at risk for CVD who otherwise would remain undetected. Several POCT devices for total cholesterol (TC) and other blood lipids are commercially available. However, their suitability and analytical performance in challenging environments, such as those observed in LMICs, is not fully known. **Methods:** Eight POCT devices for blood lipids that previously successfully participated in the CDC CVD Biomarker Standardization Programs were identified and purchased. The technical and analytical performance of all eight POCT devices was assessed. For the technical assessment, a checklist was developed and applied, encompassing the operational functionality of the devices and taking the potential environmental conditions in LMICs into consideration. Some relevant technical parameters in this checklist include: temperature and humidity requirements, power/voltage requirement (either battery or corded electric), operation language options, supplies storage condition, assay/test time and ease of use. Analytical performance parameters, such as accuracy and imprecision, were assessed by the use of single donor whole blood samples. Accuracy was compared to reference values obtained by CDC reference measurement procedures. **Results:** The operating temperature and humidity requirements were similar across all devices, ranging from 10°C to 40°C and 8% to 90%, respectively. Out of the eight devices, six can be operated with battery and two with power supply. Two devices had an option of at least five languages to choose from. Two devices needed supplies to be cooled at 2°C to 8°C. The test time ranged from 1.5 minutes to 12 minutes and the sample volume ranged from 5µL to 100µL. Preliminary results show that mean bias in whole blood samples with TC concentrations below 200 mg/dL ranged from -18% to 12% for evaluated POCT devices and mean imprecision ranged from 3.1% to 17.5%. For a sample with a TC concentration of 210 mg/dL, the individual sample bias ranged from -19% to 23%. Higher imprecision was observed on this sample. **Conclusion:** The technical characteristics of the assessed devices will be helpful in the selection of the most appropriate device in the specific LMIC setting. The analytical performance using whole blood appears different to the performance observed in serum. Further studies to verify current findings with more individual donor samples are needed.

A-068

Verification of the Siemens N Latex and Diazyme Free Light Chain Assays on the Siemens Atellica Platform and Method Comparisons with the Binding Site Freelite Assay

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Background: The International Myeloma Working Group diagnostic criteria for multiple myeloma and related plasma cell disorders recommends the use of serum free light chains (sFLCs), with the ratio of involved to uninvolved sFLC>100 constituting a myeloma defining event. sFLCs also have utility in screening, prognosis, monitoring response to therapy, and detecting recurrence. The Binding Site Freelite assay is the only sFLC assay currently included in national and international guidelines. The study objective was to evaluate the analytical performance of the Siemens and Diazyme sFLC assays on the Siemens Atellica Platform and compare their performance with the Binding Site FreeLite assay on Optilite platform. **Methods:** Serum free kappa light chain (FKLC) and free lambda light chain (FLLC) were measured using the Diazyme FLC and Siemens N Latex FLC assays on the Siemens Atellica platform (Siemens, Germany) according to manufacturer's instructions. Precision was assessed with quality control material. Method comparisons between the two assays and the Binding Site Freelite assay on the Optilite platform were performed using patient samples. For dilution validation, patient samples with known concentrations on the Optilite Freelite assay were analyzed at increasing dilution factors **Results:** Total imprecision was 2.9-4.8% and 4.4-11.9% for FKLC, and 8.3-13.1% and 4.5-5.8% for FLLC on the Siemens and Diazyme assays, respectively. The FKLC assays were linear between 2.4-66.0 mg/L and 3.8-162 mg/L on the Siemens and Diazyme assays, respectively. For FLLC, the assays were linear between 2.6-63 mg/L and 9.6-200 mg/L on the Siemens and Diazyme assays, respectively. The Siemens assay had better precision for FKLC and Diazyme had better precision for FLLC. An extended linearity was also evaluated for the Siemens assay (up to 600 mg/L and 700 mg/L for FKLC and FLLC respectively). Method comparison showed poor agreement between both assays as well as with the Optilite Freelite assay. At FKLC <100 mg/L, the Diazyme assay showed a negative bias when compared with the Siemens assay. At higher sFLC concentration (>500 mg/L) disparities were more pronounced between the methods with the Siemens FLLC assay showing significant negative bias compared to the Optilite assay, with differences of up to 50 -100% noted in multiple samples. Samples diluted using different dilution factors on the Siemens assay showed consistent results. In contrast, sample dilution reproducibly causes marked under-recovery using the Optilite assay. **Conclusions**: The Siemens N Latex FLC and Diazyme serum FLC assays showed acceptable precision and linearity, and acceptable agreement with the Optilite Freelite assay at sFLC concentrations (<500 mg/L). Significant differences in sFLC results were noted at high sFLC concentrations with the Siemens FLLC assay in particular showing significant negative bias which may impact diagnosis and lead to underestimation of monoclonal protein levels and thus disease severity. We recommend that clinicians are made aware of these differences when switching assays and assays from the same manufacturer be used to monitor patients. We also advocate for assay standardization to minimize such differences.

A-070

A Novel Liquid Chromatography Mass Spectrometry Assay for the Quantification of Hydrogen Sulfide and Other Thiols in Biological Samples

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Background: Hydrogen sulfide (H_2S) is a water soluble and colorless gastro-transmitter with a distinctive rotten egg smell. Most endogenous H_2S is produced in mammalian cells through three main enzymatic pathways. In addition, H_2S is also generated by sulfur reducing bacteria present in the intestinal flora through dissimilatory sulfate reduction pathways. The quantitative analysis of H_2S in biological samples by various analytical methods vary in orders of magnitude. While most of these methods are not based on mass spectrometry, none used isotope dilution liquid chromatography tandem mass spectrometry. Here we develop and validate a robust, sensitive, and accurate stable isotope-dilution electrospray ionization liquid chromatography with on-line tandem mass spectrometry (HPLC-ESI-MS/MS) method for the quantification of H_2S and other thiols in biological matrices, which is critically needed to further extend our understanding of this gaseous biologically active compound. **Methods:** The internal standard for H_2S ($Na_2{}^{34}S$) was prepared by using metallic sodium with isotope labelled elemental sulfur (^{34}S) in tetrahydrofuran and refluxed for 48 hours under nitrogen atmosphere. The internal standard showed to be stable for at least one year. The mass spec method was developed and fully validated to quantify hydrogen sulfide as well as cysteine, homocysteine, glutathione, glutamylcysteine and cysteinyl glycine thiols in biological matrices. All QCs, calibrators and samples were spiked with a known amount of internal standard before derivatization. The derivatized thiols were chromatographically separated on a C18 column. This method was used to examine the systemic levels of the six thiols in a clinical cohort (n=1,326). **Results:** The spike-and-recovery approach was used to generate calibration curves with seven points. All calibration curves have a squared correlation coefficient of 0.997 or higher. The limit of detection (LOD) of hydrogen sulfide, cysteine, homocysteine, glutathione, cysteinyl glycine, glutamyl cysteine is 2 nM, 51 nM, 70 nM, 250 nM, 17 nM, and 23 nM respectively, and the limit of quantification (LOQ) is 6 nM, 170 nM, 234 nM, 833 nM, 55 nM, and 78 nM respectively. Matrix effect was determined by preparing six calibration curves in six different serum pools and comparing their slopes to the average slope of six calibration curves prepared buffer. The six compounds showed minimal matrix effect. The clinical study showed that hydrogen sulfide is significantly reduced in older subjects (p= 0.032), and in male subjects (p=0.027). **Conclusion:** We have described a novel isotope dilution liquid chromatography tandem mass spectrometry method for the quantification of hydrogen sulfide and other thiols that is a robust and has high throughput.The novel method facilitated the quantification of hydrogen sulfide in a big clinical cohort for the first time. The clinical results are in agreement with published literature and our studies confirm that the levels of hydrogen sulfide are significantly lower in older and male subjects, thus can be used to further study the role of hydrogen sulfide in age-associated diseases.

A-071

A Novel Liquid Chromatography-Tandem Mass Spectrometry Assay for Quantification of C-peptide in Human Serum

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Background: C-peptide is a short 31-amino acid polypeptide that is released from pancreatic β-cells during the cleavage of insulin from proinsulin. It is a widely used biomarker of endogenous insulin secretion and is useful in the assessment of residual β-cell function in diabetes and in the diagnostic workup of hypoglycemia. Previously developed mass spectrometric methods for measuring the concentration of C-peptide in serum have analyzed the intact peptide. Unfortunately, intact C-peptide has a low ionization efficiency, requiring sample preparation methods that utilize immunoaffinity enrichment or solid phase extraction with two-dimensional chromatography to enhance sensitive detection by liquid chromatography-tandem mass spectrometry (LC-MS/MS). In this study, our goal was to develop and validate a novel assay that employs proteolysis and solid phase extraction during sample preparation to enhance the analytical sensitivity of serum C-peptide quantification by LC-MS/MS with standard chromatographic separation and no immunoaffinity enrichment. **Methods:** Since there are no arginine and lysine (trypsin cleavage sites) in C-peptide, the endoproteinase Glu-C which cleaves at the carboxyl terminus of glutamic and aspartic acid residues was used for proteolytic digestion in the method. Higher molecular-weight proteins were precipitated using acetonitrile, followed by solid phase extraction with mixed anion exchange resin to enrich C-peptide. The resulting mixture of lower molecular weight polypeptides were reduced (with dithiothreitol), alkylated (using iodoacetamide), and digested (with Glu-C). Intact isotopically labelled C-peptide was used as an internal standard and spiked in serum samples before extraction. Analysis of digested peptides was carried out on a Waters Xevo TQ-S tandem mass spectrometer coupled to Acquity UPLC. The two amino-terminal peptide fragments, EAEDLQVGQVE (EAE1) and LGGGPGAGSLQPLALE (LGG1), were monitored using multiple reaction monitoring (MRM) in positive ion mode. EAE1 and LGG1 peptide fragments were used for quantification and quality assurance respectively. **Results:** Glu-C exhibited monotonic digestion kinetics and was determined to be a reliable proteolytic enzyme. The proteolytic release of the two peptides reached a plateau within an hour under these digestion conditions. The assay was linear between 0.1 and 15 ng/mL, with a correlation coefficient (Pearson r^2) of 0.997. Total imprecision was 7.7 %CV and long-term imprecision at 0.16 ng/mL was 10.0 %CV. The lower limit of quantitation of the assay was estimated to be 0.06 ng/mL. Spike-recovery studies demonstrated a mean recovery of 98.2 % (± 9.1 %) and no interference was observed from samples with hemoglobin, triglycerides, total protein, bilirubin, and creatinine concentrations up to 0.84 g/dL, 1850 mg/dL, 11.4 g/dL, 33 mg/dL, and 10.8 mg/dL respectively. The method compared favorably with a reference measurement procedure (isotope dilution mass spectrometry) and a commercially available immunoassay (Siemens Immulite 1000). No significant carryover (0.06%) was observed up to serum C-peptide concentration of 50 ng/mL. **Conclusion:** Protein precipitation with single-step solid phase extraction and proteolysis with Glu-C is a robust sample preparation method for C-peptide quantification in human serum by LC-MS/MS.

A-073

Evaluation of a Novel Semi-Quantitative Chemiluminescent Immunoassay for the Detection of Anti-SARS-CoV-2 IgG Antibodies in COVID-19 Patients

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Background: Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a profound impact on the health and safety of populations around the world. The objective of this study is to evaluate a novel semi-quantitative chemiluminescent immunoassay (CIA) for the measurement of anti-SARS-CoV-2 IgG in serum or plasma samples from patients with confirmed COVID-19 diagnosis as well as a variety of controls. **Methods:** A total of 1199 samples were included, consisting of 71 samples from COVID-19 patients confirmed by polymerase chain reaction (PCR) test, control samples from patients with relevant respiratory illnesses or infections (n=628), and healthy individuals that were collected in the United States prior to September 2019 (n=500). All samples were tested by the novel, fully automated, QUANTA Flash SARS-CoV-2 IgG CIA on the BIO-FLASH instrument (Inova Diagnostics, San Diego, CA). Qualitative correlations were calculated, and clinical performance was assessed. Additionally, 6 serum samples of varying anti-SARS-CoV-2 IgG titer were tested twice daily over 10 days, in 2 replicates to evaluate the precision of the CIA method. **Results:** The results derived from the clinical evaluation are summarized in the tables below. Receiver operating characteristic (ROC) curve analysis demonstrated good discrimination of the assay (Table 1). The precision evaluation study showed a maximum coefficient of variability of within laboratory imprecision of 8.2%. COVID-19 samples were analyzed in tiers based on the number of days between PCR test and blood draw, and 100.0% (95% CI 91.8-100.0%) of patients who had their blood drawn ≥ 15 days after PCR were found to be reactive on this assay (Table 2).

Table 1 – Clinical performance characteristics and precision of the assay

Performance Characteristic	QF SARS-CoV-2 IgG CIA
Sensitivity in COVID-19 samples (n=71) (95% CI)	88.7% (79.3 – 94.2%)
Specificity in control samples (n=1128) (95% CI)	99.9% (99.5 - 100.0%)
Likelihood + (95% CI)	1000.9 (177.1 – 5676.4)
Likelihood – (95% CI)	0.113 (0.058 – 0.207)
Odds Ratio (95% CI)	8875.1 (1334.9 – 55739.6)
Area under the curve (95% CI)	95.2% (91.1 – 99.3%)
Total Precision (CV%)	4.1 – 8.2%

Table 2 – Analysis of grouped COVID-19 samples

COVID-19 Samples: PCR Test to Blood Draw	Samples Tested	Reactive	Nonreactive	Positive Percent Agreement (95% CI)
0 – 7 Days	10	7	3	70.0% (39.7% - 89.2%)
8 – 14 Days	18	13	5	72.2% (49.1 – 87.5%)
≥ 15 Days	43	43	0	100.0% (91.8 – 100.0%)

Conclusion: The novel QUANTA Flash SARS-CoV-2 IgG CIA demonstrated excellent clinical performance discriminating COVID-19 patients from controls. Further studies on the role of this assay in assessing immunity are warranted.

A-074

Comparison of diagnostic tests for SARS-CoV-2 infection: anti-S IgG versus neutralizing antibodies

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Background: The structure of SARS-CoV-2 involves an important glycoprotein in the trimeric envelope named protein *Spike (S) protein,* which is expressed on the viral surface. This protein is the main target of vaccines, as it binds to the host cell, coupling to the angiotensin-converting enzyme 2 (ACE2) receptor. Neutralizing antibodies are able to block the link between the *Spike* protein's RBD (Receptor-Binding Domain) and the ACE2 receptor, preventing the virus from entering the cell. A virus can induce a multifactorial immune response including, among other factors, the production of different antibodies that will act together to avoid viral infection. However, only a fraction of these antibodies are able to neutralize the virus and prevent infection of new cells. Those are considered "neutralizing antibodies", produced in response to viral infection or vaccination. This study aimed to compare the results of diagnostic tests for the detection of anti-S IgG and neutralizing antibodies to SARS-CoV-2 from clinical samples processed at a large clinical laboratory in the city of São Paulo, Brazil. **Methods:** Immunoglobulin G quantification tests were performed by Abbott® SARS-CoV-2 IgG II chemiluminescence Quant (Non-Reagent: less than 50.00 AU / mL - Reagent: greater than or equal to 50.00 AU / mL) and neutralization capacity by GenScript® cPass™ Competitive Immunoenzymatic Assay SARS-CoV-2 Neutralization Antibody Detection Kit (Non-reagent: less than 30% - Reagent: greater than or equal to 30%). Comparative analysis was performed between the methods. **Results:** A total of 86 samples were included in the study, from individuals between 4-89 years old. A total of 53.48% (n=46) were positive for both tests, while 34.88% (n=30) were considered negative. Among positive results, an increasing trend in the percentage of neutralizing antibodies were detected, when compared to the quantitative results of immunoglobulin G. When comparing the positive/negative results for both assays, a Kappa index of 78.7% was obtained, with an agreement of 89.5%. No relevant agreement was observed between anthropometric data on age results. **Conclusion:** The Abbott IgG Quantitative Anti-S and GenScript Neutralizing Antibodies assays revealed significant agreement between results.

A-075

Comparison of Vitros 5600 Free T4 Assay with Free T4 by Equilibrium Dialysis

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Background: Thyroid function tests such as thyroid stimulating hormone (TSH) and Free T4 (FT4) often require clinical investigation when the values are outside of the reference range. A common request that we receive from our Endocrinology colleagues is to send out a sample that has a normal TSH and a FT4 value that is just below the reference range for analysis by equilibrium dialysis to determine if the patient requires follow up. The low FT4 value does not fit with the clinical picture of the patient or other test results, as the TSH value indicates an euthyroid presentation. The majority of these test results are a FT4 with a value of 0.7 ng/dL, with our normal reference range being 0.8-1.9 ng/dL. A value of 0.7 ng/dL is within the allowable error of 0.2 ng/dL for our immunoassay and can be considered a normal value. To determine how many of our "low" FT4 values were also considered low by an alternate methodology, we reviewed two years of data on patients whose FT4 values were measured by both immunoassay and equilibrium dialysis. As the number of calls we receive about this issue has increased in the last couple of years, we also examined our FT4 reference range to determine if an adjustment was necessary.

Methods: Retrospective data analysis was performed on patient thyroid testing results, including TSH and FT4 performed in our laboratory and reference lab testing of FT4 by equilibrium dialysis. Reference lab testing results examined dated from October 2018 through October 2020. Reference range analysis included data from January 2018 through December 2020 and was performed in EP Evaluator. Only FT4 values from patients with normal TSH values were included in the analysis.

Results:153 orders for FT4 by equilibrium dialysis were sent to a reference lab over the time period examined. 22 of those samples did not have any thyroid testing ordered in our laboratory, so no comparisons could be done. 114 of the remaining samples had a normal TSH value (87%). 95 samples had a FT4 value below our reference range (73%), and 66 (69%) of those were samples with a value of 0.7 ng/dL. 31 samples had a low FT4 value by both immunoassay and equilibrium dialysis (33%), but only 13 of the values of 0.7 ng/dL were also low by equilibrium dialysis (20%). Reference range analysis of 19,578 samples with normal TSH values validated the low end of our FT4 reference range but performing the analysis year by year revealed that the low end of the range had shifted down over time.

Conclusion: While a broad reference range analysis validated 0.8 ng/dL as the low end of our FT4 reference range, data from 2020 indicated that our range had shifted lower. Adjusting the low end of our reference range to 0.7 ng/dL should result in a decrease in samples sent for unnecessary testing of FT4 by equilibrium dialysis.

A-076

Detection and Identification of Novel IGF-1 Variants in a High-throughput Clinical Reference Laboratory

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Background: precise quantitation of insulin-like growth factor-1 (IGF-1) levels is an important element of the assessment of a patient IGF-1 status, which may be influenced by the identification of IGF-1 variants. Although the functional consequences of many IGF-1 variants are not clear, clinical laboratories can collaborate with physicians to fulfill this gap in knowledge. Precise quantitation of insulin-like growth factor 1 (IGF-1) levels is important for assessing a patient's IGF-1 status, which may be influenced by the presence of IGF-1 variants. In our prior work, we described an automated approach for monitoring wild type IGF-1and 15 IGF-1 variants during routine clinical analysis by high resolution liquid chromatography-mass spectrometry (LC-MS). We used an "Isotopic Peak Index (IPi)" to (a) assign all variants to 1 of 4 variant groups (VG) with each VG monitored at a single m/z ratio, and (b) to distinguish between variants within groups. We also used relative retention time (rRT) to further distinguish unresolved variants. The approach was validated by DNA sequencing and resulted in the discovery of new IGF-1variants.**Methods:** herein, we describe how we implemented these methods in a high-throughput clinical laboratory. The approach, which we termed Novel Variant Detection and Identification (NVDI) is a multi-step process: (1) the software flags a potential IGF-1 variant if it detects an *m/z* of any of the four VGs; (2) the IPi of the peak is compared to known variants in its group; (3) the charge state of the variant's isotopic envelope is checked, and its chromatographic peak area is compared to that of the WT; (4) the variant's peak rRT is compared to known variants from its groups; and (5) if a variant does not match any previously identified one, it is routed to DNA sequencing for identification.**Results:** using this NVDI approach, we previously identified 6 variants from the ExAC database: P66A, A67S, S34N, A38V, A67T, and A70T; 2 previously reported V44M and A67V variants; and discovered 6 unreported variants: Y31H, S33P, R50Q, R56K, T41I, and A62T. Monitoring and identification of potentially new IGF-1 variants is performed during routine operations using the NVDI approach. Several potentially novel variants were identified recently. Having passed Steps 1-4 of the discovery workflow, these variants do not match characteristics (IPi and rRT) of any previously identified variants. DNA sequence analysis of the variants is now underway. The established NVDI method is in use continuously to monitor IGF-1 assay results from clinical patient testing. The variants' MS spectra, chromatographic peaks, and DNA sequencing are shown for each variant.

Conclusion: the number of reported IGF-1 variants has significantly grown in recent years. The discovery of new variants indicates that IGF-1 sequence variation exceeds that reflected in ExAC database, probably because of its limited size. By monitoring the large number of specimens submitted for diagnostic measurement of IGF-1, we expect additional discoveries in the future.

A-077

Analysis of Saliva Swabs to Determine Their Usefulness in the Diagnosis of Late Onset CAH

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BACKGROUND Congenital adrenal hyperplasia (CAH) is a rare disease that affects both men and women at a rate of around 1:1000 people. Late onset CAH is a disease that presents itself later in childhood or early adulthood and is due to a 21-hydroxylase deficiency. People experience symptoms of androgen excess such as acne, premature development of pubic hair, accelerated growth, advanced bone age, and reduced adult

height. These symptoms can be shared by other common diseases such as poly-cystic ovarian syndrome (PCOS). For proper diagnosis, hormone testing is needed. The best diagnosis tool is salivary hormone analysis. The hormones that lead to the most accurate diagnosis include 17-hydroxyprogesterone, cortisol, 21-deoxycortisol, and 11-ketotestosterone. Unstimulated passive drool is the gold standard but can be difficult in children. Saliva swabs are the next choice as they are easier to use, but interferences and high background in some of the analytes needed can be problematic. In this study we look at two different saliva swab collection devices to assess which is best to use when screening for CAH. **METHODS** A total of 20 steroids and 4 pharmaceuticals were selected for comparison of recoveries from swabs that have the potential to be used for analysis in children. Two different collection swabs from oral fluid collection devices were analyzed. LCMS water was used to mimic oral fluid in the preparation and testing of interferences. Pooled saliva samples were used for recovery determinations. These pooled samples have known concentrations of analytes. To test for non-specific binding of the collection devices, pooled saliva was pipetted into the collection devices without swabs. To test interference from swabs or plastic collection devices, two additional experiments were performed. Analyte free LCMS water was collected on the swab or the plastic container using the same procedure as the recovery experiment. Recoveries from oral fluid collection devices were tested in two ways, collection device recovery and swab recovery. **RESULTS** For the detection method all analytes were within their linear range. 7-point calibration curves for all analytes exceeded $R^2 = 0.99$. None of the analytes showed any non-specific binding to the plastic collection devices from either manufacturer. After soaking the swabs in water and SPE extraction, the Salimetrics swabs had 2 of the 23 analytes report a "blank" value greater than the lowest calibration point. None of the analytes showed any values above the lowest calibration point for the Oasis swabs. After storing LCMS water in the plastic collection devices and SPE extraction, no analytes from either manufacturer showed levels above the lowest calibration point. For the Salimetrics swabs, no significant analyte loss (recovery values between 80% and 120%) was reported for 13 of the 23 analytes. The Oasis swabs showed no significant analyte loss for 17 of the 23 analytes. Neither of the plastic collection devices showed any significant analyte loss for any of the analytes. **CONCLUSIONS** Oasis Diagnostics saliva collection device performed the best for all analytes and was the only device to test well for both 17-hydroxyprogesterone and cortisol.

A-078

Wastewater as a Public Surveillance Tool for SARS-CoV2

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Abstract:*Background* Wastewater-based epidemiology (WBE) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be a vital source of information for coronavirus disease 2019 (COVID-19) management during and beyond the pandemic. Environmental surveillance as a part of WBE of SARS-CoV-2 can provide an early, cost-effective, unbiased community-level indicator of circulating COVID-19 in a population. We present a fully validated analytical droplet digital PCR method for the detection and quantification of SARS-CoV2 in environmental wastewater specimens. *Methods*Composite sampling was performed by local utilities using a standardized approach. Raw wastewater specimens were concentrated by either centrifugation or electronegative filtration. Viral RNA extraction was performed using an automated 96 well magnetic particle purification. Analysis was achieved with a droplet digital PCR platform. Absolute viral load was used to approximate infection rate in the community served by the utility. *Results*The droplet digital PCR method was superior to qPCR methods for the analysis of SARS-CoV2 in wastewater because of reduced inhibitors, absolute quantification, and the lack of need for calibrators. Controls for wastewater analysis are difficult to source and should be carefully considered. The validation included and evaluation of sensitivity, accuracy, precision, and specificity. Challenges to wastewater testing include supply chain, sensitivity, sample inhibitors, and data interpretation. *Conclusion*Wastewater based surveillance of SARS-CoV2 is proving to be a valuable component of understanding the epidemiology of the coronavirus pandemic. It may be considered as one more tool in the public health laboratory arsenal to monitor corona virus outbreaks. Further, wastewater surveillance frameworks developed for estimating COVID-19 prevalence could be readily adapted to help to identify areas where vaccination is lacking and for long-term monitoring of disease prevalence. Uncovering such vaccination deficiencies will be crucial in ensuring the goal of herd immunity is expeditiously achieved.

A-079

Performance Evaluation of the Atellica IM SARS-CoV-2 Total Assay*

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Background: Siemens Healthineers has developed a fully automated SARS-CoV-2 Total Assay (COV2T) for the Atellica® IM Analyzer. The COV2T assay is a total antibody capture 1-step antigen sandwich immunoassay using acridinium ester chemiluminescent technology. The assay uses recombinant SARS-CoV-2 virus S1RBD antigen for the qualitative and semi-quantitative detection of SARS-CoV-2 antibodies in serum or plasma. Methods: The performance of the Atellica IM COV2T Assay was evaluated with samples collected from subjects with a clinical diagnosis of COVID-19 based on a positive polymerase chain reaction (PCR) method, serial draw samples obtained from SARS-CoV-2 PCR positive individuals and samples collected prior to the COVID-19 outbreak (before November 2019). Potentially cross-reactive samples were also evaluated. Results: COV2T assay results were reported as reactive for samples with ≥ 1.00 Index and nonreactive for samples with < 1.00 Index. The LoD and LoQ is 0.50 Index and 0.60 Index, respectively. The clinical sensitivity of the assay at ≥ 21 days post positive PCR result was 100% (66/66). Seroconversion sensitivity was measured in 13 panels with 2 or more nonreactive blood draws and 2 or more reactive blood draws, the first reactive draw ranged between 3 and 16 days post positive SARS-CoV-2 PCR. Clinical specificity was 99.82% (1089/1091). No cross-reactivity was detected with SARS-CoV-1 IgG or MERS-CoV IgG. Conclusions: The Atellica IM COV2T Assay demonstrates robust sensitivity and specificity.

*This test has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. Claims for semi-quantitation have not been reviewed by the FDA and are not available in the U.S. This test has been authorized only for detecting the presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner. Product availability may vary from country to country and is subject to varying regulatory requirements.

A-080

Development and Analytical Performance of a New Research Use Only (RUO) hCG+β Immunoassay for the Alinity i®and the ARCHITECT® $i1000_{SR}$ Analyzers

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Introduction: The hCG+β assay is used as an aid in the early detection and monitoring of pregnancy and as an adjunctive test in the diagnosis and management of gestational trophoblastic disease (GTD) and germ cell tumors of testicular, ovarian, and extragonadal origin. It is well known that the hCG+β subunit circulates in many forms including a nicked version or a core fragment version (Berger et al 2014). Development of this Research Use Only (RUO) assay included critical selection of antibodies that suitably detected hCG+β and its variants. The RUO Alinity i® and ARCHITECT® $i1000_{SR}$ hCG+βeta immunoassay has been developed for the quantitative determination of the sum of human chorionic gonadotropin (hCG) plus the free hCG β-subunit in human serum and plasma

Methods: This newly formulated hCG+β RUO assay is a two-step sandwich chemiluminescent microparticle immunoassay. The assay is standardized against the newest WHO 6th International Standard for hCG (18/244) and reports the concentration of hCG in pmol/L and in IU/L in parallel. Analytical performance of this RUO assay was assessed on the Alinity i® and included evaluation of hCG isoform recovery, precision, sensitivity, and linearity.

Results: An assessment between the WHO 4th International Standard and the newest WHO 6th International Standard for hCG on the RUO reported significant differences in hCG concentration (up to 40%) due to the higher purity of hCG in the WHO 6th Standard. Further isoform testing showed how this RUO assay recovers each isoform (hCG, hCGn, hCGβ, hCGβn and hCGβcf) relative to recovery of total hCG. Testing showed over recovery for hCGβ at 143% relative to hCG. hCGn and hCGβn were within 10% of the hCG isoform at 94.7% and 108.1% respectively. The core fragment, hCGβcf, 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 ≤ 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 ≤0.1 mIU/mL for low end samples) for our analytical measuring interval (LOQ - 10,000 mIU/mL).

Conclusions: An analytically robust RUO immunoassay has been developed for Abbott's Alinity i® and ARCHITECT® $i1000_{SR}$ instrument systems.

A-081

Validating the analytical reproducibility of a new chemiluminescence-based point-of-need platform for multiplex protein measurements across pg/ml to µg/ml range: Key® and BV®

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Background: MeMed BV® is a test for differentiating between bacterial and viral infection. It is based on computational integration of circulating levels of TNF-related apoptosis-induced ligand (TRAIL), interferon gamma-induced protein-10 (IP-10), and C-reactive protein (CRP). The test result is a score between 0 and 100 that correlates with increasing likelihood of bacterial infection, as established in multiple clinical validation studies. Precise and simultaneous measurement of these three proteins within minutes is a challenge as they range in concentration from pg/ml (TRAIL) to µg/ml (CRP). With this goal in mind, a chemiluminescence-based analyzer called Key® was developed to run the BV test in 15 minutes (Figure). **Methods**: The reproducibility studies for each BV measurand and the score were conducted using a panel of 4 clinical serum samples representing infectious bacterial, infectious viral, equivocal and healthy scores. In total, 90 repeat runs were performed for each clinical sample over 5 non-consecutive days on six Key analyzers, located in three laboratories: Johns Hopkins University School of Medicine, Baylor College of Medicine (Texas Children's Hospital) and MeMed. Studies were performed in accordance with CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures. **Results**: The reproducibility results complied with the pre-established acceptance criteria for the score and individual analytes. The reproducibility coefficient of variation % range observed over the 4 samples was 9.7-12.7%, 4.6-6.2%, and 5.0-11.6% for TRAIL, IP-10 and CRP respectively. The reproducibility standard deviation range for the score was 0.4-9.4 score units. **Conclusion**: Key can quickly, precisely and in parallel measure proteins in the pg/ml-µg/ml range, supporting its applicability to patient triage. Future studies will address real world use of this platform and test.

A-083

Validation Study Summary of "Semi-quantitative Allergy Testing Solution" of Three Panels using Immunoblot Technology.

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Background: Diagnostic allergy testing is used to determine whether symptoms are caused by IgE antibodies exposure to the specific allergen. Currently available allergen-specific IgE assays include Phadia, Siemens, and Hycor platforms which are based on chemiluminescent immunoassays. These provide quantitative individual allergen-specific IgE antibodies in human serum. The running cost of the select panel or group of allergens using these platforms is high, due to individual allergens being analyzed singly. Currently, allergy panel testing is available to detect sensitivity to specific allergens. The National Reference Laboratory is a referral laboratory facility in U.A. E that aims to provide cost-effective alternative solutions to health care providers without compromising the quality and accuracy of the test results**. Objective:** To evaluate the performance characteristics of the Euroimmun allergy panel immune blot assay strips (Gulf country-specific allergy panels (-Food, Inhalation, Pediatric) using EUROBlotOne platform and EUROLineScan for the semiquantitative evaluation of allergens for routine clinical laboratory use. **Method:** The Euroimmun allergy panel immune blot assay is a semiquantitative method to determine allergen-specific to IgE (sIgE) in serum. The test is a multiparameter immune blot assay containing optimized combinations of relevant allergens enabling the simultaneous analysis of sIgE against these different allergens using enzyme conjugate catalyzing a color reaction. The test kit contains test strips coated with more than 20 common different allergens. When using EUROLineScan the intensity of the bands is calculated in Enzyme-Allergo-Sorbent Test classes of 0 to 6 and represent the concentration grades identical to the well-known RAST system (Radio-Allergo-Sorbent Test) used in allergy diagnostics. **Results:** The inter-assay variation and intra-assay variation were determined. The intensity of the bands was within the specified range and showed excellent inter-and intra-assay reproducibility. The same method comparison studies showed > 90% agreement between for Turkey food panel (18 runs, with 33 Allergens), Inhalation panel (20 runs with 20 Allergens) & Pediatric panel (20 runs with 27 allergens) using Concentration (kU/L) and international allergy classification based on the degree of reaction with specific allergen-specific IgE. The comparison studies (Analytical Specificity and Sensitivity) were also done for the Phadia-ImmunoCAP system (quantitative Flouroenzyme Immunoassay methodology) using 10 runs both for Turkey Food and Inhalation panels and showed excellent Negative concordance. However, allergen classification differs when compared to the Phadia-ImmunoCAP system. These differences can be explained by multiple factors including the heterogeneity in IgE antibody-mediated responses amongst different patients, differences in the composition of the allergen " extract-based reagents" used, and the calibration system of the assay. The specificity of the EUROLINE compared to the Phadia-ImmunoCAP system is 100% for all of the tested allergens. **Conclusion:** Allergy panel testing can be a reliable and affordable alternative solution for allergy testing. The solution can improve workflow and be utilized as a first line testing approach. We recommend positive results to be reflexed to a quantitative testing platform for precise allergen classification and to improve patient outcome.

A-084

Development of a Novel LC/MS Assay for the Simultaneous Identification and Quantitation of multiple classes of drugs and Metabolites in Urine encompassing positive and negative ions utilizing/using MedTest Dx's Clinitox Line of Calibrant, Controls, and Internal Standards

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Background: MedTest Dx developed a test method that simultaneously identifies and quantitates multiple classes of drugs and metabolites using MedTest Dx's Clinitox™ calibrator, control, and internal standard stock solutions for 50-65 compounds commonly of interest with pain management samples (both prescribed and illicit). The use of a single injection in a simultaneous assay would have the potential for efficient sample processing in toxicology confirmation laboratories reducing the need for splitting samples for multiple assays.

Methods: The evaluation studies included an assessment of accuracy, linearity, precision, matrix effects, interference, LLOQ, and carryover. Accuracy was assessed by assaying proficiency samples. Within run precision for three levels was assessed over 20 injections in a single run. Day-to-day precision for three levels was assessed over multiple days with 100 total injections. Linearity was assessed using nine levels in

one day with three injections per level. Matrix effects were analyzed by comparing responses in spiked pooled and single donor urine samples to responses in spiked diluent of the same concentration. Interference from common over-the-counter drugs were assessed at low and high levels. Carryover was assessed by injecting blank samples after samples at concentrations 10 times the cutoff and 100 times the cutoff. LLOQ was assessed by assaying 40 replicates of three low concentration samples over five runs. Instrument conditions: A C18 column was used for separation with a gradient mobile phase system consisting of buffered acetic acid (A) and acetonitrile (B). Ionization is simultaneously performed in both positive and negative ion modes and detected using a dynamic MRM acquisition method. The method is easily adaptable to many commercially available LC/MS platforms. Sample Preparation: Urine samples are loaded into a 96 well plate followed by Clinitox internal standards, a hydrolytic enzyme solution and buffer. The hydrolysis reaction is facilitated by the addition of heat and agitation, and then quenched with a trichloroacetic acid solution. After the reaction is quenched, the sediment and cleaved glucuronides are separated by centrifugation. Each sample is then further diluted prior to injection.

Results: Within run precision study yielded results ranging from 0.90-14.30% CV, with most of the results at less than 5% CV. Carryover results were comparable to instrument manufacturer claims. Linear performance was obtained up to ten times the cutoff for each compound. Matrix effects, interference, accuracy, LLOQ, and day to day precision studies are ongoing.

Conclusion: The novel method reliably produces accurate results for the identification and quantitation across a broad range of common drugs of abuse and metabolites in urine. The method provides high efficiency through the use of 96 well plates for sample preparation using a single injection for analysis of both positive and negative ions. For Research Use Only.

A-085

Expanding the Clinical Application Field for Targeted Liquid Chromatography Mass Spectrometry Methods - The Development of a Flexible SARS-CoV2 Detection Method as a Proof-of-Concept

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Background: The SARS-CoV-2 virus has disrupted daily life worldwide, even forced many countries to impose a lockdown. The answer to this was found, amongst others, in widespread diagnostic testing to prevent reoccurrence of outbreaks. Millions of reverse transcription polymerase chain reaction (RT-PCR) based tests are performed every day worldwide. Although these are efficient, relatively simple and acceptably cheap, it is not justified to rely on one type of technology, thereby voiding regional, as well as global capacity. As many other viruses, SARS-CoV-2 produces virus-like particles that contain proteins in relatively high concentration, but no RNA. These virus-like particles are very attractive targets from a liquid chromatography mass spectrometry (LC/MS) detection and characterization perspective. In this way, LC/MS based detection possibly provides an orthogonal approach to RT-PCR, using other reagents that are relatively inexpensive and widely available, as well as orthogonally skilled personnel and different instruments.

Methods: The Cov-MS consortium was established as a community-based effort to develop and validate a generic and broadly applicable MS-based assay which complements the current diagnostic strategies. Fifteen academic labs and several industrial partners combined their efforts and expertise to increase applicability, accessibility, sensitivity and robustness of MS-based SARS-CoV-2 detection.

Results: Based on high-resolution MS data 17 biomarker peptides from a trypsin digest from two SARS-CoV-2 proteins (Nucleocapsid (NCAP) and Spike (SPIKE)) were selected as targets. These 17 signature peptides were then incorporated in a dedicated multiple reaction monitoring (MRM) assay, combined with an 8 minute LC-run. In addition to those signature peptides, the MRM method also includes for each peptide the transition of a stable isotopically labeled analogue, and of 4 different histone peptides that allow confirmation of proper sampling. A method comparison between this LC/MS-based assay and RT-PCR on 20 nasopharyngeal swabs from patients with varying viral load, demonstrated its feasibility, with a similar score for all samples. Further improvements were made in terms of optimal conditions for digestion and the development of very specific antibodies for peptide immunopurification (SISCAPA). This removed matrix effects and hence increased the limit of detection manifold, i.e. down to 80 amol on column, which corresponds to a Ct value of 28-30, regardless of the sample storage medium. The added value compared to a classic RT-PCR is the versatility of MS and already Cov-MS is looking at the detection of variants of concern and multiplexing by including other viruses like influenza.

Conclusion: The Cov-MS consortium demonstrated the feasibility to develop a flexible LC/MS-based screening method for SARS-CoV-2, with the potential to be expanded to other types of viruses. This proof of concept shows the capabilities and strengths of the application of LC/MS-based techniques in viral screening and sets a template for the rapid development of new viral assays.

A-086

Virus Counter: Rapid Detection of Individual Viral Pathogens

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Interferometric Reflectance Imaging Sensor (IRIS) is capable of detecting and counting individual viral pathogens in a complex medium without requiring tedious sample preparation (Fig.1 for the concept). This approach does not rely on virus labeling, instead utilizing the mass of the virus particle itself to generate a discernable optical signal that serves as a distinguishable signature of the physical properties of the virion allowing for an orthogonal identification via size discrimination. Unlike discrete resonant devices, this platform (Single Particle IRIS) has sensitivity and response that are independent of the binding location on the sensor surface, making it easy to detect and size an individual particle bound anywhere on the entire sensor surface, effectively yielding 10^5 - 10^6 parallel sensing elements.We have demonstrated the single virus detection in real time as the virions are captured on the corresponding antibodies [1] and developed disposable cartridges [2]. Recent improvements in capture efficiency of intact virions using DNA-conjugated antibodies have reduced the LOD to 43 PFU/ml [3]. The "Virus Counter" represents a different paradigm and has significant advantages over the current and emerging conventional technologies: **•Detecting intact virions:** Enumerates the intact virions, with potential to correlate to the infectivity of patients. **•Little or no sample preparation requirement**. **•Fully automated POC instrument operated with little training and resources**. **•Low-cost scalable chips** will be manufactured using mature Si technology with exquisite quality control and scalability. **•Multiplexed and configurable with robust universal DNA functionalized sensor chips.**

[1] SM Scherr SM, et al., "Real-time capture and visualization of individual viruses in complex media," ACS Nano (2016)

[2]SM Scherr SM, et al., "Disposable cartridge platform for rapid detection of viral hemorrhagic fever viruses. Lab Chip. (2017)

[3]E Seymour, et al., "Configurable Digital Virus Counter on Robust Universal DNA Chips,"ACS sensors (2021)

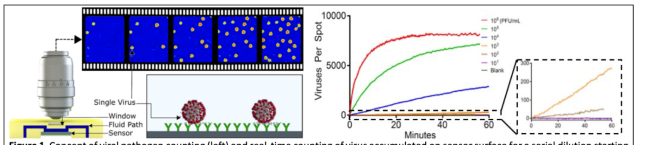

Figure 1. Concept of viral pathogen counting (left) and real-time counting of virus accumulated on sensor surface for a serial dilution starting from 1 × 10^6 PFU/ml. Expansion of lower concentrations reveals a limit of detection (LOD) of 100 PFU/ml [1,2]. Detection threshold is reached in <3min at 10^3PFU/ml. Improved capture efficiency of intact virions have reduced LOD to <50 PFU/ml [3].

A-087

Free Light Chain Assays using Nephelometry and ELISA: a Reference Lab Experience for AL amyloidosis Cases

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Background: Serum kappa and lambda free light chains (FLC) are elevated in two thirds of patients with light chain amyloidosis (AL). This monoclonal gammopathy is rare and difficult to diagnose. Elevated FLC precede the diagnosis for several years, and may prompt a biopsy if found in the setting of heart failure. The most common FLC assays utilized in the US are nephelometric/turbidimetric immunoassays (Freelite, The Binding Site). A novel ELISA method (Sebia Inc.) is being evaluated. Both assays use polyclonal antibodies against FLC. The Sebia FLC assay was compared to Freelite nephelometric FLC assay on BNII to determine if the new assay is suitable for routine diagnostics and follow-up of AL patients.**Methods:** 216 serum specimens from patients clinically characterized with AL were analyzed for kappa and lambda FLC using a Siemens BNII nephelometer with Freelite assay and manually performed Sebia's ELISA-based immunoassays. Reference intervals (RI) for Freelite were deter-

mined by the laboratory, while Sebia's were provided by the manufacturer. Method comparison was performed using Passing-Bablok (PB) regression and concordance analysis for all, only diagnostic and only follow-up samples. **Results:** The AL cohort had 92 unique subjects (34% females, ranged 39- 89y, median 63y) and obtained at diagnosis (n=92) or follow-up (n=124). In all samples, kappa PB regression was y=0.453x +2.663, Spearman correlation r_s=0.859 and lambda y=0.417x +6.848, r_s=0.912. Using each method's RI, samples were classified as low/normal/high. All samples had an overall 65.7% concordance for kappa, 84.2% for lambda and 73.5% for the κ/λ FLC ratio. The rates of agreement increased when only diagnostic samples were evaluated at 73.9%, 92.4%, and 92.4%, respectively for kappa, lambda, ratio. Overall, the two assays have similar diagnostic sensitivity based on the FLC ratio for diagnosing AL: Freelite at 88.0% (95% CI: 79.8%, 93.2%), Sebia at 90.2% (82.4%, 94.8%).

The follow-up samples showed an overall analytical concordance of 59.7%, 78.0%, and 59.3% for kappa, lambda, and ratio, respectively. When clinical performance between assays was assessed using an amyloid response criteria internationally adopted (PMID 23091105) which uses an absolute delta between involved and uninvolved free light chains of 40 mg/L, the response category the patients would be placed with the Sebia test was concordant to Freelite in 82.1% of cases. **Conclusion:** FLC testing is first-line testing for evaluation of patients with suspected AL. The concordance between the Sebia and Freelite assays is appropriate for AL diagnosis and statistically equivalent. Even with lower analytical concordance of the tests for follow-up, the clinical category of response would still be the same in 82.1% of cases. FLC testing does not have a reference material for manufacturer development of new assays. The agreement between tests is remarkable considering the different platforms, methods and antibodies employed in the reactions, along with recent Freelite RI drifts. Sebia eliminates polymerization over-quantitation, a known phenomenon with Freelite at very high concentrations. As new assays enter the market, clinical studies like this one will help laboratories understand if there is need of re-baselining AL patients when switching assays.

A-088

SimpleFlow™- a Next Generation Non-Enzymatic Tissue Dissociation Technology Generates Single Cells at Cold Temperature with Unperturbed Cell Surface Markers and Transcriptome Signature

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Background: To fully understand the cellular specificity and complexity of tissue microenvironment under physiological conditions such as tumors and diseased conditions, it is necessary to measure molecular signatures with single cell resolution. Single Cell sequencing is an established method to study genomics, transcriptomics, proteomics, metabolomics and understand cell to cell interactions at the single cell level and has now become an integral part in drug and biomarker discovery. Many well-developed methodologies and multiple commercial systems available for tissue dissociation heavily rely on enzymatic dis-aggregation of tissues at 37°C and take a minimum of 1 h processing time. Dissociated single cells from such technologies recently have been demonstrated to impair cell surface markers as well as express inflammatory and or stress-related mRNAs, thus changing its transcriptome signature from the natural state of the tissue.

Methods : Cellsonics has developed a rapid next generation SimpleFlow™ System including a single use cartridge for tissue dissociation. It uses a novel Bulk Lateral Ultrasonic (BLU™) technology to dissociate single cells from solid tissues at 8°C in less than 10 min processing time. The SimpleFlow System was used to dissociate a wide range of fresh tissues from mouse and pig ranging from 10 mg to 500 mg to obtain a heterogenous suspension of viable cells. The quantity, quality and transcriptomic integrity of cells generated with the SimpleFlow™ System were compared with a traditional enzymatic method. The integrity of cell surface markers between these dissociation techniques were determined by FACS analyses, and the transcriptomic integrity was determined with Bulk RNA-Seq and Single cell RNA-Seq analysis (10x Genomics Chromium). With 10x Genomics support software, Cell Ranger and Loupe browser, the cells were interrogated for specific cell-markers and grouped into 10 distinct clusters. Loupe was further used to highlight differentially expressed genes in these clusters.

Results: Integrity of dissociated tissue cells, for e.g. Hepatocytes, is maintained in single cells dissociated with SimpleFlow™ System.Yield (live cells/mg) from various tissue sizes and types from pig and mouse dissociated with SimpleFlow™ System were comparable to those dissociated with Enzymatic cocktail.With BulkRNAseq analysis, >200 genes from Pig Liver and >90 genes from a CD45+ single-cells from mouse tumor were differentially expressed in single cells dissociated with Enzymes (both dissociation methods were compared to frozen tissue that served as control).Single Cell-RNAseq analysis of tumor dissociated with enzyme cocktail revealed >4-6 log fold increase in heat and oxidative stress-related *HSPA1A* and *HSPA1B* transcripts, Cxcl2 and Tnfsf11 transcripts indicative of T-cell activation, and Nr4a1 and Atf3 transcripts indicative of stress conditions imposed by conditions of tissue digestion than as compared to tumor dissociated with SimpleFlow™ System.

Conclusion: SimpleFlow™ System can rapidly dissociate solid tissue to viable, heterogeneous single cells at cold temperatures from a biopsy-size to 500 mg tissue. Cellular and molecular data analysis of single cells demonstrate that proprietary BLU™ energy has no, or infinitesimal effect on the molecular footprint of the native tissue, for e.g. surface molecules as well as their multiomes (transcriptome/methylome/proteome).

A-089

Developing a Quick and Robust Mass Spectrometry-based Method for the Detection of SARS-CoV-2

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Background: SARS-CoV-2 is a highly infectious virus that has created a global COVID pandemic. Attempts to contain the virus have only had limited success, partly due to its spread through unknowingly asymptomatic carriers, emphasizing the need for widespread and regular testing. Real time reverse transcription polymerase chain reaction has proven to be the gold standard method in the detection of COVID infections via SARS-CoV-2 viral RNA. Although PCR has demonstrated high sensitivity (> 80 %) and specificity (> 98 %), a shortage of reagents and trained scientists has resulted in a backlog of tests and inconsistent processing times. This highlights the need to develop orthogonal methods to create a robust and economic system capable of sufficient testing. SARS-CoV-2 particles also contains numerous copies of spike glyco- (P0DTC2), envelope (P0DTC4), membrane (PODCT6) and nucleocapsid (P0DTC9) proteins, all of which are biomarkers of a COVID infection. Therefore, any technique that can reliably detect the presence of these components could be used to test for infection. One approach capable this is bottom-up mass spectrometry (MS). Proteolytic digestion of proteins results in peptides, which can be separated by liquid chromatography (LC). These peptides are easier to identify than intact proteins (due to their size) and numerous peptides can be searched for from each protein, decreasing the chances of a false positive. **Methods:** A mass spectrometry-based SARS-CoV-2 peptide quantification method was developed using a Thermo Fisher Scientific™ Vanquish™ Flex UHPLC system and a Thermo Fisher Scientific TSQ Altis™ mass spectrometer. Recombinant P0DTC2 and P0DTC9 proteins (Invitrogen™) were spiked into pooled (n > 2) nasal fluids and saliva. The spiked nasal fluids were pipetted onto a nasopharyngeal swab, which was added to viral transport media (VTM), whilst the spiked saliva was added directly to VTM. Samples were then precipitated, centrifuged and enzymatically digested (Thermo Fisher SMART Digest™ Trypsin Kit). The resulting peptides were separated using a 4-minute LC gradient with a Hypersil GOLD™ C18 column (2.1 x 50 mm, 1.9 µm), coupled with a single reaction monitoring method that utilized Skyline™ transition predictions. The six peptides demonstrating the lowest levels of detection were optimized. All assays were then repeated in triplicate and data was analyzed using Thermo Fisher Scientific TraceFinder™ software. **Results:** Low detection and quantification limits were observed for each peptide (low/sub-femtomole on column), demonstrating the viability of using the TSQ Altis for COVID testing. The acquisition of calibration curves demonstrated strong linearity ($R^2 > 0.99$) across analytical measurement ranges (AMR) between 0.25 and 100 femtomoles on column for the best performing peptides. A coefficient of variation less than 20 % was achieved for every peptide at each concentration within the AMR. **Conclusion:** In summary, an LC-MS assay has been developed to accurately quantify SARS-CoV-2 proteins in saliva and nasal fluids. This robust, quick and easy-to-implement method could allow for the reliable detection of SARS-CoV-2 infections.

A-090

A Sensitive and Rapid UPLC-MS/MS Method for Simultaneous Quantitation of Five Newer Generation Antiepileptic Drugs and Two Active Metabolites

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Background: Epilepsy is one of the most common disorders of the central nervous system. It affects approximately 50 million people worldwide, nearly 450,000 of which are under 17-year-old in America only. Antiepileptic drugs (AED), especially

newer generation AEDs are clinical effective, and may offer better safety to these patients. However, they can still result in rare and destructive toxicities. Due to the narrow therapeutic ranges associated with these drugs, therapeutic drug monitoring (TDM) of AEDs is often necessary to optimize pharmacotherapy by maximizing efficacy and minimizing toxicity. We herein report a very simple, rapid liquid chromatography-mass spectrometry (UPLC-MS/MS) method that could simultaneously measure five AEDs and two active metabolites including levetiracetam (LEV), lacosamide (LCM), zonisamide (ZON), lamotrigine (LMT), 10-hydroxycarbazepine (OXC-M1, the main oxcarbazepine metabolite), clobazam (CLO) and N-desmethyl clobazam (N-CLB, clobazam metabolite) in serum. **Method:** To extract five drugs and two metabolites simultaneously, only 20 µl of serum was used and 80 µl of acetonitrile with respective labeled internal standards was added for protein precipitation. After 5-minute centrifugation, 780 µL of HPLC water was combined with 20 µL of supernatant. After brief vortex, 2 µl was injected for LC-MS/MS analysis. LC-MS/MS analysis was performed on a Sciex Exion UPLC system connected to a 6500 triple Quad (Citrine) mass spectrometer in MRM and positive ion mode. Separation was performed on a C18 reversed-phase liquid chromatography column using a gradient (ammonium formate pH-8 in water and 3.59% formic acid in acetonitrile). The five drugs and two active metabolites were eluted over a 4.5-minute gradient. **Results:** The method was validated for linearity, recovery, precision, and accuracy, etc. in human serum according to CLIA and CAP regulation. The assay was linear over the concentration ranges 1.56-100 µg/mL for LEV, 0.47-30 µg/mL for LCM, 1.25-80 µg/mL for ZON, 0.47-30 µg/mL for LMT, 1.09-70 µg/mL for OXC-M1, and 7.81-2000 ng/mL for CLO, 62.5-20000 ng/mL for N-CLB, respectively, with correlation coefficient great than 0.99. Recovery was 99-107% for all seven compounds. With-in run and between-run precision for three levels of quality controls were 4.9-11.7%. The accuracy was evaluated with a clinical reference laboratory (n=40) and CAP proficiency survey materials, and a correlation coefficient great than 0.95 was observed. The absence of matrix effects was also confirmed. **Conclusion:** We have developed and validated a very simple, rapid, and cost-effective UPLC-MS/MS method for simultaneous quantitation of five AEDs and two active metabolites in serum within four and half minute analysis time. It has been implemented in our Children's hospital, which provides same day turnaround time and addresses the clinical needs of pediatric patients.

A-092

Development and Validation of a Liquid Chromatography Mass Spectrometry Method for Simultaneous Measurement of 25-OH D3, epi-25-OH D3, 25-OH D2, Vitamin A, α-Tocopherol, and γ-Tocopherol

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Background: Circulatory fat-soluble vitamin levels are commonly measured to identify deficiencies that may lead to rickets, osteomalacia, night blindness, and reversible motor and sensory neuropathies. We developed and validated a rapid and robust LC-MS/MS method that simultaneously measures 25-OH D3, epi-25-OH D3, 25-OH D2, vitamin A, α-tocopherol, and γ-tocopherol for clinical use.

Methods: 100 µL of serum was mixed with isotope-labeled internal standard and extracted using a 96-well supported-liquid extraction plate with 1.5 mL of hexanes/isopropanol (90/10) (v/v). Dried eluate was reconstituted with 100 µL of methanol/water (90/10) (v/v) and analyzed by LC-MS/MS with a 10-minute gradient. Accuracy was assessed using NIST Standard Reference Materials SRM972a and SRM968f, patient comparison analysis with a LC-MS/MS method at a reference lab, and spike-recovery studies using patient sera and vitamin D-depleted serum. Analytical measurement range (AMR) was determined by spiking 6 analytes into vitamin D-depleted serum to give 7 specimens at varying concentrations. The lower limit of the measuring interval (LLMI) was assessed using 6 pooled specimens with varying low concentrations of each analyte over 20 days. Precision (repeatability and reproducibility) was assessed using quality control materials. Interference studies were performed using pooled patient specimens spiked with varying concentrations of hemoglobin, bilirubin, or intralipid. Matrix effect was assessed by post-column infusion and by matrix dilution with saline.

Results: The method was linear covering physiological concentrations with $r^2 > 0.99$. Repeatability and reproducibility were <15% CV at all QC levels. LLMI for 25-OH D3, epi-25-OH D3, 25-OH D2, vitamin A, α-tocopherol, and γ-tocopherol were 4 ng/mL (15% CV), 4 ng/mL (15% CV), 4 ng/mL (18% CV), 1 µg/dL (20% CV), 0.2 µg/mL (20% CV), and 0.2 µg/mL (8% CV). Recoveries for NIST Standard Reference Materials were between 92 - 111% and between 81 - 122% for spike-recovery studies. Passing-Bablok analyses for vitamin D total (n = 41) , vitamin A (n = 34), and α-tocopherol (n = 37) demonstrated slopes between 1.04 and 1.11 and r^2 between 0.94 and 0.96. Minimal matrix effect was observed.

Conclusion: We have developed and validated a comprehensive and rapid LC-MS/MS method for the simultaneous measurement of 25-OH D3, epi-25-OH D3, 25-OH D2, vitamin A, α-tocopherol, and γ-tocopherol for clinical use.

A-093

Accurate Quantification of Monoclonal Immunoglobulins Migrating in the Beta Region on Protein Electrophoresis

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Background: Concentration of monoclonal immunoglobulins (MIg) in neoplastic monoclonal gammopathies is generally measured by densitometric scanning of monoclonal peaks on gel electrophoresis, or by the measured peak area guided by immunosubtraction (ISUB) on capillary electrophoresis (CE). For MIg migrating in beta region, densitometric measurement is complicated by interference of major beta-region proteins, transferrin, and C3. Accurate measurement of MIg is important in the diagnosis of light chain predominant multiple myelomas.

Methods: C3 interference in densitometry was abrogated by heat treatment of serum and MIgs were quantified by densitometry of the residual band. Inactivation at 56° C for 30 minutes and at 37° C for 24-hours were tested. Immunochemical measurement of transferrin was converted to its densitometric equivalent quantity by assaying serum samples without MIg in the beta region. For MIg co-migrating with transferrin, contribution of the latter was removed by subtracting the converted transferrin concentration from the combined densitometric quantification of the band. With CE, MIg was measured by using ISUB to guide demarcation.

Results: Of the 32 samples analyzed, MIg in beta region overlapped transferrin and C3 bands in 10 and 20 cases, respectively. In two instances MIg band overlapped both transferrin and C3. Because heat inactivation of C3 at 56°C denatured normal proteins as well as MIg, inactivation at 37° C was used. Results by C3 depletion and transferrin subtraction method were lower, yet comparable to the results from using CE measurement guided by ISUB. Difference in the results of two methods are insignificant (P= 0.43). Correlation coefficient between the two methods is 0.98. As expected, results from both methods were lower than those from a perpendicular drop measurement of the peak or by nephelometric assay of the involved isotype.

Conclusion: The method described here improves accuracy of measurements for MIg migrating in the beta region, without the need for special reagents or equipment. In addition to the general improvement in accuracy of laboratory measurements, accurate measurement of beta migrating immunoglobulins is essential for proper diagnosis of light chain predominant multiple myelomas (LCPMM). Serum free light chain concentration per gram of MIg is an essential measure for the diagnosis of LCPMM. Identification of this sub-type has gained importance with the recognitions that LCPMM patients have higher burden of renal disease and shorter survival.

A-094

Evaluation of the Stanbio β Hydroxybutyrate Reagent on the Beckman Coulter DxC 700 AU Analyzer

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Background:β-Hydroxybutyrate (b-OHB) is the primary ketone produced in ketoacidosis. Many labs use a handheld b-OHB method according to the American Proficiency Institute participants. Very few automated chemistry platforms provide their own branded b-OHB reagents. This study defines and summarizes the analytical performance of the Stanbio b-OHB reagents on the Beckman Coulter DxC 700 AU chemistry analyzer attached to the Power Express automation line.

Methods: Performance characteristics included: precision, analytical measurement range, on-board reagent and calibration stability, correlation of the Beckman Coulter DxC 700 AU with the Stanbio Sirrus, and Abbott Architect analyzers, specimen stability and reference range verification.

Results: The Stanbio b-OHB reagent showed acceptable performance criteria. The analytical measurement range was shown to be 0.0 to 8.0 mmol/L which is beyond the manufacturer's claim of 0.0 to 4.5 mmol/L. Strong correlations for patient results were found with a slope of 0.96, intercept of 0.055 and R^2=99.9% for each comparison (Stanbio Sirrus and Abbott Architect). Intra and Inter-run precision of QC were less than 1.0%CV for each level, respectively. The reagent blank stability, calibration stability and on- board reagent stability were established during the precision study. The reference range of 0.02 - 0.27 mmol/L was verified.

Conclusion: This assay allowed our laboratory to automate b-OHB testing with minimal intervention by technical staff. The analytical performance of the Stanbio b-OHB reagent on the DxC 700 AU analyzer exhibited an acceptable performance for high volume testing laboratories with automation. The linearity was proven beyond the manufacturer's designated range. Reagent blank and calibrations were stable for 30 days and on-board reagent stability was 60 days. Specimen stability was verified up to 8 days refrigerated.

A-095

The development of a point of care test for the quantitative measurement of active neutrophil elastase in sputum

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Background: Sputum neutrophil elastase (NE) is a recognised biomarker of disease severity in various respiratory disorders including chronic obstructive pulmonary disease (COPD) and bronchiectasis; with its routine measurement, at the point of care (PoC), being of considerable interest to clinicians to assist in patient triaging. In 2017, ProAxsis Ltd registered NEATstik®, a lateral flow test for the qualitative detection of sputum NE, with a CE mark; however, the need for a quantitative device was highlighted by a recent study (Shoemark, A. *et al* ERJ 2019;53:1900303). Here the authors reported NEATstik's potential utility in identifying patients at highest risk of developing complications. The principal aim of this study was, therefore, to evaluate the potential of rendering NEATstik® more quantitative, through the combined use of a CMOS reader device and NE calibration standards.

Methods: NE calibration solutions (173 ng/ml-10,000 ng/ml) and sputum samples (n=19) were prepared in NEATstik sample dilution buffer. Sputum samples were initially diluted x10, followed by a x2 serial dilution. NE Calibrants and samples were then transferred (70µl) to a NEATstik device; with a CMOS reader device used to measure signal intensity at both control and test-lines. Sputum sol was prepared simultaneously for subsequent analysis on ProAxsis' Activity based Immunoassay (ABI) for NE, to determine the correlation coefficient between NEATstik and the ABI.

Results: CMOS readings revealed that a pronounced matrix effect persisted, for each sputum sample, beyond the initial x10 dilution; with statistical analysis (Kruskal-Wallis; Dunn's Multiple Comparison Test) indicating that a further x8 dilution is necessary for the quantitative measurement of active NE, in sputum, with NEATstik. Moreover, a highly significant correlation (spearman r value = 0.86) was observed between NE levels when measured on these two platforms.

Conclusion: Improved accuracy in the quantitative determination of sputum NE may assist in the identification of key disease thresholds i.e., subclinical infection/impending exacerbations, and thus, enable pre-emptive medical intervention thereby improving the standard of care received by patients with respiratory disease.

A-096

Analytical performance of the NOVEOS immunoassay for detection of allergen-specific IgE to M006 Alternaria alternata) to aid in the diagnosis of allergic diseases

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Background: Allergic reactions are a result of the immune systems hyper reactive response to genetic or environmental triggers. These allergic responses are mediated by Immunoglobulin E (IgE) which serve a pivotal role in diagnosing specific allergies. The fully-automated and innovative NOVEOS immunoassay instrument utilizes chemiluminescent, paramagnetic micro-particle technology, and a 4µL sample per test to aid in diagnosing allergic diseases. We demonstrate the analytical performance of the new NOVEOS specific IgE (sIgE) test for detection of sensitivity to M006 (Alternaria alternata) allergen in this study.

Methods: The NOVEOS instrument precision and functional sensitivity was assessed over 20-days per CLSI EP15-A3/CLSI EP05-A3 and CLSI EP17-A2/CLSI EP05-A3, respectively. Diagnostic and relative agreement was evaluated in accordance with CLSI EP12-A2. Method comparison between NOVEOS System and another device was conducted in accordance with CLSI EP09C. Limit of Blank (LoB) and Limit of Detection (LoD) were completed per CLSI EP17-A2 by testing multiple instruments. Carryover was also established by testing on multiple instruments per CLSI I/LA20-A3. The linearity assessment included multiple serial dilutions at different concentrations and was assessed per CLSI I/LA20-A3. In addition, the following studies were evaluated using the paired-difference design in alignment with CLSI I/LA20-A3: interference, competitive inhibition, and cross reactivity against the non-IgE immunoglobulin classes.

Results: The NOVEOS sIgE test for M006 (Alternaria alternata) showed good repeatability and within-lab precision across 20-days with < 10%CV and < 12%CV, respectively; functional sensitivity was determined to be less than 0.35kU/L. In addition, LoB, LoD and carryover were found to be less than 0.10kU/L across multiple instruments. The NOVEOS sIgE assay also demonstrated a strong correlation when compared to clinical findings, confirmed by skin prick test, and another commercially available device. The assay also exhibited good linear regression over multiple concentrations. Lastly, interference (endogenous and exogenous substances, including biotin), cross-reactivity and competitive inhibition were found to have minimal or no impact to the NOVEOS sIgE performance.

Conclusion: The NOVEOS immunoassay for detection of allergen-specific IgE for M006 (Alternaria alternata), demonstrated a strong analytical performance to aid in the diagnosis of allergic diseases.

A-098

Validation of a novel quantitative anti-SARS-CoV-2 antibody assay specific for the receptor binding domain

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Introduction: The recent SARS-CoV-2 pandemic remains a global problem. Waning antibody titers and re-infection cases have raised considerable concern regarding the protective capacity of humoral immunity. Progress in the critical study of the temporal evolution of the humoral response necessitates a quantitative antibody assay. **Objective**: To develop and validate a quantitative anti-SARS-CoV-2 antibody assay specific for the receptor binding domain (RBD) of the novel coronavirus. **Methods**: This assay was developed using electrochemiluminescent technology to quantitatively measure anti-RBD IgG antibodies specific to SARS-CoV-2 spike protein. To create a solid phase RBD antigen, biotin-conjugated recombinant RBD antigen is added to streptavidin bound carbon-coated microtiter plate. The first incubation is with patient sera or controls. Following wash steps, ruthenium-conjugated anti-IgG antibodies are added for the second incubation, binding to anti-RBD IgG complexed to the solid phase RBD antigen. Unbound material is removed with another wash step followed by measurement of relative light units. Affinity purified human IgG is used to plot a standard curve of response versus anti-IgG antibody concentration from which the unknown antibody concentration can be calculated. Lower limit of quantification (LLOQ), accuracy and precision, dilution linearity, cross-reactivity and interference studies were performed using SARS-CoV-2 antibody positive human serum samples. A total of 1020 pre-pandemic human serum samples were used to establish the assay cut point and clinical specificity. A method comparison was performed against EUA-approved Roche Elecsys Anti-SARS-CoV-2 nucleocapsid assay using 49 negative and 162 PCR confirmed positive individuals. **Results**: Limit of detection was 0.6 µg/mL, and lower and upper limits of quantification were 1.0 and 100 µg/mL, respectively. Method validation results showed within-run and between-run accuracy of ≤±10% for low, medium, and high samples. Within-run and between-run imprecision was ≤12% for low, medium, and high samples. The assay was free from interference from hemolysis, icterus, lipemia, rheumatoid factor and biotin up to 500 mg/dL, 20 mg/dL, 3000 mg/dL, 100 IU/mL and 5000 ng/mL, respectively. 1011 of 1020 pre-pandemic samples tested were below the cut point and 9 of 1020 results were above the cut point, resulting in clinical specificity of 99.1% with 95%CI. Method comparison to Roche's

nucleocapsid assay resulted in 49 of 49 negative samples and 161 of 162 PCR-confirmed positive samples, yielding a 99.5% agreement between the two methods and clinical sensitivity of 99.4%. **Conclusion**: We developed and validated a highly quantitative and reproducible method for measuring anti-SARS-CoV-2 IgG antibodies. High performance serology test like demonstrated here will be a valuable tool for studying the temporal evolution of the humoral response to SARS-CoV-2 infection and vaccination.

A-099

Simultaneous quantification method of cortisol and cortisone in human saliva by a liquid chromatography - mass spectrometry

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Background The quantification of cortisol in different biological materials (serum, urine and saliva) is essential in the diagnosis and management of diseases of the hypothalamic-pituitary - adrenal axis, such as Cushing's syndrome and adrenal insufficiency. The quantification of serum cortisol is a measurement of total (free and bound) cortisol and in conditions of altered carrier proteins (cortisol binding globulin and albumin) concentrations it may be misleading. Cortisol in saliva accurately reflects serum-free cortisol, as it is unbound and in equilibrium with circulating free cortisol. Salivary cortisone is formed predominantly from serum free cortisol, by the enzyme 11bhydroxysteroid dehydrogenase type 2, as the latter passes through the salivary gland. Total and free cortisol levels are higher than total and free cortisone levels in the serum, yet the ratio is reversed in the saliva, with salivary cortisone to cortisol ratio of 4:1 to 10:1, thus allowing more accurate measurement of cortisone, especially at low serum total cortisol levels. The analysis of these 2 steroids in saliva has been increasingly used due to this physiological advantage of assessing the free fraction of steroids and to their painless and ease of collection performed by the patient at home**Objective** This study aimed to develop a fast and robust quantification method for detecting cortisol and cortisone in a single analysis and sample of saliva by liquid chromatography coupled to a mass spectrometer**Methods** Using an UPLC-MS/MS Waters Xevo TQS, 20 µl of internal standard and only 200 µl of saliva, we developed a simultaneous analysis of cortisol and cortisone, without derivation, in positive mode. A C18 column was used in a 4-minute analysis, in addition to the SPE column. Only 10 uL is needed for injection in the UPLC**Results** With linearity from 10 to 1000 ng/dL, the calibration curve obtained correlation coefficients greater than 0.99, for cortisol we obtained a correlation coefficient of $r^2 = 0.9977$, while for cortisone the correlation coefficient was $r^2 = 0.9975$. Precision and accuracy tests were performed, the intra and inter-assay studies showed a variation of ± 15%. No significant interference from the matrix components was observed during the analysis of the analytes of interest. The carryover study demonstrated that there was no significant contamination from samples previously analyzed**Conclusion** A sensitive and robust method for simultaneous analysis of cortisol and cortisone by liquid chromatography coupled to a mass spectrometer was developed using a small amount of saliva sample, without the need for derivatization, in addition to a short run time when compared to other methodologies. Measurement of salivary steroids with liquid chromatography-tandem mass spectrometry (LC-MS/MS) enhances the sensitivity by 10- to 100-fold and it improves the specificity by eliminating cross-reactivity among various metabolites.

The analysis of these 2 steroids in saliva has important clinical uses with the physiological advantage of assessing the free fraction of steroids and to their painless and ease of collection, that can be performed by the patient at home.

A-100

Grass and Tree Allergen Components Specific IgE Assay on the NOVEOS™ Immunoanalyzer: Evaluation of Inter-method Comparison

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Background: IgE sensitization to pollen allergens affect up to 30% of the industrialized population and continues to increase due to the intensifying pollen season. The current *in vitro* diagnostic method for pollen sensitization involves the utilization of crude allergen extracts. These extracts contain extensive pollen cross-sensitization, cross-reactive carbohydrate determinants (CCD), and other non-allergenic molecules that may deter from accurately determining the symptom-eliciting pollen source. However, molecular-based testing, which utilizes recombinant allergen components, can address the issues of crude extracts and provide more effective immunotherapy strategies. This study evaluates the performance of grass and tree components on the NOVEOS™ Immunoanalyzer assessing inter-method comparison.**Methods**: A total of 107 patients sensitized to at least one of the components found in grasses and trees were tested on the NOVEOS Immunoanalyzer and Phadia 1000 System against grass (rPhl p 1, rPhl p 5b, rPhl p 7, rPhl p 12), birch (rBet v 1, rBet v 2), and olive (rOle e 1) components. In addition, a panel of 10 negatives patients for each component were also tested.

Doc MKX 157, Rev A, DCC 20-010

Results: The overall agreement between the two systems was 95% (Cohen's kappa = 0.89; 95% confidence interval [CI] 0.80 - 0.99) with positive linear correlation (r2 from 0.72 to 0.99, and Spearman's rho from 0.79 to 0.97) across all components. With the exception of rPhl p 12, Passing-Bablock regression analysis resulted slopes from 0.74 (95% [CI] 0.61 - 1.05) to 1.16 (95% [CI] 0.88 - 1.52). rPhl p 12 resulted a slope of 1.94 (95% [CI] 1.47 - 3.12), indicating a higher sensitivity with the NOVEOS sIgE assay. 10 discordant patients (ImmunoCAP positive, NOVEOS negative) were observed, of which 6 had sufficient volume to test for anti-CCD IgE reactivity. All 6 patients were confirmed to be positive for anti-CCD IgE. Patients with anti-CCD IgE antibody may show falsely elevated results on the ImmunoCAP sIgE assays, which employs a cellulose-based matrix that can contain CCD epitopes, despite using recombinant components. Disregarding CCD positive patients, the overall agreement between the two systems improves to 98% (Cohen's kappa = 0.96; 95% [CI] 0.89 to 1.02).**Conclusion**: The NOVEOS Immunoanalyzer capably detects sIgE to grass and tree allergen components and provides a strong agreement with ImmunoCAP sIgE assays not demonstrating CCD interference.

A-101

IgG antibody response after Sars-CoV-2 Pfizer-BioNTech vaccination

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Background: Our previous study showed that after natural exposure to the SARS-CoV-2 virus, IgG nucleocapsid antibody levels stayed elevated for at least 3 months in the majority of participants. To address the same question after vaccination, we needed a reliable test to measure IgG antibody levels against SARS-CoV-2 spike protein, as the majority of vaccine producers used the spike protein as an immunogenic target.

Methods: The Beckman Company released a semi-quantitative Access Sars-Cov-2 IGG-2 antibody test which was designed to measure antibodies directed against SARS-CoV-2 viral spike protein after natural exposure. The purpose of this study was to demonstrate the utility of this test in monitoring antibody response after vaccination. We utilized sera from lab employee volunteers who donated blood samples during the vaccination process. We collected 14 samples on or before the day of the first dose of Pfizer vaccine (group 1), 20 samples during the week of second dose (group 2) and 30 samples between 10 to 21 days after the second dose of the Pfizer vaccine (group 3).

Results: In group 1, the antibody levels ranged from 0.01-9.0 AU/ml with a mean of 1.02 AU/ml. In group 2, levels ranged from 3.03 - 162 AU/ml with a mean of 41.5 AU/ml. In group 3, levels ranged from 86 to greater than 450 AU/ml (upper limit of the test). 10 out of 30 samples in group 3 had antibody levels above 450 AU/ml. We did not perform dilutions at that time. If we used the upper limit of AMR of the test (450 AU/ml), the calculated mean for group 3 was 310.1 AU/ml. 8 samples with results above 450 AU/ml were tested for antibodies against SARS-CoV-2 nucleocapsid protein (Abbott IGG test) and the results were negative.

Conclusion:

This data suggest that in these 8 particular cases all antibodies measured by the Beckman test were developed due to vaccination. In future studies, a dilution protocol will be developed for high level antibody measurements and the Beckman IGG-2 test will be used to monitor post vaccination anti-spike antibodies.

A-102

Evaluation of urinary CXCL-10 as a screening test for early identification of renal graft rejection by Meso Quickplex SQ 120

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Background: Kidney transplantation is the best treatment for children with end-stage kidney disease which improves survival, growth, cognitive development and quality of life. However, long-term allograft survival remains unacceptably compromised by acute or chronic forms of rejection. Currently, the non-invasive monitoring is serum creatinine (Cr) – but insensitive, and the gold-standard for accuracy is surveillance kidney biopsy – but invasive. CXCL-10 is a 10.8 kDa protein that are secreted by infiltrating inflammatory cells, renal tubular cells, and mesangial cells. IFN-γ induces the production of CXCL-10, which acts as a chemotactic agent for leukocyte recruitment in renal allograft rejection, and also mediates the Th1 helper response. Recently, urinary CXCL10/Cr has been shown to be a sensitive and specific biomarker for the diagnosis and prognosis of acute kidney transplant rejections in both adults and children. In order to bring this research biomarker into clinical practice, we fully evaluated the performance of CXCL-10 in urine samples based on the clinical and laboratory

standards institute (CLSI) guideline. **Methods:** CXCL-10 by Meso Quickplex SQ 120 (Meso Scale discovery) is a two-step, electrochemiluminescence immunoassay in a 96-well plate format. Within-run and between run precision tests were performed with both the quality control (QC) material from the company and urine samples from children post kidney transplant. The range of linearity was tested by mixing a high and low urine CXCL-10 sample at various ratios, and the recovery was assessed by spiking a urine sample with three levels of CXCL-10 calibrators. Freeze and thaw cycles, interference by hemoglobin, triglyceride, conjugated bilirubin and protein as well as lot-to-lot comparisons were also evaluated. Patient comparison was assessed using urine samples from both children and adults post kidney transplant by both the company calibrator and by the PeproTech calibrator. All samples were run in duplicate and had been tested with 3 different operators. **Results:** Within-run and between-run precision was <7% and <15% for both QC and patient urine samples, with the latter being slightly larger due to matrix effects. Linearity was up to 900 pg/L with a limit of quantitation of 2.45 ng/L. The recovery for urinary CXCL-10 ranged from 94% to 102%. CXCL-10 in urine was stable for up to 6 freeze and thaw cycles. No interferences were detected with proteinuria up to 5 g/L and conjugated bilirubin up to 475 umol/L. Only severe hematuria (Hb=5 g/dL) was able to interfere with CXCL-10 measurements, while triglyceride tended to interfere with CXCL-10 even at 1.5 g/L. The lot-to-lot variations showed %bias ranging from 5% to 22%. The correlation between Mesoscale and PeproTech showed R^2=0.9754 (CXCL-10 by Mesoscale=3.1198 *CXCL-10 by PeproTech - 0.0517) (n=290). **Conclusions:** Urinary CXCL-10 measured by Meso Quickplex SQ 120 is a robust and reliable test with good reproducibility. It is ready to be offered as a diagnostic biomarker for a real time implementation clinical trial to assess patients post kidney transplant. The strong correlation between the Mesoscale and PeproTech would allow a smooth transition for researchers who are used to interpreting CXCL-10 measurements by PeproTech calibrators.

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High-Sensitive Serum Biotin Method Development by Micro-liquid Chromatography Tandem Mass Spectrometry

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Background Biotin, also known as vitamin B7, is an essential coenzyme needed for many important biochemical reactions involving carboxylase enzymes. Biotin is ubiquitous in common foods and the recommended daily intake (RDI) for biotin is 0.03 mg[1]. People also use biotin food supplements. These over-the-counter products can contain up to 10 mg of biotin per serving. Biotin can be a potential interference for certain immunoassay. To determine biotin status in people and to assess potential interferences with immunoassays, a sensitive, accurate and reliable method is needed. The objective of this work is to develop a highly sensitive LCMS-MS method that can accurately detect biotin in serum over a wide concentration range. **Method** Sample preparation involves dissociation of biotin from binding proteins, isolating biotin via extraction and quantitation of biotin via liquid chromatography mass spectrometry. Protein dissociation was achieved by incubating 300 µL serum with 4% TFA for one hour at room temperature, followed by protein precipitation with methanol. Quantitation was performed using a stable isotope-labeled internal standard. Chromatographic separation of biotin was achieved within a 5 min run on a Waters nano-Ease M/Z HSS T3 1.8um 300um x 50mm column and followed by tandem mass spectrometry on a Thermo Electron triple quadrupole Altis mass spectrometer in positive electrospray ionization mode. The precursor and product ions of biotin and biotin-IS were monitored in selected reaction monitoring mode using the following transitions: m/z 245→227 (quantitation ion (QI) for biotin), m/z 249→231 (QI for biotin-IS), m/z 245→123 (confirmation ion (CI) for biotin) and m/z 249→194.8 (CI for biotin-IS). **Results and Conclusion** The measurement range of the method is 0.8 ng/ml - 450 ng/ml. The developed method has a 10-fold improvement in sensitivity compared to regular UPLC by utilizing microflow (10ul/min). This allows for measuring of biotin in supplement users and non-supplement users. The method demonstrates excellent linearity (R2 = 0.991-0.997), reproducibility and sensitivity. The presented LC-MS/MS method allowed for higher sensitivity than currently published MS-based method. The method allows for accurate measurement of biotin in the general population. The developed method will support large biomonitoring studies to establish correlation between increased biotin levels and potential interference when measuring other biomarkers using IAs.

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ELISA Quantitation of Creatine Kinase Isoform MM

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Background: Creatine Kinase (CK) is a dimeric enzyme that exists as three isoforms: BB, MM and MB. These isoforms are found in brain tissue, skeletal muscle and cardiac muscle, respectively. Levels of CK-MM are elevated in the serum of patients with muscular dystrophy. Elevated levels of CK-MM can be used as a biomarker for muscular dystrophy screening. **Methods:** A two-site sandwich immunoassay was developed with two commercially available anti-human CK-MM monoclonal antibodies (MAb). The capture MAb was passively coated to a 96-well microtiter plate. The open sites on the plate were blocked with a nonreactive protein solution. A second MAb was biotinylated and paired with streptavidin-horseradish peroxidase (HRP) conjugate for detection. Human CK-MM antigen was used as the substrate for determining linear range, limit of detection (LOD), limit of quantification (LOQ) and precision. Dilution linearity and spike recovery assessments were performed with human serum specimens (n=3) and sample diluent (n=3) prepared with human CK-MM antigen. Human CK-MB and CK-BB antigens were used to assess cross-reactivity at concentrations ranging from 0.20 ng/mL to 800 ng/mL. Dilutions of human CK-MM in sample diluent (n=5) were used to assess precision (intra-assay and inter-assay) and were assayed in four replicates on five different days. Recovery samples were diluted 100-fold and spiked with human CK-MM antigen. **Results:** The standard curve was generated via the 5-parameter logistic model within the plate reader software. This assay exhibited a linear range (R^2=0.9854) from 0.08 ng/mL to 80 ng/mL. The LOD was 0.02 ng/mL. The LOQ was 0.14 ng/mL. Intra-assay variation (repeatability) ranged from 4.5% to 10.5% with a mean of 7.2% (n=25). Inter-assay variation (reproducibility) ranged from 10.1% to 17.2% with a mean of 14.1% (n=25). Percent recovery for dilution linearity ranged from 92.8% to 109.0% with a mean of 103.3% (n=6). Spike recovery ranged from 65.5% to 110.0% with a mean of 85.8% (n=6). Cross-reactivity with human CK-MB averaged 8.7%. Cross-reactivity with human CK-BB was not observed. **Conclusion:** This study demonstrates that human CK-MM concentrations can be determined with a highly sensitive non-competitive ELISA without reliance upon measurement of CK enzymatic activity. It should be possible to use this method for muscular dystrophy screening in newborns, monitoring disease progression or monitoring response to therapy.

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Performance Evaluation of the VITROS® NT-proBNP II Assay* on the VITROS® ECi/ECiQ and 3600 Immunodiagnostic and VITROS® 5600/XT 7600 Integrated Systems

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Background:

The VITROS Immunodiagnostic Products NT-proBNP II assay quantitatively measures N-terminal pro Brain Natriuretic Peptide (NT-proBNP) in human serum and plasma (EDTA or heparin) on the VITROS® ECi/ECiQ and 3600 Immunodiagnostic and VITROS® 5600/XT 7600 Integrated Systems to aid in the diagnosis of heart failure (HF) and for the risk stratification of acute coronary syndrome and HF. The test is further indicated as an aid in the assessment of increased risk of cardiovascular events and mortality in patients who have stable coronary heart disease and in the assessment of HF severity in patients diagnosed with HF.

Method:

In this assay NT-proBNP present in the sample binds to horseradish peroxidase (HRP)-labelled detection antibody which is captured by biotin-conjugated capture antibody bound to Streptavidin coated microwells. The amount of bound HRP conjugate, which is directly proportional to the concentration of NT-proBNP, is measured by a chemiluminescent reaction.

Results:

The measuring range of the VITROS NT-proBNP II assay is 20.0 -30,000 pg/mL. In a CLSI-EP05-A3 precision study, 11 precision pools with mean NT-proBNP concentrations ranging from 30.9 to 24,700 pg/mL had within-run percent coefficient of variation (%CV) of 1.0% to 2.6% and within-laboratory %CV of 2.1% to 6.6%. Accuracy was evaluated consistent with CLSI document EP09 for 153 patient samples ranging from 24.5 - 23,400 pg/mL. A comparison of the VITROS NT-proBNP II assay on the VITROS 3600 with the commercially available Elecsys® proBNP II immunoassay using Passing-Bablok regression resulted in a correlation of VITROS 3600 = 0.95 *

Elecsys - 8.46 pg/mL with a Pearson correlation coefficient of 0.989. The VITROS NT-proBNP II assay Reference Interval (RI) was established as described in CLSI EP28-A3c for six subgroups based on age and gender from 385 female and 374 male healthy donors. Analysis of the central 95% was calculated using the Robust method. The overall RI lower limit is < 20.0 pg/mL and the RI upper limit is 217 pg/mL. A multicenter prospective study of 20 sites across the US (1020 subjects age ≥ 22 yrs) was conducted to establish the clinical performance characteristics of the VITROS NT-proBNP II assay for diagnosis or exclusion of HF in patients presenting to emergency departments with suspicion of acute or acute decompensated HF. Age-dependent HF rule-in cutoffs of 450, 900, and 1,800 pg/mL and an age-independent rule-out cutoff of 300 pg/mL, as recommended by the 2016 European Society for Cardiology guidelines, were evaluated versus independent cardiologist adjudicated diagnosis. An overall rule-in sensitivity of 91.53% (95% CI: 88.86 -93.73%) and specificity of 76.94% (95% CI: 72.89 - 80.65%) and an overall rule-out sensitivity of 99.08% (95% CI: 97.86 - 99.70%) and specificity of 50.52% (95% CI: 45.94-55.10%) were obtained.

Conclusion:

The VITROS NT-proBNP II assay has demonstrated acceptable analytical and clinical performance, excellent correlation to the Elecsys® proBNP II immunoassay, and robustness to interferences (≤10% bias). including hemoglobin and biotin.

*CE marked product. Product availability is subject to fulfilment of regulatory requirements in each market. Product is not commercially available in the United States.

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Antibody-free Quantification of Serum Chromogranin A by Targeted Mass Spectrometry

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BACKGROUND Chromogranin A (CgA) is a 48-kDa protein that serves as a sensitive, but nonspecific, serum biomarker for neuroendocrine tumors (NETs). Levels of CgA are increased in the presence NETs, making CgA the most practical, important, and useful serum marker for monitoring patients with NETs. Circulating levels of serum CgA are proportional to tumor burden, providing prognostic information in treatment response. Currently, serum CgA is measured using various immunoassays. However, limitations due to a lack of calibrator material and antibody standardization, and a reduced dynamic range requiring sample dilution, pose hurdles in implementing such tests in diagnostic laboratories. In contrast to immuno-based assays, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become an increasingly popular tool for protein quantification in the clinical laboratory. Commonly used techniques for protein quantification by LC-MS/MS include enzymatic digestion followed by selected-reaction monitoring (SRM) of a "proteotypic" peptide. This approach allows for measurement of the total amount of a protein, irrespective of post-translational modifications, truncations, or oligomerizations that may hamper detection by traditional immunoassay techniques. Mass spectrometry also has the ability to quantitate over a larger dynamic range than traditional immunoassays, decreasing the need for repeat analysis of specimens after dilution. Our goal was to develop and evaluate an antibody-free, LC-MS/MS-based method for CgA without these limitations.

METHODSCgA was extracted from 100 µL of serum using a mixed-mode anion exchange solid-phase extraction (SPE) plate, digested with trypsin, and analyzed by LC-MS/MS using well-characterized CgA calibration standards. The assay was analytically validated and compared with the CISBIO immunoassay using 200 serum specimens previously submitted for CgA analysis. Specimens with discordant results were reanalyzed by high-resolution mass spectrometry (HRMS)-based methods to assess the contribution of truncated and post-translationally modified forms of CgA.

RESULTS The assay had a linear range of 50 to 50,000 ng/mL, recoveries between 89% and 115%, and intra- and inter-assay imprecision <10%. The LC-MS/MS assay results showed a Pearson's r correlation of 0.953 with the CISBIO immunoassay, with CgA values 2- to 4-fold higher on average. Concordance for CgA between the 2 assays was 80.9% (95% CI 72.8%-89.2%), showing substantial agreement. Specimens that were discordant between the two assays (below the cutoff point with the CISBIO assay but 5- to 9-times above the cutoff point with the LC-MS/MS assay) showed elevations in all peptides spanning the full length of the CgA protein. However, truncation and post-translational modification, including 2 phosphorylation sites that had not been previously observed or predicted, did not appear to contribute directly to discordance between the 2 assays.

CONCLUSION Quantification of CgA by LC-MS/MS provides an analytically sensitive and reproducible alternative to commercially available immunoassays. The ability to quantitate over 4-orders of magnitude prevents the need to repeat samples on dilution, allowing for a greater than 2-fold increase in sample throughput per batch when compared to the current CISBIO immunoassay.

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Performance characteristics of an immunoassay for SARS-CoV-2 neutralizing antibodies detection

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Background: The outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally despite the extensive implementation of preventive health measures. The complex pathophysiology of COVID-19 seems to consist of multiple mechanisms, of which the immunopathological response to SARS-CoV-2 infection became a major area of interest. The innate immune system provides the first line of immunological defense against SARS-CoV-2 infection. A robust serological test to detect neutralizing antibodies (Nabs) to SARS-CoV-2 is urgently needed to determine not only the infection rate and humoral protection, but also vaccine efficacy during clinical trials and after large-scale vaccination. The current gold standard is the conventional virus neutralization test (cVNT) requiring live pathogen and a biosafety level 3 laboratory. **Objective:** To validate the performance of a Nabs detection ELISA assay in serum samples.

Methods: We validate the performance of the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript, USA) that detects total immunodominant NAbs targeting the viral spike (S) protein receptor-binding domain. Were tested five different cohorts of sera samples: 20 negative and 31 positive SARS-CoV-2 RT-qPCR samples, 18 samples with known levels of SARS-C0V-2 by cVNT, 10 pre-pandemic samples collected from healthy donors and 20 samples positives for other virus (HIV, dengue virus, cytomegalovirus and Epstein-Barr virus). The cutoff % inhibition was 20% as recommended by the manufacturer.

Results: The samples from patients with COVID-19 confirmed by PCR were collected between 5 - 63 days after the onset of symptoms. Among 31 participants with positive RT-qPCR, 19 (61.3%) had positive neutralization test results**.** The test reached 51.6% overall sensitivity. However, obtained 73% and 100% sensitivity for samples collected 14 and 20 days after the onset of symptoms, respectively. Thirty blood samples from negative control group (donors healthy and negative RT-qPCR) were negative for SARS-CoV-2 neutralizing antibodies as expected, yielding an overall specificity of 100%. There was only 44.4% agreement between the results of cVNT and NAbs, especially for higher % inhibition in the cVNT test. Possible decay of neutralizing antibodies due to the stability of the sample, whose collection and conservation conditions are unknown, could be an explanation for such divergences. We detected the potential cross-reactivity of antibodies against SARS-CoV-2 with HIV and cytomegalovirus.

Conclusion: In summary, we have addressed a serological test with an approach that enables the detection of NAbs in an easy, safe and rapid manner with high specificity and sensitivity. The sensitivity of the kit increases proportionally to the time of symptoms of the patient, being maximum 20 days after onset of symptoms, similar to the conventional serological kits.

A-112

Analytical Evaluation of Abbot Alinity Procalcitonin Assay in a Pediatric Hospital

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Background: Procalcitonin (PCT) is a marker of systemic bacterial infection and has been used to aid in the diagnosis of neonatal sepsis as well as antibiotic stewardship. The Alinity i clinical chemistry analyser (Abbott Laboratories) has recently been introduced as a next-generation in vitro diagnostics system. An assessment of the analytical performance of the Alinity PCT assay has not been reported in the literature. As part of a pilot project to assess the value of PCT testing in a pediatric emergency department, we completed an analytical evaluation of the PCT assay on the Alinity platform and compared it to the PCT assays on Abbott ARCHITECT, as well as the reference method, Thermofisher's Kryptor. The next step in this project is to evalu-

ate the performance of Abbott's PCT assay in clinical evaluation of febrile neonates presenting to the emergency department and compare its clinical value to C-reactive protein (CRP).

Methods: Precision, reference range verification, limit of blank (LOB), limit of detection (LOD) and lower limit of quantification (LLOQ) were verified according to CLSI guidelines. Residual sera with normal and elevated PCT were used to assess linearity across the analytical measurement range (AMR). By measuring 46 and 68 patient sera with PCT values spanning the AMR, pairwise method comparisons were performed between Alinity vs ARCHITECT, and Alinity vs Kryptor, respectively.

Results: At 0.20, 1.9, and 66 µg/L QC levels, the within-laboratory CVs for Alinity were 2.6%, 2.8% and 3.4%, respectively. PCT was ≤0.1 µg/L for 90% of serum samples collected from healthy volunteers. The Alinity assay was linear (slope=1.02, intercept=-0.33, R^2=0.999). In method comparisons, Alinity had Passing-Bablok slopes of 0.96 and 0.84 (intercepts=0.00 and -0.07; R^2=0.984, 0.933), against ARCHITECT and Kryptor, respectively.

Conclusion: The Alinity PCT assay met the manufacturer claims for precision, linearity, LOD, and LLOQ. The Alinity PCT assay performs very similarly compared to the PCT assay on its predecessor, ARCHITECT. However, it has a significant negative bias compared to the Kryptor assay. Since PCT's clinical decision limits have been obtained using the Kryptor assay, appropriate cut-offs for the Alinity and ARCHITECT assays need to be established to ensure accurate interpretation. The project on neonatal sepsis has been initiated and the results will be reported at a future conference.

A-113

Kappa and Lambda Immunofixation Screen versus Urine Protein Electrophoresis for the Initial Investigation of Monoclonal Proteins in Unconcentrated and Concentrated Urine.

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Background: Initial detection of a monoclonal protein (MCP) occurs in urine, before or instead of serum, In a small number of patients. A common approach is urine protein electrophoresis (UPE) followed by urine immunofixation (uIFE:G,A,M,K,L) when indicated. Samples may be concentrated ("c") or unconcentrated ("uc", neat). Alternatively, urine immunofixation screening (uIFS) may be performed initially, using a penta-antibody, combined or separate light chain (LC) approach. With UPE, interpretive comments may describe the protein pattern (e.g. glomerular); while uIFS usually reports MCP detected or not detected. Urine investigation remains useful, as serum free light chain testing is not 100% sensitive or specific. This study compares our current uc-UPE approach to uc-uIFS and c-uIFS for the initial investigation step; c-uIFS is used as the comparator (i.e. true positive) as uc-uIFE reports were retrospectively available for only 25% of patients.

Methods: 55 urines were selected based on their interesting UPE or case history. The uIFS had 3 lanes: total protein, kappa, lambda (8 samples per Sebia gel). The uc-UPE, uc-uIFS, and c-uIFS gels were circulated in unmatched sets to 7 biochemists, for three potential interpretations: negative (neg), positive (pos), questionable; where positive and questionable were both considered presumptive positives requiring uIFE. Consensus-neg or -pos represents 100% agreement (7/7).

Results: Table1 highlights the value of uc-uIFS in Grps4&5. Based on UPE alone, 35 patients would have uIFE ordered; as c-uIFS is confirmatory for LC "not detected in this sample", the same or fewer uIFEs (29 for Grps3-6) would be predicted when c-uIFS is the initial investigation. The heterogeneity of positive urine findings in different patients, and in the same patient over time, was evident in this study.

Conclusion: Immunofixation screening using concentrated urine and separate light chain lanes is preferrable for the initial investigation of MCP.

Table1: Unconcentrated UPE versus unconcentrated and concentrated uIFS

	Notes (pos = pos or questionable)	# samples (%) of 55	uIFE on UPE	uc-UPE consensus neg or pos	uc-uIFS consensus neg or pos	c-uIFS consensus neg or pos	Known MCP	uc - uIFE reported
Mean				32% pos	27% pos	48% pos		
Sensitivity	versus c-uIFS			34%	44%			
PPV	versus c-uIFS			51%	78%			
NPV	versus c-uIFS			53%	62%			
Grp1	uc-uIFS neg; c-uIFS neg	6 (11%)	12%	2 neg	6 neg	6 neg	1 (16%)	
Grp2	uc-uIFS mainly neg; c-uIFS mainly neg	20 (36%)	33%	6 neg; 2 pos	12 neg	0 neg	2 (10%)	4 - neg
Grp3	uc-uIFS mainly neg; c-uIFS 50% pos	10 (18%)	39%	4 neg; 2 pos	4 neg		2 (20%)	2 - neg
Grp4	uc-uIFS mainly neg; c-uIFS mainly pos	4 (7%)	54%	1 neg; 2 pos	1 neg		3 (75%)	1 - neg; 1 - pos
Grp5	uc-uIFS mainly neg; c-uIFS pos	7 (13%)	16%	5 neg; 1 pos	4 neg	5 pos	7 (100%)	1 - neg; 2 - pos
Grp6	uc-uIFS pos; c-uIFS pos	8 (15%)	38%	2 neg; 1 pos	8 pos	7 pos	8 (100%)	3 - pos

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Quantification of Plasma Catecholamines using LC-MSMS

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Background: The quantitation of plasma catecholamines (epinephrine, norepinephrine, and dopamine) in clinical diagnostic laboratories has traditionally employed liquid chromatography-electrochemical detection methods. However, these methods are susceptible to interference and may not achieve the desirable sensitivity for all 3 analytes simultaneously (low nmol/L endogenous levels). The objective of this study was to develop a quantitative LC-MS/MS method for plasma catecholamines that is fast, reproducible, sensitive and robust. **Methods:** Plasma specimens were prepared as follows: 50 uL of a mix containing heavy isotope-labelled catecholamines (D6-epinephrine, D6-norepinephrine, and D4-dopamine) in 0.1 M perchloric acid, followed by 20 mg of acid washed aluminium oxide, and adsorption buffer containing 14.3 mM Na_2EDTA and 1 M Tris (pH 8.6) were added to 500 uL of EDTA plasma. Samples were vortex mixed for 10 min and centrifuged, supernatant was discarded and alumina washed twice with ultrapure water. Catecholamines were extracted with 100 uL of 2% formic acid in water and 40 uL of the final extract was injected for LC-MS/MS analysis. Multiple reaction monitoring method was developed on a TQ 6500+ LC-MS/MS system (Sciex) in positive electrospray mode. ExionLC AD liquid chromatograph equipped with a Phenomenex Luna Omega Polar C18 column (150 x 2.1 mm, 3 um) was used for analyte separation. Mobile phase consisted of 0.2% formic acid in water (A) and methanol (B). Chromatographic separation was achieved in 7 minutes using gradient elution (0 - 100 % B over 5 minutes, followed by a column wash and equilibration). Calibrators (0.1 - 25 nM) were prepared in 1% BSA in phosphate buffer, Lyphochek Endocrine Control (Bio-Rad, Hercules, CA) was used for quality control. Two transitions per analyte (quantifier and qualifier) were monitored and normalized using the corresponding internal standard. Imprecision, linearity, dilution recovery, sensitivity, method comparison, carryover, interferences and stability were assessed. **Results:** Catecholamines were baseline separated with the following elution order: norepinephrine, epinephrine, followed by dopamine. The method was shown to be linear ($r^2 > 0.99$) in the 0.1 to 100 nM range (for each catecholamine). No significant carryover was observed. The lower limit of quantification (LLOQ) was 0.1 nM for epinephrine, and norepinephrine, and 0.05 nM for dopamine. Excellent reproducibility was observed for all compounds (CV < 5%). Among samples subject to interference on the in-house HPLC-ECD method, the LC-MS/MS method yielded no interference and an accurate result was obtainable. **Conclusions:** A quantitative

method for plasma catecholamines using LC-MS/MS and a very simple, well known extraction procedure was developed. The described method achieves the required sensitivity, is cost effective, and can be easily implemented in clinical diagnostic service.

A-115

Performance evaluation of a newly developed multiplex specific IgE detection immunoassay system using the AdvanSure™ MAX108

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Background: The identification of clinically meaningful specific immunoglobulin E (sIgE) is essential for the diagnosis and management of various allergic diseases. The multiple allergen simultaneous tests (MAST) is increasingly used as a screening tool for allergic diseases in Korea recently. This study aimed to examine the performance of a newly developed version of AdvanSure™ MAX108 with 107 different allergens compared to an old version, as well as the ImmunoCAP. **Methods:** Remnant sera of 120 patients for whom the conventional MAST were requested, were collected and stored at - 20°C. AdvanSure™ MAX108 (LG Chem, Seoul, Korea) with 107 allergens using AlloView 2.0 analyzer, were compared to the old version, Advansure™ AlloScreen (LG Chem, Seoul, Korea) with 96 allergens using AlloStation Smart II. The concentrations of sIgEs to each allergen in two different multiplex assays were categorized from class 0 to 6. Results higher than class 1 were defined as positive. Results of two or more class difference between two different assays for each allergen were regarded as disagreement. The ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) sIgE single-plex assay for selective allergens was performed to assess the sensitivity of newly added fifteen allergens in AdvanSure™ MAX108, or resolve discrepant results between two different MASTs. **Results:** In qualitative comparisons, the total agreement between two multiplex sIgE immunoassays showed overall 97.4% (kappa=0.78) for 92 matched allergens. Agreement rates for each allergen ranged from 80.0% to 100.0%. Regarding newly added fifteen allergens in the Advansure™ MAX108, total agreement with ImmunoCAP were ranged from 71.4% to 100.0%. Among the total of 11,040 allergen sIgE results, 455 (4.1%) results showed discrepancy between two different MASTs. Among the available 330 (3.0%) comparison tests using ImmunoCAP sIgE, Advansure™ MAX108 showed higher agreement (80.0%) than Advansure™ AlloScreen (43.3%). Qualitative agreement for total IgE (cut-off, 100 IU/mL) between two MASTs was 95.9%. **Conclusion:** The performance of the newly developed AdvanSure™ MAX108 with 107 different allergens is a powerful tool for its better sIgE detection capability than the old version, with expanded panel in short assay time.

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Comparing two rapid SARS-CoV-2 antigen detection assays for laboratory diagnosis of COVID-19 in Brazil.

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Background: The COVID-19 pandemic continues to spread across the world. Because of this, tests must be increasingly faster, simpler and more accurate for the detection of SARS-CoV-2 infection. Rapid tests (RTs) for qualitative detection of SARS-CoV-2 antigen (Ag) could play a critical role in the fight against COVID-19, contributing to disease and outbreak management by enabling prompt and accurate public health surveillance, prevention and control measures. **Objective**: This work aimed to compare the analytical performance of two RT for the qualitative detection of SARS-CoV-2 Ag available in the Brazilian market regarding their analytical performance.

Methods: This was an observational, analytical, and concordance study, in which previously defined positive and negative samples, based on their RT-PCR (gold standard) results. The kits Panbio™ COVID-19 Ag Rapid Test Device (Abbott, Germany) and COVID-19 Ag ECO Teste (ECO Diagnóstica, Brazil) were assessed for their analytical performance and the results compared to RT-qPCR (golden standard) technique. Statistical analyses were calculated, considering a 95% CI and $p<0.05$.

Results: The mean age of patients was 39.8 (± 15.6) years, with 50.5% was female and 49.5% was male. Data collection was performed when patients had an average of 2.95 (± 3.75) days of symptoms. Among positives patients in gold standard test, 70% male and 30% was female. Panbio™ COVID-19 Ag Rapid Test Device kit has 55.6% sensitivity and COVID-19 Ag ECO Teste kit obtained 72.7%. Both kits reached 100% specificity, positive predictive value (PPV) = 1.0 and negative predictive value (NPV) = 0.93. Considering that the tests evaluated are intended to be used for the diagnosis of the disease, they must therefore be highly sensitive, which was not found in any of the tests evaluated. These low sensitivity values founded could be explained by a methodology limitation, that is less sensitive than RT-qPCR (gold standard method).

Conclusion: An accurate diagnosis is essential to prevention, control measures and public health surveillance in these times of pandemic. Evaluated test did not reach the sensitivity to be used as a diagnosis test, even with an excellent specificity.

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Evaluation of rapid antigen test for detection of SARS-CoV-2 virus in saliva

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Background: Detection of SARS-CoV-2 on patient specimens is the first crucial step for the guidance of treatment, effective infection control in the hospital and control of infection in the community. Screening of infection in suspected cases with real-time PCR (RT-qPCR) in respiratory specimens is the gold standard test recommended by the WHO. Although this diagnostic test is useful in the identification of patients with COVID-19, the process of collecting upper and lower respiratory tract specimens increases the risk of exposure to viral droplets, and there is a patient burden. Therefore, an alternative specimen, which can be self-collected, to diagnose COVID-19 is desirable for the clinical management of COVID-19 during this pandemic era. Antigen (Ag) rapid test directly identify proteins produced by SARS-CoV-2 virus in respiratory secretions. In contrast to the RT-qPCR, the Ag rapid test are relatively inexpensive, simple to perform, do not require infrastructure, and enable obtaining point-of-care results within a few minutes. **Objective:** To evaluate the performance of a commercially available kit for the qualitative detection of SARS-CoV-2 Ag in saliva samples.

Methods: We evaluated the performance of the Covid Ag Oral Detect Kit (Eco Diagnóstica, Brazil). Patients >18 years old with symptoms of Covid-19 were invited to participate in this study. Saliva and nasopharyngeal swabs specimens were collected on the same day. The saliva Ag rapid tests were performed according to the manufacturer's instructions and the results were compared with the RT-qPCR results. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated to assess diagnostic performance.

Results: Between February and March 2021, 29 patients were recruited. The mean age of patients was 39.4 (± 13.9) years, with 69% was female and 31% was male. Data collection was performed when patients had an average of 3.52 (± 2.27) days of symptoms. Using nasopharyngeal swab RT-qPCR as the reference standard the sensitivity and specificity of Covid Ag Oral Detect Kit were 66.7% and 100%, respectively. Directly comparing the sensitivities of tests using saliva and other upper or lower respiratory specimens is difficult because the viral loads in the clinical specimens must vary with time. The PPV and NPV were 100% and 86.7%. The clinical usefulness of saliva specimens for diagnosing COVID-19 remains controversial because the reported diagnostic sensitivity varies widely between studies.

Conclusion: Saliva might be an alternative specimen for the diagnosis of COVID-19. This method could facilitate the diagnosis of the disease, given the simplicity of specimen collection. However, due to the low sensitivity of this kit for a diagnostic test, it is necessary to increase the sample size to better evaluation.

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Performance evaluation of automated immunoassays for the detection of anti-SARS-CoV-2 antibodies

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Background: Efforts to understand and control the coronavirus disease 2019 (COVID-19) pandemic have led to the detailed characterization of the humoral response to SARS-CoV-2 infection. Serological tests can be used for symptomatic individuals for which RT-PCR testing was either not performed at the time of acute illness or for which nasopharyngeal swab result was found to be negative, and also for epidemiological studies. However, the relevance of serological tests is highly related to their clinical performance, hence antibody assays with good sensitivity and specificity are needed. **Objective:** The aim of this study was to assess the analytical performances of six automated tests for detecting SARS-CoV-2 IgM and IgG antibodies in human serum or plasma.

Methods: Samples were tested using the following commercially available automated immunoassays according to manufacturer's instructions: Alinity i SARS-CoV-2 N IgG and IgM (Abbot, USA), LIAISON® SARS-CoV-2 S1/S2 IgG and IgM (Diasorin,

Italy) and Access SARS-CoV-2 RBD IgG and IgM assay (Beckman-Coulter, USA). The tests were performed according to the manufacturer's instructions. This study included serum samples from patients with RT-PCR confirmed SARS-CoV-2 infection, patients with negative RT-qPCR results and pre-pandemic samples collected from healthy individuals (negative control group. The sample size varied between kits (80 - 300 samples). To assess the sensitivity we choose the real time RT-qPCR method as the gold standard. Time from onset symptoms also was used to determine sensitivity. Specificity and sensitivity for each serological test were estimated considering results of the negative control group and positive RT-qPCR samples, respectively. Serum samples with a potential cross-reaction to the SARS-CoV-2 immunoassays were investigated.

Results: Performance of Abbott assay showing 85% overall sensitivity for IgM for patients >8 days after symptoms onset and 100% specificity. Sensitivity for IgG was 96 % more than 15 days post symptom onset and 99.3 % specificity. Performance of Diasorin assay showing 78.5% overall sensitivity for IgM for patients >8 days after symptoms onset and 96% specificity. Sensitivity for IgG was 86.7 % more than 15 days post symptom onset and 100 % specificity. The Beckman assays showed overall sensitivity for IgM of 80 % for patients > 8 days after symptoms onset and a specificity of 98 %. Sensitivity for IgG was 79.4 % more than 8 days post symptom onset and 99.2 % specificity. All patients with positive RT-qPCR and >20 days after symptoms onset were positive for serological assays. We detected the potential cross-reactivity of IgM antibodies against SARS-CoV-2 with dengue virus (Abbott) and high rheumatoid factor (Beckman). Potential cross-reactivity of IgG antibodies against SARS-CoV-2 was detected with Epstein-Barr virus, Herpes virus and chikungunya virus (Beckman).

Conclusion: These data show the high sensitivity and specificity of the CLIA assays, especially of the Abbott assays. When interpreting sensitivity values, the time from onset of illness in COVID-19 patients needs to be considered for. Studies showed that the timeline for the detection of immune-response antibodies is critical to give accurate serological test results. Inappropriate diagnostic timing may lead to false-negative results, especially at an early stage of infection.

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Development and validation method to identify 17α-hydroxyprogesterone in serum samples by LC-MS/MS

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Background: 17α-hydroxyprogesterone is an endogenous progestogen steroid hormone produced by the adrenal cortex and gonads, and the enzyme 17α-hydroxylase is responsible for synthesis of this compound from progesterone. When levels of 17α-hydroxyprogesterone are elevated and the levels of androstenedione (A4) and testosterone (T) are elevated too, is indicative of congenital adrenal hyperplasia (CAH) in the newborn. Moreover, it can be present in non-classical CAH, which occurs in children and adolescents. Since it is necessary a very sensitive and selective technique to analyze 17α-hydroxyprogesterone and other steroids in serum, Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been used to determine these compounds. **Objective:**To develop and validate a method for quantify que presence of 17α-hydroxyprogesterone in serum samples by LC-MS/MS.

Methods: The analysis was performed in LC-MS/MS using Solid Phase Extraction (SPE). For this extraction, was used an Oasis Prime HLP 96-Well micro elution plate. The solutions used to the extraction were, as the first mobile phase, 2 mM of ammonium fluoride in water, and the second mobile phase was constituted of 2 mM of ammonium fluoride in methanol. An Acquity UPLC BEH C18 130Å column (1.7 µm, 2.1 mm X 100 mm) by Waters was used for this analysis. The performance was compared with a reference laboratory and 30 real samples was analyzed.

Results: The current method was linear in a range of 5 to 20,000 ng/dL. It was observed that the matrix had an important effect on the method precision. For this reason, the calibration curve in the matrix was adopted to quantify the routine samples. Carryover was not observed. The recovery range achieved was between 93.7 and 103.5%. The limits of detection and quantification were 8 and 11 ng/dL, respectively. The intra and inter-assay precision values were between 0,7 and 13.3%, and 3.7 and 13.2%, respectively. The method showed a good correlation with reference laboratory ($r > 0.99$).

Conclusion: The analytical method in this study proved to be adequate for 17α-hydroxyprogesterone analysis in serum samples since it attended to all required criteria. The method showed good results for all parameters evaluated.

A-120

An analytical method by LC-MS/MS for identification of excessive ethanol consumption in hair samples: a development and validation.

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Background: There is a particular interest in forensic and clinical medicine related to alcoholism since alcohol is a psychoactive substance widely used. Ethyl glucuronide (EtG) is a phase II direct ethanol metabolite and has been used to detect alcohol abuse and monitor alcohol abstinence. Since it is necessary high sensitivity and selectivity to the analysis of this compound, the enzymatic and hematological alcohol markers, which are traditionally used, cannot be considered satisfying to the assessment of alcoholism. In this sense, the use of sensitive techniques, for instance, Liquid Chromatography-Tandem Mass Spectrometry, to detect low concentrations of EtG incorporated in hair, can be an important tool for this assessment. **Objective:** to develop and validate an analytical method for evaluate the presence of Ethyl Glucuronide (EtG) in hair samples.

Methods: The analysis was performed in Sciex 6500+ LC-MS/MS QTrap. The extraction procedure involved the addition of an extraction solvent, internal standard (EtG-D5), milling, heating in an ultrasonic bath, and filtration using the Collar Vacuum System. The solutions used in the UHPLC system were an aqueous solution with 0.1% of formic acid as the first mobile phase and 0.1% of formic acid in acetonitrile as the second mobile phase. The column used was a Poroshell 120 PFP (Agilent Technologies) for the efficient chromatographic separation.

Results: The current method is selective since it does not suffer any significant interference related or not with the evaluated analyte. It was observed that the matrix had an important effect on the method precision. For this reason, the calibration curve in the matrix was adopted to quantify the routine samples. The method was linear in a range of 15.0 and 176.0 pg/mg. All the replicates analyzed in concentrations above and below the cut-off showed a coefficient of variation smaller than 20%, which confirms the technical competence of the method in reaching the legal cut-off value for the wide-ranging toxicological examination detection window. The repeatability, reproducibility, and instrumental repeatability showed results below 20%. The recovery of the method showed a range between 80 and 120%. The limits of detection and quantification were 10.7 and 15.0 pg/mg, respectively. It was not identified the presence of carryover in samples until 2,000 pg/mg.

Conclusion: The analytical method in the study proved to be adequate for EtG analysis in hair samples since it attended to all required criteria. The method showed adequate selectivity, sensitivity, and linearity that contemplated the cut-off recommended for chronic ethanol consumption. The repeatability, reproducibility, and accuracy were satisfactory in the limit of quantification and all over the cut-off, reinforcing its applicability as a confirmatory test.

A-121

Reliable Blood Grouping With a Smartphone-Powered Quick Response Microfluidic Card

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Background: Rapid and reliable blood grouping plays an essential role in various biomedical and forensic scenarios. However, identification of weak blood group agglutinations remains challenging due to the inevitable environmental interference and low precision during the process of result readout. **Methods**: Herein, we invented a disposable and handheld microfluidic platform of flipping QR-code identification with prompt error-discrimination (FLIPPED) features for accurate blood grouping by using a commercially available smartphone. For better identification of weak agglutination samples, a linear regression curve between the blood HCT value and resultant color change was established *via* machine learning. Additionally, an automatic correction algorithm was designed to remove any potential errors from scanning angles and ambient light intensities. **Results**: A reliable blood typing platform was devised, which can detect ABO, Rh, MNS, P, Kell, Kidd, and Lewis blood groups within 30 s to 3 min. An accuracy of 100% was observed in a total of 450 clinical blood samples in varied ambient conditions, validating the robustness of the proposed FLIPPED assay. Additionally, no any mistyping was observed for blood samples with different HSA concentrations, bilirubin levels, and total cholesterol levels. **Conclusion**: The proposed FLIPPED blood grouping assay possesses the characteristics of enhanced and simplified workflow along with wireless transmission, offering the potential for being developed into a highly compact, automatic, efficient, and low-cost universal platform for quantitative analysis of a variety of biological and pathological biomarkers.

A-122

Tracking Single Small Extracellular Vesicles via Surface Self-Assembly

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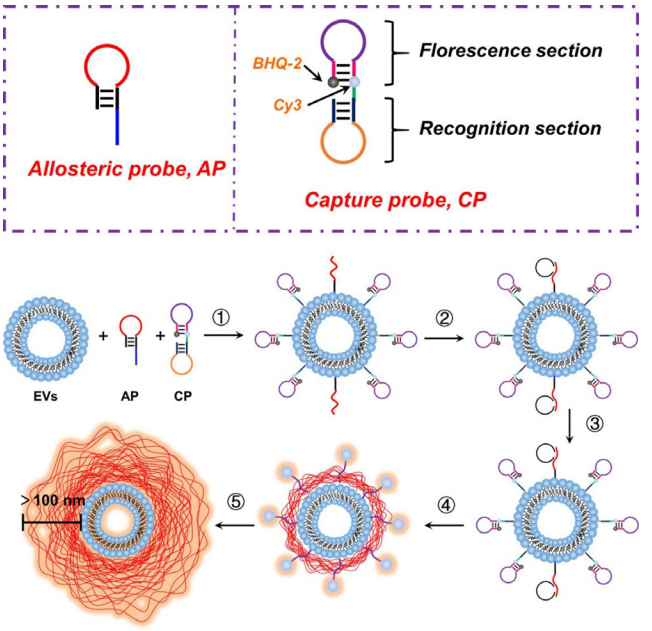
Background: Simultaneous determination of the amount and analyze membrane surface proteins of small extracellular vesicles (sEVs) whit a diameter among 30-150nm is of great significance to both fundamental medical researches and translational researches. But no existing methodologies could reliably track single sEV and analyze its surface protein profiles in a reliable manner. **Methods:** We established an RCA-assisted Surface Self-assembly (RaSSa) technology for detection of reliable detection of EVs with diameters less than 50 nm. In the proposed method, rolling circle amplification (RCA) technology was utilized to self-assemble multilayers on the surface of sEVs in the presence of Mg2+, by which the particle size of EVs could be enlarged to a level that can be recognized by commercial flow cytometry. We then investigated its analytical performance, including the linear range and sensitivity by using A549 cell secreted sEVs as a model. We also studied the relationships between sEVs and several cancers.**Results:** The RCA products could automatically self-assemble into a nanoshell on the surface of sEVs after binding to CD9 and CD63 that are over-expressed in the sEV membrane. Meanwhile, according fluorescence increases could be observed conveniently after hybridization. We noticed a single sEV could increase its size from 50nm to 150nm after 2 h self-assembly, by which it could be conveniently sorted by commercial flow cytometer. Eventually, a detection linear from 102 to 106 particles/μl was observed. Furthermore, we have observed the amounts of HSP70+ sEVs extracted from lung cancer patients were significantly higher than those from volunteers (P<0.05). **Conclusion:** We depicted here a novel RaSSa strategy for simultaneously analyzing the amounts of sEVs and its surface membrane proteins. By integrated recognition of two membrane proteins so as to assure high specificity, the proposed assay could be developed for liable sEVs tracking in clinical diagnosis.



A-123

Performance of the Abbott Architect immunochemiluminometric NTproBNP assay

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Background: NTproBNP is an important cardiac biomarker for heart failure (HF). HF diagnostic thresholds for NTproBNP derived from the Roche assay are age-related - 450 (<50y), 900 (50-75) and 1800pg/mL (>75y) respectively. We have evaluated the performance of the Abbott NTproBNP assay on the Architect i2000 analyzer against the Roche electro-chemiluminescence immunoassay on the Cobas e801 analyzer.

Methods: The Abbott NTproBNP is a two-step chemiluminescent microparticle immunoassay. Test imprecision was assessed on 3 levels of Abbott controls. Limit of Blank (LoB) and Limit of Quantitation (LoQ) were determined. Correlation studies were performed on leftover sera from 297 consecutive patients (age 21-107 years, mean 73.0±14.7; F=144) on whom NTproBNP had been tested. NTproBNP (Abbott) was measured in another 388 (M=202) relatively well ambulatory subjects (<50y 50-69 yrs=105, F 61; >70 yrs=115, F 56) without heart disease or renal dysfunction (eGFR >90mL/min). Measuring range verifications were performed on serum pools run in duplicates for each level. Statistical analyses were performed with MedCalc Statistical Software v19.8 (Ostend, Belgium).

Results: Inter-assay precision (CV%, level) were: 4.1% (139.5pg/mL), 3.7% (521.6) and 4.5% (5053) respectively. LoB was 1.2pg/mL (claimed 2.6) and LoQ was 5.5pg/mL (claimed 8.2). The assay demonstrated linearity from 1.2-41,500pg/mL. Passing-Bablock regression analyses between the Cobas and Abbott NTproBNP over a concentration range of 12.3-32068pg/mL gave a slope of 1.18 (95% CI 1.17-1.19) and intercept of 1.217 (95% CI -1.497 to 4.329); Spearman correlation coefficient was 0.999 (p<0.0001). There is an age-related increase in Abbott NTproBNP for cardio-renal healthy subjects (Figure), the 99th percentile of which are 399 (<50y, n=145), 846 (50-74y, n=183) and 1831pg/mL (>75y, n=60) respectively.

Conclusion: The Abbott NTproBNP assay performs well, agrees closely with Roche results and the Roche HF thresholds can be equally applied to the Abbott values. The Abbott assay provides laboratories with an alternative for NTproBNP measurement.

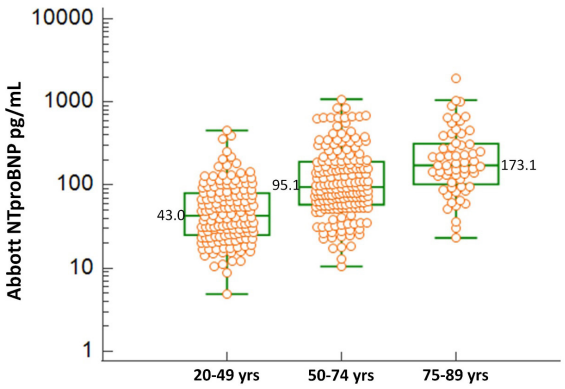

Figure. Distribution of NT-proBNP in cardio-renal healthy subjects (n=388)

A-124

The POC assay PATHFAST hs-cTnI provides interchangeable and comparable results in whole blood and plasma

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Background: The numbers of patients presenting to emergency departments with suspected myocardial infarction (MI) are still increasing. It is very important to determine cTn in the shortest possible time upon admission of the patient. It has been shown that the POC assay PATHFAST hs-cTnI allows a similar diagnostic performance for rule-in and rule-out of MI using the ESC 0/1 hour protocol like the Abbott Architect hs-cTnI assay (Clin Chem 2019; 65(12):1592-1601.) However, the clinical performance of PATHFAST hs-cTnI was evaluated by testing batch-wise from frozen plasma aliquots, and not from fresh whole blood specimen. According to experience, the main application in practice in the emergency department is POC testing from whole blood. The aim of the present study was to compare PATHFAST hs-cTnI concentrations measured from fresh plasma and whole blood directly.

Methods: We compared whole blood and plasma in parallel by using the PATHFAST hs-cTnI, (LSI Medience, Japan), which received FDA approval for the use as a POC test system. The test-specific limit of detection (LoD) measured in plasma is 2.33 ng/L, the 99th percentiles are 28 ng/L (90% CI, 20-30), overall; 20 ng/L (90% CI, 13-30), 352 women; and 30 ng/L (90% CI, 21-37), 382 men. The measurable percentage of healthy subjects between LoD and 99th percentile was 66.3% (Clin Biochem. Jun;2018;56:4-10).

Results: Of 318 paired samples (EDTA whole blood and EDTA plasma), 94 samples were not eligible due to missing hs-cTn values, or hs-cTnI values below the LoD. The remaining 224 sample pairs were used for method comparison. The Passing-Bablok regression equation showed a linear correlation described by the equation y = 0,218 + 0,824 x . The comparison between whole blood and plasma yielded a correlation coefficient (r) of 0.99. The bias measured for whole blood versus plasma were between +52 and -82%. In the Bland Altman plot the limits of agreement (LoA) did not exceed the maximum allowed difference of 90% between methods. Results from plasma and whole blood were considered to be in agreement, indicating that they can be used interchangeably. Inter-rater agreement between elevated and normal plasma and whole blood based on the 99th percentile URL yielded a weighted Cohen´s Kappa of 0.93 (95%CI: 0.89-0.98) which indicates a very good agreement (>0.90) between methods. Precision was evaluated according to CLSI ep 15 by serial measurements in parallel in whole blood and plasma on four different days with different cTnI concentrations by using suitable selected patient samples. Whole blood coefficient of variation was 17.7 and 5,2% between mean concentrations of 3.8 and 16.4 ng/L, respectively. Of particular note is the finding that precision was higher in whole blood than in plasma.

Conclusion: For convenience and in order to avoid the time-consuming preparation of plasma, the ability of a POCT analyser to process whole blood samples gains importance. Our data show that whole blood can be used interchangeably with plasma for testing of hs-cTnI by using PATHFAST hs-cTnI.

A-125

Detection of SARS-CoV-2 in multi-sample pools: a fundamental tool to response efforts to pandemic

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Background: The recently-emerged SARS-CoV-2 is responsible for a current pandemic of unprecedented scale. The assertive and reliable laboratory diagnosis of this virus is today a global public health priority. Due to the high demand of molecular tests for the diagnosis of SARS-CoV-2, some countries experience an acute shortage of reagents important for the performance of the tests, with the possibility of interruption of the tests. The pooling diagnostic test that employs a set of samples is usually used in cases such as this, aiming at reducing the costs of reagents and consumables to perform the examination. The objective is to establish ideal parameters for sample group testing for the detection of SARS-CoV-2. **Methods:** The study design was performed according to FDA recommendation that includes testing each sample individually and using your proposed pooling strategy. The individual samples, as well as the pools, were included in the same RNA extraction protocol, and the same aliquot of sample was used for testing individual samples, as well as creating the pools for RNA extraction. The pools in this study were create with n=5, of which negative pools containing five negative samples and positive pools containing one positive and four negative samples. The validation included 20 negative pools, and 30 positive pools that were separated into three groups based on the Cycle Quantification values (Cq): ≤20, 21-30 and >31 (group A, B and C, respectively). **Results:** A total of 50 pools were evaluated in this study. This included a total of 250 nasopharyngeal swab samples which had already been tested for SARS-CoV-2 using established methods. Of these, all negative and positive pools were confirmed by individual sample testing (100% concordance), including all groups (A, B and C). On an average, Cq values obtained with samples pooled exceeded individual sample by 1.87±1.56 cycles. The Cq value range of positive samples included in pools and individual samples ranging from 16.46 to 38.36 cycles and 13.3 to 35.64 cycles, respectively, demonstrated excellent reaction efficiency. **Conclusion:** The results showed that the described pooling method for COVID-19 can be applied immediately in current clinical testing laboratories. The high sensitivity of optimized real-time RT-qPCR assays makes pooled testing a potentially efficient strategy for resource utilization particularly when positivity rates for particular regions or groups of individuals are low. Such implementation would enabling the expansion of detection in the community, as well as in specific groups, such as hospitals, schools, industry or factory shifts.

A-126

ID NOW COVID-19 platform: a new molecular detection of SARS-CoV-2 by isothermal nucleic acid amplification technology.

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Background: The emergence of acute respiratory disease caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that potentially impact public health worldwide has been a topic of interest in clinical research. Therefore, molecular methods are now an important tools in all laboratories for routine detection of this virus. The ID NOW COVID-19 assay performed on the ID NOW Instrument is a molecular diagnostic test that uses isothermal nucleic acid amplification technology for the qualitative detection of SARS-CoV-2 viral RNA nuclei in direct nasal, nasopharyngeal or throat swabs of individuals suspected of COVID-19 contamination. **Objective:** To evaluate the performance of molecular detection of SARS-CoV-2 by the ID NOW COVID-19 platform (Abbott).

Methods: A total of 43 nasopharyngeal swabs were selected from symptomatic patients from laboratorial routine of Hermes Pardini Institute (IHP). All samples were performed “blindly” and the results were compared with those of RT-qPCR. The collected samples were kept at room temperature and processed within a maximum of 2 hours after collection, according to the manufacturer’s instructions.

Results: Agreement analysis was performed between the ID NOW and RT-qPCR and 100% agreement was found. Of the 43 samples analyzed, 11 positive samples on RT-qPCR were positive on ID NOW COVID-19 and 32 negative samples on both methodologies.

Conclusion: The ID NOW COVID-19 assay showed excellent performance and can be an interesting option of choice in hospitals and patient testing environments due to the speed and accuracy of the results. However, it´s essential to highlight that this validation was performed in a pandemic period, where there was a high rate of circulating viral load, since the literature reports low sensitivity (reaching up to 47.0% false-negatives) of the ID NOW test for samples with low viral load. Therefore, this information must be taken into account as the circulating viral load decreases.

A-127

Validation and verification of Alinity m SARS-CoV-2 Assay (Abbott)

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Background: A novel severe acute respiratory syndrome-related coronavirus, SARS-CoV-2, is the virus behind the global COVID-19 pandemic. Testing for acute SARS-CoV-2 infection is a fundamental tool in the public health measures taken to control the COVID-19 pandemic. Polymerase chain reaction assay with real-time reverse transcription (RT-PCR) is considered the gold standard used for the confirmatory diagnosis of symptomatic patients, as well as, increasingly, for the screening of asymptomatic contacts and individuals at risk. Alinity m SARS-CoV-2 is a RT-PCR indicated for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal swabs from patients suspected of being infected with COVID-19. Objective: This study aims to validate and verify the accuracy of the Alinity m SARS-CoV-2 Assay (Abbott, USA), comparing with other routine tests.

Methods: The assay was performed according to the manufacturer's instructions. A total of 335 previously tested samples by TaqPath COVID-19 RT-PCR (Thermo Fisher, USA) (n=189) and by Allplex 2019-nCoV-2 Assay (Seegene, Republic of Korea) (n=156) were selected and analyzed by Allinity m assay. Positive samples were separated into groups according to Cts value (Group 1: Ct<24, Group 2: Ct 25-30 and Group 3: Ct>30).

Results: Comparing the Alinity m with other kits, it was observed that the negative agreement with TaqPath was 76% (60/79) and Allplex was 97.3% (73/75). The positive agreement with TaqPath was 96.2% (51/53), 91% (10/11) and 94.4% (34/36) in group 1, 2 and 3, respectively. Regarding the Allplex, the positive agreement was 100% in all groups. The global positive agreement was 95% and 100% to TaqPath and Allplex, respectively.

Conclusion: The Alinity m demonstrated a high positive agreement with the others kits, and a higher frequency of positive results when compared with the TaqPath. However, two factors must be taken into accounts such as samples with low viral load and the difference in genetic targets surveyed. The Alinity and Allplex kits detect the RdRp gene, a target that is not detected by the TaqPath kit, which could justify the greater negative agreement among the former. Nevertheless, the Alinity m SARS-CoV-2 assay showed good accuracy for the SARS-CoV-2 molecular detection and can be used in the laboratory routine for diagnosing COVID-19.

A-128

Saliva is a novel sample matrix for the molecular diagnostic of COVID-19?

J. d. Silva, D. A. G. Zauli. *Grupo Pardini, Vespasiano, Brazil*

Background: The assertive and reliable laboratory diagnosis of SARS-CoV-2 is today a worldwide public health priority. Most samples are collected from patients with clinical suspicion of infection using a nasopharyngeal and oropharyngeal swab. However, this collection is extremely uncomfortable for the patient, some countries suffer from problems of lack of swabs in market, and there is a high rate of errors in collection that adversely affects the accuracy of test. The use of saliva as a sample for molecular diagnosis of SARS-CoV-2 has some advantages. Saliva can be easily supplied by the patient; it does not require specialized personnel for its collection. In addition, the comfort of the procedure is significantly greater when compared to the procedure with swab or nasopharyngeal sputum. **Objective:** To evaluate performance of the molecular diagnosis of SARS-CoV-2 by RT-qPCR in saliva as a sample matrix. **Methods:** A total of 101 swab samples with previous RT-qPCR results were used. The samples were collected randomly and at same time from patients who were performed SARS-CoV-2 test by nasal swab in Grupo Pardini laboratory. The paired samples were extracted with MagMAX Viral/Pathogen Nucleic Acid Isolation Kit and RT-qPCR was performed with TaqPath COVID-19 CE-IVD RT-PCR Kit (both Thermo Fisher) with evaluation of viral targets: ORF1ab, N and S gene. Among total, there were 74 negative and 27 positive samples with a Cq (Cycle Quantification) range between 13.63 and 32.43. **Results:** The molecular diagnosis of SARS-CoV-2 by RT-qPCR in saliva demonstrated a sensitivity of 90% (24/27) and specificity of 100% (74/74) compared to nasal swab samples. Although the sensitivity of test was affected by samples with a low viral load (Cq>30), the test obtained an optimal efficiency of reaction. The precision demonstrated optimal repeatability and reproducibility.**Conclusion:** This validation demonstrated an equal performance for the viral targets detected in both swabs and saliva samples. Therefore, saliva has become an important sample matrix for detecting infectious diseases including SARS-CoV-2. Molecular testing of saliva using RT-qPCR, a more comfortable and quick method, is useful for the diagnosis of COVID-19, without great loss of sensitivity and specificity compared to nasal swab samples.

A-129

Does more sensitive thyroid hormone assays improve the pituitary thyrotropin-free thyroxine relationship?

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Background: Sysmex Corporation Japan recently introduced thyroid hormone assays - thyroid-stimulating hormone (TSH) and free thyroxine (fT4), on its HISCL-5000 immunoassay analyser. The HISCL-5000 is a fully automated immunochemistry analyzer that employs a 1-step chemiluminescent enzymometric immunoassay (CLEIA) using CDP-Star (chloro-dioxetane-chloro-phenyl-phosphate) as substrate. The claimed Sysmex assay sensitivity for TSH (0.002 mU/L) and fT4 (3.2 pmol/L) is better than that for the Abbott Architect TSH (0.004 mU/L) and fT4 (5.5 pmol/L). We wondered if the improved Sysmex assay sensitivity will result in better pituitary TSH-fT4 relationship. **Methods:** The HISCL-TSH is a sandwich assay. Alkaline phosphatase-labelled anti-TSH reacts with TSH in the sample followed by addition of streptavidin-coated microparticles. When biotinylated anti-TSH is added, it forms a complex with the microparticles and the labelled TSH. After separation of the bound from the free and addition of CDP-Star, alkaline phosphatase on the microparticles will activate CDP-Star to produce luminescent signal. The HISCL-fT4 is a competitive assay. Biotinylated anti-T4 reacts with fT4 in the sample and binds to the streptavidin-coated microparticles. Alkaline phosphatase-labelled-T3 cross reacts with unbound antibodies. After separation of the bound from the free and addition of CDP-Star, alkaline phosphatase on the microparticles will activate CDP-Star to form a luminescent signal. We verified that the functional sensitivity of the HISCL TSH and fT4 assays is indeed 0.002 mU/L and 3.2 pmol/L respectively. We then proceeded to determine the HISCL TSH and fT4 from 437 Architect samples comprising 276 euthyroid (TSH 0.42-3.96 mU/L, fT4 10.1-19.0 pmol/L), 72 hyperthyroid (TSH <0.004-0.36 mU/L, fT4 11.6-41.9 pmol/L) and 89 hypothyroid (TSH 10.6-157 mU/L, fT4 <5.5-9.9 pmol/L) values. Samples with results below the detection limit of the HISCL TSH (n=59) and fT4 (n=11) were excluded. Statistical analyses were performed on MedCalc Statistical Software v19.8 (Ostend, Belgium). **Results:** There were 367 pairs of TSH and fT4 results comprising 278 euthyroid (TSH 0.2-4.4 mU/L, fT4 9.4-25.5 pmol/L), 11 hyperthyroid (TSH 0.002-0.16 mU/L, fT4 10.9-47.4 pmol/L) and 78 hypothyroid (TSH 8.5-98.4 mU/L, fT4 3.2-12.5 pmol/L) values. A significant inverse log/linear relationship between serum TSH and fT4 (log TSH = 1.762 - 0.106 fT4; r = -0.76) was apparent. From this derived statistical relationship a 2-fold increase in fT4 from 15 to 30 pmol/L would be expected to be accompanied by an approximate 40-fold reduction in TSH from 1.49 to 0.038 mU/L. **Conclusion:** This study confirms the response of pituitary thyrotroph to changes in circulating fT4 and the utility of TSH in evaluating thyroid disorders. However, this TSH-fT4 relationship depends on the individual performance of TSH and fT4.

A-130

Development of an automated highly sensitive Chemiluminescence Enzyme Immunoassay for SARS CoV-2 nucleocapsid antigen

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Background Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is known to be a highly transmissible and pathogenic coronavirus that emerged in late 2019. Since then, SARS-CoV-2 has rapidly spread around the world, causing numerous deaths. Therefore, it is necessary to establish a method for measuring SARS-CoV-2 quickly and easily. We have developed a highly sensitive and quantitative chemiluminescence enzyme-immunoassay (CLEIA) for SARS-CoV-2 Ag on fully-automated LUMIPULSE system, which was approved as an IVD product in June 2020, and its performance is evaluated.

Methods SARS-CoV-2 Ag assay for LUMIPULSE is a two-step sandwich CLEIA with a specific treatment process to extract the nucleocapsid (N) Ag from the virus during the 1st immunoreaction. The resulting reaction signals are derived within 30 minutes/test, and are proportional to the amount of N Ag in the sample allowing quantitative determination of N Ag in nasopharyngeal swab, nasal swab and saliva samples.

Results Limit of detection (LoD) and quantitation (LoQ) were 0.19 pg/mL and 0.60 pg/mL, respectively. A 20-day precision study was performed during a 31-day period using two controls and four panel specimens, and the imprecision for positive specimens was ≤ 2.5% total CV. Linearity was demonstrated over the range 0.36 to 6,056.64 pg/mL in nasopharyngeal swab. The assay showed the cross reactivity with SARS-CoV N Ag, but it did not exhibit cross reactivity to coronavirus (HCoV-229E, HCoV-OC43, HCoV-NL63, or HCoV-HKU1), and MERS-Coronavirus. All measurements of negative samples gave negative result. The correlation coefficient between SARS-CoV-2 Ag assay and the number of copies by RT-PCR was 0.85 (N=30) in nasopharyngeal swab. The specificity showed 100% in negative nasopharyngeal swab (N=198), when the each cut off value was set to 1.34 pg/mL for nasopharyngeal swab.

Conclusion The performance of SARS-CoV-2 Ag assay for LUMIPULSE system was satisfactory. Especially its sensitivity allows the test for asymptomatic subject using nasopharyngeal swab, nasal swab and saliva samples. Throughput of assay with LUMIPULSE G1200 or LUMIPULSE L2400 analyzer performs 120 or 240 tests/hour, respectively. The use of this assay will help to identify the SARS-CoV-2 infection including asymptomatic persons through large-scale test such as airport quarantine, not only routine test using. Moreover, since the assay can measure the viral load quantitatively with high-accuracy, it might be useful for the evaluation for person's contagiousness or for the severity of disease. Finally, it has a contribution in diagnosing COVID-19 from the spread of infection all over the world.

General Clinical Chemistry

A-131

Routine laboratory testing to determine if a patient has COVID-19

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Background: Specific diagnostic tests to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and resulting COVID-19 disease are not always available and take time to obtain results. Routine laboratory markers are used to assess the clinical status of a patient. The ain is to demonstrate that the routine laboratory test, such as the case of white blood cell count, platelet count, measures of anticoagulation, ferritin level, serum albumin, C-reactive protein (CRP) and procalcitonin , allows a more selective and efficient diagnosis to detects SARS-CoV-2. **Methods:**

Retrospective cohort study including 80 patients who presents COVID symptoms. All patients underwent basic biochemistry test as part of the diagnosis and reverse transcriptase polymerase chain reaction (RT-PCR) to confirm the presence of SARS-CoV-2. **Results:** We included 80 patients (N=80) in our study, 51 men and 29 women. The RT-PCR was positive in 15 patients. We observed an increase in the levels of inflammation and infection markers in patients with a COVID-19 diagnosis confirmed by RT-PCR: ferritin (373,06 vs 341,23 ng/ml, p=0,384), C-reactive protein (81,73 vs 57,55 mg/l, p=0,02) and procalcitonin (0,7 vs 0,25 ng/ml ,p=0,125). In addition, this patients with SARS-CoV-2 diagnosis presents elevated levels of coagulation markers or d-dimers: 8923,7 vs 1573,4 , p=0,03). On the other hand, we conclude a decrease in the levels of white blood cell count (7,88 vs 9,27/µl p=0,335, platelet count (251.300 vs 260.400/µl , p=0,331) and albumin (2,9 vs 3,2 g/dl, p=0, 390). **Conclusion:** These easy-to-measure, time-saving and very low-cost parameters have been shown to be effective in the early prediction of the COVID-19 severity. Their use at the early admission stage can improve the risk stratification and management of medical care resources in order to reduce the mortality rate.

A-134

Evaluation of Analytical Performance of the MedFrontier Intact FGF23 Immunoassay in Serum.

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Background: Fibroblast growth factor 23 (FGF23) is a major regulator of phosphate homeostasis. Clinically, measurements of FGF23 are useful in the diagnosis of x-linked hypophosphatemia (XLH), tumor induced osteomalacia (TIO), and familial tumoral calcinosis with hyperphosphatemia. Different types of FGF-23 immunoassays are available, those targeting the intact form (iFGF23), and those detecting c-terminal fragments (cFGF23). Intact FGF23 assays have been reported to be more sensitive and specific than c-terminal assays in the evaluation of FGF23 concentrations in patients with XLH and TIO. Objective: Evaluate the performance characteristics of the MedFrontier intact FGF23 immunoassay in serum for use in the diagnosing of XLH and TIO.Methods: The MedFrontier intact FGF23 assay (Eagle Biosciences) is an immunoenzymatic assay that uses two anti-human FGF23 mouse monoclonal antibodies. One antibody is coated onto microtiter wells and the other is alkaline phosphatase labeled. The signal generated is proportional to the concentration of intact FGF23 in the serum sample. The concentration of intact FGF23 is determined by means of comparison against a multipoint calibrator curve. Data reduction is performed with MikroWin 2010 software (Labsis Laborsysteme GmbH). The performance characteristics of the assay were established using at least two different reagent kit lots. Method validation included determination of stability, assay imprecision, limits of detection (LOD) and quantification (LOQ), analytical measurement range (AMR), accuracy, interferences, cross-reactivity with cFGF23, effect of antigen excess (hook-effect), and establishment of reference intervals. Results: iFGF23 was stable for 24 hours ambient, 14 days refrigerated, 90 days frozen and through 3 freeze/thaw cycles. Intra and inter-assay imprecision in serum, expressed as % coefficient of variation (%CV), ranged from 2.1-7.8% and 4.5-6.7%, respectively. The LOQ was 14pg/mL (%CV=5.9%) with a LOD of 2.4 pg/mL. The AMR of 10.7-3038.6 pg/mL was verified by running a different lot of calibrators as unknowns (slope of 0.97 and r^2 of 0.98). Accuracy was determined by spiking 8 serum samples with recombinant iFGF23 (R&D systems), and an average recovery of 100% (range 86-116%) was observed. The assay was not substantially affected by the presence of hemoglobin up to 1000 mg/dL, triglycerides up to 1000 mg/dL, or bilirubin up to 60 mg/dL. No cross-reactivity with c-terminal FGF23 was observed up to a concentration of 230,680 pmol/L. No hook effect was noted up to 30,000 pg/mL. The reference intervals based on the 95th percentile of healthy normophosphatemic subjects (119 pediatrics and 120 adults) were established as <52 pg/mL for pediatrics and <59 pg/mL for adults. Conclusion: The analytical performance characteristics of the MedFrontier intact FGF23 immunoassay were established and are sufficient for clinical application. Further studies to establish the assay's clinical sensitivity and specificity for the identification of patients with XLH and TIO are ongoing.

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Immunoassay Equivalency between the Atellica® IM 1600 and Atellica® CI 1900* Analyzers

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Introduction: The Atellica® CI 1900 Analyzer* is an integrated analyzer that uses Atellica® IM and CH assay reagents. The purpose of this investigation was to evaluate the analytical performance of the Atellica® IM Alpha Fetoprotein (AFP), Hepatitis B core IgM (aHBcM), High-Sensitivity Troponin I (TnIH), Total human Chorionic Gonadotropin (ThCG), and Thyroid Stimulating Hormone 3-Ultra™ (TSH3UL) Assays on the Atellica CI 1900 analyzer and compare it to the performance of the same assays on the commercial Atellica® IM 1600 Analyzer.**Methods:** Performance testing included precision and method comparison. Precision was run over five days with five replicates per sample per day, for a total of 25 replicates per sample for the Atellica CI analyzer. Method comparison and precision were evaluated based on CLSI documents EP09-A3 and EP15-A2, respectively. Precision for assays run on the Atellica IM analyzer was taken from the commercial instructions for use and is based on 20-day studies.**Results:** Results on the Atellica CI 1900 analyzer were generated using Atellica IM reagents and calibrator products. Within-lab precision for the Atellica IM AFP Assay on the Atellica CI 1900 analyzer ranged from 2.4 to 7.1% CV. AFP method comparison for the Atellica CI 1900 analyzer versus Atellica IM 1600 analyzer using weighted Deming regression yielded a slope of 1.05, y-intercept of –1.07 ng/mL, and r of 1.00, with 44 samples. Within-lab precision for the Atellica IM aHBcM Assay on the Atellica CI analyzer ranged from 2.9 to 6.8% CV. aHBcM method comparison for the Atellica CI 1900 analyzer versus Atellica IM 1600 analyzer yielded 100% positive agreement and 100% negative agreement, with 45 samples. Within-lab precision for the Atellica IM TNIH Assay on the Atellica CI 1900 analyzer ranged from 1.5 to 3.5% CV. TNIH method comparison for the Atellica CI 1900 analyzer versus Atellica IM 1600 analyzer using weighted Deming regression yielded a slope of 0.96, y-intercept of 0.6 pg/mL, and r of 1.00, with 50 samples. Within-lab precision for the Atellica IM ThCG Assay on the Atellica CI 1900 analyzer ranged from 1.7 to 4.0% CV. ThCG method comparison for the Atellica CI 1900 analyzer versus Atellica IM 1600 analyzer using Deming regression yielded a slope of 1.02, y-intercept of –5.54 mIU/mL, and r of 0.99, with 50 samples. Within-lab precision for the Atellica IM TSH3UL Assay on the Atellica CI 1900 analyzer ranged from 2.7 to 4.8% CV. TSH3UL method comparison for the Atellica CI 1900 analyzer versus Atellica IM 1600 analyzer using Deming regression yielded a slope of 1.03, y-intercept of –0.007 uIU/mL, and r of 1.00, with 51 samples.**Conclusion:** When evaluated on the Atellica CI 1900 Analyzer, the AFP, aHBcM, TNIH, ThCG, and TSH3UL assays demonstrated equivalent performance to that observed on the Atellica IM 1600 analyzer. These assays demonstrated acceptable

precision and method comparison. Performance of these assays on the Atellica CI 1900 Analyzer supports equivalent assay performance for laboratory settings where comparison to the Atellica IM 1600 analyzer is desired.*Product under development. Not available for sale. Future availability cannot be guaranteed.

Unrestricted

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Evaluation and Method Comparison of an Automated Squamous Cell Carcinoma Assay

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Background: Squamous cell carcinoma antigen (SCC) is expressed in normal epithelium and epithelial tissues. The antigen consists of over ten protein fractions which are generally divided into two groups: the acidic with isoelectric points <6.25, and the neutral of 6.25 or greater. While the neutral form of SCC normally remains inside the cell, acidic SCC is easily released from the cell and is often elevated in squamous cell carcinomas or other nonmalignant squamous cell lesions. As a result, SCC levels in the serum are used for diagnosing and managing carcinomas of various tissues including uterine cervix, lung, skin, head and neck, esophagus, urothelium, anal canal and vulva.

The B·R·A·H·M·S SCC KRYPTOR® assay quantitatively measures SCC in human serum using the KRYPTOR family of automated analyzers. Although the assay has been used regularly in other markets, it is relatively new to the US. The purpose of this study was to compare the KRYPTOR SCC to a well-established in-house ELISA (Erickson et al., 2010). Performance characteristics were also assessed and validated.

Methods: Deidentified residual serum specimens sent to ARUP Laboratories for routine testing, as well as serum specimens from healthy volunteers, were collected under University of Utah's Institutional Review Board approved protocols. KRYPTOR SCC was measured according to the test kit manufacturer's protocol, and the ELISA according to the established in-house protocol. KRYPTOR performance characteristics evaluated were analytical sensitivity, linearity, precision, and analyte stability and carryover. A reference limit was also established.

Results: A method comparison study against an established ELISA generated a slope of 1.048, intercept of -0.003, and r^2 of 0.830 (n = 23, Deming regression). The limit of detection was 0.1 ng/mL (22 determinations each of blank and low level serum). Linearity was established by combining serum specimens with high and SCC concentrations at different ratios to create as set of 10 specimens, each of which were tested in duplicate. Regression analysis produced a slope of 0.968, intercept of -0.778 and r^2 of 0.995, with percent recoveries ranging 91.1 - 104.0%. Precision was determined from two serum pools of differing SCC concentrations tested over 5 days, four replicates per pool per day. Repeatability and within-laboratory CVs were 4.3 and 5.0% at 1.1 ng/mL, and 0.8 and 2.3% at 66.0 ng/mL, respectively. SCC was stable in serum for minimums of 48 hours at room temperature, 14 days at 4 - 8 °C, 2 months frozen at -20 °C, and over a minimum of 3 freeze/thaw cycles. Carryover was insignificant at -0.025 ng/mL. A reference limit was established a 1.7 ng/mL (nonparametric analysis, 95th percentile, n = 124).

Conclusions: The B·R·A·H·M·S SCC KRYPTOR demonstrates acceptable performance for quantifying SCC human serum and compares favorably to a well-established ELISA. A reference limit of 1.7 ng/mL was also established. Moreover, analyte stability and carryover were found quite acceptable.

A-137

Plasma ACE activity in hospitalized patients with COVID-19

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Background: The renin-aldosterone angiotensin system (RAAS) is a major regulator of the cardiovascular system and homeostatic fluid balance. COVID-19 has been shown to affect the RAAS pathway, specifically by using the angiotensin converting enzyme-2 (ACE2) as a viral entry point. There has been concern about whether RAAS blockers worsen, protect or are neutral to COVID-19 disease onset and progression. There is an urgent need to understand how COVID-19 infection modulates the RAAS pathway and whether current therapeutic strategies require modification based on RAAS blocker usage.

Methods: Analysis of remnant clinical samples were approved by the UCSF Institutional Review Board. Thirty-two patients with a positive COVID-19 infection and 32 patients with a negative COVID-19 infection were investigated. Remnant plasma samples were sampled from both COVID-positive and COVID-negative patients at baseline (48 hours within admission) and from COVID-positive patients at terminal (48 hours within discharge). Plasma ACE activity was performed using a Siemens ADVIA 1800 chemistry analyzer. Statistical analysis was conducted using generalized linear models and paired and unpaired t-tests. Significance was set at $p<0.05$.

Results: Among 64 COVID-positive patients, 22 (69.0%) were male and 10 (31.0%) were female while 16 (50.0%) males and 16 (50.0%) females represented the COVID-negative patients. There was decreased plasma logACE activity among COVID-positive patients at baseline ($p<0.0004$) compared to COVID-negative patients at baseline. Plasma logACE activity increased in COVID-positive patients at the terminal timepoint ($p<0.007$) and at that point was similar to logACE activity in COVID-negative patients ($p=0.17$).

Conclusion: Plasma ACE activity was significantly lower at the start of hospitalization among COVID-19 patients compared to the end of their hospital stay, and compared to COVID-19 negative patients. Our data suggest that circulating ACE may be uniquely involved with COVID-19 disease and disease progression. More studies are warranted to investigate the full impact of COVID on the RAAS pathway.

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Spectrum of Thyroid Function Test among Type 2 Diabetics Patient in a Rural Tertiary Health Institution in Southwest Nigeria: A Hospital Based Study

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Background: Thyroid disease is a pathological state that can adversely affect glycemic control in diabetics and has the potential to affect the health. The report showing the association between diabetes and thyroid dysfunction were first published in 1979. Thyroid disease is found commonly in diabetes and various studies have demonstrated a higher prevalence of thyroid dysfunction in diabetic patients than the general population. The occurrence of thyroid dysfunction through thyroid function test among diabetes patients in our Diabetic clinic has not been determined. This hospital based study is aimed at determining the spectrum of thyroid function test profile among diabetics' patients attending the diabetic clinics in our rural tertiary hospital.**Methods:** The Federal Teaching Hospital is located in IdoEkiti an agrarian rural community in the Ido/Osi local government area of Ekiti State. Fifty-six (56) T2DM patients with good glucose control were recruited from the Diabetic Clinics of the Hospital and twenty-two (22) non-diabetic persons were recruited from the general population. Age, educational level, occupation, marital status and duration of DM extracted from the questionnaire. Weight, height, BMI, waist and hip circumferences, and waist-hip ratio measured for all participants. Blood samples were assayed for fT3, fT4, and TSH. Assay was performed on Autoplex 2nd Generation Autoanalyzer using chemiluminescence immunoassay (CLIA) AccuLite CLIA Microwells reagent kits; both produced by Monobind Inc., 100 North Pointe Drive, Lake Forest, CA 92630, USA. The limit of detection for TSH is 0.0062μIU/ml, for FT3 is 0.742pg/ml, and for FT4 is 0.28ng/dl, defined as; 2 standard deviation in the measurement of zero doses with this method in our laboratory. SPSS version 20 statistical computer package was use to obtain the mean, standard deviation, frequencies, proportions, Student's t-test and chi square test. Ethical approval was obtained from the Health Research Ethics Committee of the Federal Teaching Hospital, Ido-Ekiti, before commencing the study.**Results:** There were 67.9% and 59.1% of female participant in the diabetic subjects and the non-diabetic subjects respectively. More than 43.6% of the participant had tertiary education and above; about 37.2% of the participants were skilled or professional workers; while close to 70% of the participant were married.About seventy-eight percent (78.6%) of the diabetic patients had Euthyroid biochemical pattern; 21.4% showed abnormal biochemical pattern of Euthyroid Sick Syndrome (8.9%), Subclinical Hyperthyroid (7.1%) and Subclinical Hypothyroid (5.4%). None of the diabetic subjects have overt hypothyroid or hyperthyroid thyroid function test. More than fifty percent of the diabetics were diagnose less than five years ago. The value of TSH was increasing with the duration of DM but not statistically significant. **Conclusion:** Thyroid function test in diabetes may identify those that have altered thyroid function for early review and management. Unidentified thyroid dysfunction may alter the metabolic controls in patients with diabetes. The early recognition and treatment of thyroid disorder in diabetic patients may benefit glycemic control, attenuate cardiovascular risk, and improve general wellbeing.

A-139

Six Sigma Performance of the Alinity c HbA1c Assay

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Background: HbA1c measurements have been widely used to monitor long-term blood glucose control. The Abbott Hemoglobin A1c (HbA1c) test automatically lyses red blood cells and utilizes an enzymatic method that specifically measures *N*-terminal fructosyl dipeptides of the *beta*-chain of HbA1c. Two separate measurements are made: glycated hemoglobin (A1c) and total hemoglobin (THb) which are used to determine the percent HbA1c [NGSP] units or the hemoglobin fraction in mmol/mol [IFCC] units. Sigma metrics are an efficient way for evaluating product quality, by comparing a combination of two metrics, precision and bias, relative to a total allowable error goal. A higher sigma metric corresponds to a higher quality product where six sigma is considered a world class quality performance metric. This study used NGSP samples to compare the sigma metrics for several comparator systems.

Methods: Key precision performance data were analyzed across 3 Alinity c instruments and 3 reagent lots to assess the accuracy and bias performance of the constituent assays per Clinical and Laboratory Standards Institute (CLSI) protocols. The assay bias was assessed using NGSP reference samples (n = 40). The relationship of the constituent assays to the % HbA1c results were assessed using patient data acquired from Alinity c instruments from the period of Jan 2019 to Jan 2020. Using CAP precision and bias performance data (GH5-B and GH5-C C 2020) on 5 NGSP proficiency samples tested on several comparator methods, a relative Sigma score was determined (TEa of 5%).

Results: Within-lab (total) imprecision for the two constituent assays, THb and A1c assays, was ≤ 3.9% CV for panels spanning the assay range of 4.0 to 14.0% HbA1c. Conversion of the individual constituent results to %HbA1c resulted in a within-lab (total) imprecision ≤ 1.7% CV. An analysis of 2.4 MM worldwide patient data showed that %HbA1c values were independent of the THb result and widely distributed across the THb range of 1250 to 8000 µmol/L. It was observed that 0.04% worldwide (0.026% US) of the samples were associated with a very low hemoglobin value of < 1250 µmol/L (< 5 g/dL). Severe Anemia is defined as 8 g/dL suggesting samples with low THb levels (< 1250 µmol/L) should be excluded from testing. Evaluation of 40 NGSP reference samples, showed the Abbott Alinity c HbA1c assay has a low bias of ≤ -1.7% (Slope 1.0, r=0.995). A six sigma estimate of comparator assays was obtained using NGSP proficiency samples. A limitation of using this NGSP data is that the derived result is an estimate because of the variability in laboratory sites reporting results, in addition to the limited number of replicates and limited number of comparator systems. The Abbott Alinity c HbA1c assay has a low mean imprecision (2%) and bias (1%) for the proficiency samples. Using a relative six sigma performance score, the Abbott systems with a score of 2.2 to 3.3 ranks in the top tier of comparator systems for the individual proficiency samples.

Conclusion: The Alinity c Hemoglobin A1c assay is a precise and accurate method for measuring HbA1c.

A-140

Sigma Metrics for Next Generation Clinical Chemistry Assays

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Background: A sigma metric is a simple measurement of assay quality comparing the precision and bias performance of an assay to a total allowable error (TEa) goal. A higher Sigma metric corresponds to higher quality product where 6-sigma is considered a world-class quality performance metric. This study was conducted to determine sigma performance for 8 ARCHITECT and 6 Alinity *c* system clinical chemistry assays across several therapeutic areas. This study used reference standards to establish accuracy and bias to ensure traceability to an established reference. Additionally, precision profile charts were created for a subset of assays to compare the precision performance of the assays tested on the ARCHITECT and Alinity *c* systems.

Methods: A sigma metric was calculated for each assay and were plotted together on a single method decision chart. The sigma metric was calculated using the equation: sigma = (TEa - bias)/precision. The TEa goal was based on a recognized standard (*e.g.* CLIA, the 2014 allowable limits of performance from the Royal College of Pathologists of Australasia (RCPA) etc.); precision was based on the within-laboratory standard deviation (SD) or percent coefficient of variation (%CV) for the sample with a concentration closest to a medically-relevant concentration level as estimated from a 20-day study performed per CLSI EP05-A2; bias was based on the difference from the predicted value of a reference.

Results: The next generation clinical chemistry assays showed excellent precision across the measuring interval. A majority of the ARCHITECT and Alinity *c* clinical chemistry assays demonstrated at least 5-sigma performance (see Table for Alinity results), and all of the assays had at least 4-sigma performance at a medically-relevant concentration level.

Conclusion: Sigma metrics can be a useful tool for laboratorians to use to compare and monitor assay performance to ensure high quality healthcare for patients

Alinity ci System Assay Decision Levels, TEa Goals and Sigma Metrics

Alinity Assay	Level	TEa	Sigma
Alb BCG2	3.72	14	6.6
Alb BCP2	3.70	14	9.5
Amy2	309.2	15.7	13.5
Chol2	211.6	10	13.4
Protein2	6.96	10	7.2
Uric Acid2	6.78	13	10.5

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Performance Characteristics of the Anti-Müllerian Hormone Assay on the ADVIA Centaur XP Immunoassay System

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Background: Anti-Müllerian hormone (AMH) in vitro measurements are increasingly being used in the assessment of ovarian reserve, prediction of response to controlled ovarian stimulation in women undergoing assisted reproductive technologies, as an aid in the diagnostic evaluation of polycystic ovary syndrome, and prediction of menopause. The objective of this study was to evaluate the performance characteristics of an AMH assay from Siemens Healthineers on the ADVIA Centaur® XP Immunoassay System.

Methods: A single-pass sandwich immunoassay for the detection of AMH has been developed using direct chemiluminescent technology, which uses two mouse monoclonal antibodies to AMH: The first antibody is biotinylated and coupled to streptavidin-coated magnetic particles, and the second antibody is detection antibody labeled with acridinium ester. A direct relationship exists between the amount of AMH present in the patient sample and the amount of relative light units (RLUs) detected by the system. The following studies were performed to evaluate assay performance on ADVIA Centaur XP system: method comparison to the predicate assay using 120 female serum samples; 20-day precision; limit of bank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) determination; linearity; hook effect; and ambient temperature sensitivity assessment.

Results: Method comparison between the ADVIA Centaur XP AMH Assay and a commercially available assay using 120 female serum samples (AMH concentration range: 0.08-22.0 ng/mL) showed a Passing-Bablok regression slope of 1.04 (95% confidence interval: 1.02-1.06), intercept of -0.03 ng/mL, and Pearson correlation coefficient of 0.99. In precision studies, repeatability coefficients of variance (CV) for eight serum samples (AMH concentrations: 0.1-16 ng/mL) were found to be ≤1.8%, and within-lab precision CVs were ≤4.3%. Assay linearity was demonstrated from 0.018 to 26.3 ng/mL. LoB was determined to be 0.003 ng/mL, LoD 0.009 ng/mL, and LoQ 0.020 ng/mL. No hook effect was observed up to at least 1150 ng/mL. Ambient temperature fluctuations between 18 and 30°C had no significant impact on sample dose recoveries (n = 7, concentration range: 0.2-18 ng/mL), with an average dose change of 3.2% compared to the reference (24°C) temperature condition.

Conclusion: The results from the studies have demonstrated reproducible assay performance that is comparable to a reference method, linearity through the analytical measurement range, robustness to ambient temperature fluctuations, and detection capability suitable for assessment of low ovarian reserve. *Disclaimer: This product is under development and not commercially available. Its future availability cannot be ensured.

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Analytical Performance Assessment of a Newly Formulated REACH compliant gamma-Glutamyl Transferase Assay (GGT2) for Abbott's Alinity c and ARCHITECT c Systems

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Introduction: Gamma- glutamyl transferase (GGT) is elevated in many forms of liver, bile ducts and gallbladder disease. GGT levels are elevated earlier and are more pronounced than those with other liver enzymes in cases of obstructive hepatobiliary disease as in pancreatitis and metastatic neoplasms. It is also used to help with diagnosis of chronic alcoholic liver disease. A high performing Research Use Only assay (RUO) that is REACH compliant has been developed on an automated clinical chemistry platform for the quantitative determination of GGT in human serum.

Methods: The newly formulated GGT assay is an IFCC traceable, calibrated, two-reagent photometric assay. Specifically, the assay method and calibrator are traceable to the IRMM/IFCC-452 standard. The assay methodology involves the GGT enzyme catalyzing the transfer of the gamma-glutamyl group from L-gamma-glutamyl-3-carboxy-4-nitroanilide to the glycylglycine acceptor to yield 3-carboxy-4-nitroaniline. The rate of absorbance of 2-carboxy-4-nitroaniline is proportional to the GGT concentration in the sample. Reagent formulation was also designed to ensure REACH compliance. Performance testing was performed on automated clinical chemistry analyzers, following CLSI guidelines.

Results: Total within laboratory imprecision was < 3.0 %CV for the serum application with linearity up to 7500 U/L. The Limit of Quantitation (LOQ) was 5 U/L. Reagent onboard stability of at least 30 days was observed. This GGT assay meets the CLSI EP 07, 3rd ed. criteria for endogenous interferent levels at GGT concentrations of 40 U/L and 150 U/L. Specifically, this new assay showed robustness to hemoglobin interference up to 250 g/L at 40 U/L GGT and > 1000 mg/dL at 150 U/L GGT. Both the conjugated and unconjugated bilirubin robustness was demonstrated at ≥ 60 mg/dL at both 40 U/L and 150 U/L levels. In addition, nineteen common drug interferences were tested at 40 U/L and 150 U/L. Acetaminophen ≥ 160 mg/L exhibited no detectable interference as well as Acetylcysteine at ≥ 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, Cefoxitin ≥ 6600 mg/L, Cyclosporine ≥ 2 mg/L, Doxycycline ≥ 20 mg/L, Sodium Heparin ≥ 4 U/mL, Ibuprofen ≥ 220 mg/L, Levodopa ≥ 8 mg/L, Methyldopa ≥ 25 mg/L, Metronidazole > 130 mg/L, Phenylbutazone ≥ 330 mg/L, Rifampicin ≥ 50 mg/L, Theophylline (1,3-dimethylxanthine) ≥ 60 mg/L, and Cefotaxime ≥ 53 U/L. Method comparison to an on-market predicate assay showed a slope of 1.08 with correlation coefficient of 1.00 across the measuring interval range of 5 – 7653 U/L.

Conclusions: A REACH compliant, IFCC traceable, calibrator based GGT assay has been formulated for use with automated clinical chemistry analyzers. This robust GGT assay has demonstrated analytically sound performance, when tested per the most recent editions of CLSI guidelines.

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Impact of Unconjugated Estriol Assay Interference on Prenatal Screening Tests

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Background: Unconjugated Estriol (uE3) is an important biomarker in prenatal screening for trisomy 21(Down syndrome), trisomy 18, and Smith-Lemli-Opitz Syndrome (SLOS). It is one of four components of the maternal quadruple (Quad) screen, which is measured during the second trimester. **Objectives:** Immunoassays for the Quad screen can be performed on the Beckman Coulter UniCel DxI 800 analyzer. The uE3 assay is a competitive binding assay that utilizes an enzyme-linked conjugate, estriol-alkaline phosphatase (ALP). Previous studies from our laboratory identified rare interference in the uE3 assay due to anti-ALP antibodies, which could be mitigated with a scavenger (inactivated) ALP. In the current study, we analyzed 160 de-identified patient samples previously submitted for the Quad screen with uE3 multiples of the median (MoM) of ≤0.5 to further investigate potential interference. We employed a reagent pack spiking study with heat-inactivated ALP (hALP) to determine if the interference could be corrected in a scalable manner. For samples with interference, the change in risks due to the corrected uE3 measurements was analyzed. **Methods:** Inactivated ALP was prepared using lyophilized bovine-ALP (Sigma Aldrich). A 500 mg/dL solution of ALP in water was heat inactivated overnight at 80ºF, centrifuged to pellet precipitated protein, and re-concentrated using an ultraspin column to approximately 165 mg/dL of hALP. The hALP was spiked into a uE3 reagent cassette and mixed for 30 minutes. The 160 samples were measured using two reagent packs (lot #920861), one with and the other without spiked hALP. Additionally, the samples were tested on a new reagent formulation from the vendor known to address the previously identified interference issues (lot #922579). Samples were suspected to have interference if the % difference in uE3 measurements was ≥50%. Pseudo-risks were calculated using a test patient environment to understand the screening impact due to the change in uE3 result. **Results:** Seventeen of the 160 samples had uE3 results that were ≥50% different between the hALP spiked and non-spiked reagent pack. These results were confirmed by analyzing the samples on the new lot, which corrected the interference. The average % difference with standard deviation was -362% ± 504% for the uE3 measurement and -144% ± 130% for the uE3 MoM; the interference negatively affected the uE3 concentrations. Analysis of screening risks using a test patient environment showed that assay interference could result in false positives for one SLOS, one trisomy 21, and three trisomy 18 post-test risk calculations.

Conclusion: We investigated the interference caused by anti-ALP antibodies in pregnant patients. Our experiment of reagent pack spiking with hALP produced similar uE3 results to a reformulated reagent designed to address potential interference, demonstrating that this is a feasible strategy to screen for samples with interference in a scalable manner. Our studies suggest that a small number of screening tests could have false positive results due to anti-ALP interference. The new, vendor-provided reagent formulation addresses anti-ALP interference risk. Such approaches can improve the performance of prenatal screening using maternal serum.

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Introduction of a New and Improved Software v5.24 (G8 v5.24) for Hemoglobin A1c Testing on the Tosoh HLC723-G8 Cation Exchange HPLC Analyzer

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Background: The measurement of HbA1c is well established as an accurate index of the mean blood glucose concentration in monitoring glycemic control in patients with diabetes mellitus. A well accepted method for HbA1c measurement is the separation and quantitation of HbA1c in whole blood by Ion Exchange HPLC on the Tosoh G8 (Tosoh Bioscience, San Francisco, CA). The Tosoh G8 program is based on chromatographic separation of HbA1c on cation exchange column. The non-porous nature of the column makes it a very efficient method for separation of peaks of the chromatogram. The separation is optimized to reduce interferences from commonly occurring hemoglobin variants and Fetal Hb thus enhancing the performance of the G8. **Objectives:** The study was carried out to evaluate the Tosoh Automated Glycohemoglobin Analyzer HLC723-G8 with the v5.24 (G8 v5.24) software modification to assess interference when measuring %HbA1c in the presence of hemoglobin (Hb) variants and HbF. The %A1c results obtained on the G8 v5.24 were compared to the results from the Trinity Biotech Ultra2 analyzer for the (HbAS, HbAC, HbAD, & HbAE) variants; and to results from the Bio-Rad Variant II Turbo 2.0 analyzer for HbF. Methods: A set of 25-30 specimens each with a pre-determined %A1c value ranging 4-10% with a variant concentration anywhere between 20-40% HbAC, HbAD, HbAS, & HbAE were run and the results obtained on the G8 v5.24 were compared with Trinity Biotech Premier Hb9210™ HbA1c Analyzer. Similarly, for HbF, a set of 21 samples were run and results were compared with the Bio-Rad Variant II TURBO analyzer. The HbF samples were prepared by using a 10:0, 6:4, 5:5, 4:6 and 0:10 spiking protocol to achieve concentrations between 1- 40%. **Results:** The study concluded that the performance testing results met their pre-determined acceptance criteria. Accurate and reportable HbA1c% results in the presence of HbC (39%), HbD (39.5%) and HbS (39%), HbE (31%) and HbF of up to 25%. Clinically significant interference is defined as >/= ± 6% relative difference in the results from a comparison method at 6 and 9% A1c. The results obtained were less than ± 6% relative difference in the results from a comparison method at 6 and 9% A1c. **Conclusion:** All performance testing results met their pre-determined acceptance criteria. The modified software, version 5.24, reduces interference from commonly occurring variants and Fetal Hb when measuring %HbA1c.

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Evaluation of the New Enzymatic VITROS® A1c Chemistry Products Slides* on the Ortho VITROS 5600/XT 7600 Integrated and 4600/XT 3400 Chemistry Systems

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Background: VITROS® A1c Chemistry Products Slides* are being developed for the measurement of glycated hemoglobin A1c on the VITROS 5600/XT 7600 Integrated and VITROS 4600/XT 3400 Chemistry Systems. The analytical performance of VITROS A1c Slides* have been evaluated for method comparison, precision, and interference to common hemoglobin variants. The VITROS A1c Slides* are being developed using VITROS MicroSlide dry technology as a homogeneous test element for use with whole blood that is sampled directly from standard EDTA collection tubes without any pre-treatment step. The VITROS INTELLICHECK® technology confirms that adequate red cells are sampled to deliver accurate results. The VITROS A1c Slides* have a high throughput of approximately 180 tests/hr, are compatible to use with the VITROS Automation Solution track and have ≥ 6 months calibration interval.**Method:** The total within-lab precision, repeatability, and analyzer to analyzer precision was calculated using an ANOVA across VITROS 4600/XT 3400 Chemistry and 5600/XT 7600 Integrated Systems with quality control and whole blood hemolysate fluids using two replicates per day, twice per day over 10 days (total n=40). EDTA venous whole blood patient samples spanning the measuring range of 4-14% A1c were evaluated on a VITROS XT 3400 Chemistry System (n=85, 4.8 – 13.3 %A1c) and VITROS 5600 Integrated System (n=85, 4.8 – 13.3 %A1c) against the NGSP secondary reference laboratory Tosoh G8 HPLC instrument. The data was analyzed following CLSI EP09c guidelines using a Passing-Bablok regression. Interferent bias with hemoglobin variants HbAC (25.4 – 37.4%), HbAD (33.9% - 42.1%), HbAE (22.7 – 40.3%) and HbAS (32.3 – 39.3%) was determined versus the Trinity Primus Ultra2 (HbAC, HbAS) or Trinity Premier (HbAD, HbAE).**Results:** The total within lab precision from a single ANOVA across all four instruments was less than 2% CV for all fluids (mean 3.8% A1c, 1.55% CV; mean 7.2% A1c, 1.98% CV; mean 10.1% A1c, 0.95% CV; mean 11.3% A1c, 1.15% CV), the analyzer to analyzer precision was less than 1.6% CV for all fluids (mean 3.8% A1c, 1.02% CV; mean 7.2% A1c, 1.53% CV; mean 10.1% A1c, 0.55% CV; mean 11.3% A1c, 0.46% CV), and the repeatability was less than 0.7% CV for all fluids (mean 3.8% A1c, 0.47% CV; mean 7.2% A1c, 0.70% CV; mean 10.1% A1c, 0.37% CV; mean 11.3% A1c, 0.53% CV). The method comparison study for the VITROS A1c Slides* showed excellent correlation with the Tosoh G8 HPLC A1c method: VITROS A1c = 0.995 * Tosoh A1c + 0.04; (r) = 0.994 on the VITROS XT 3400, and VITROS A1c = 0.987 * Tosoh A1c + 0.09; (r) = 0.991 on the VITROS 5600. Hemoglobin variants HbAC, HbAD, HbAE, and HbAS have no clinically significant interference with VITROS A1c Slides*.**Conclusion:** The data presented here demonstrate that the new VITROS A1c Slides* on the VITROS Integrated and Chemistry Systems show excellent analytical performance and enhanced ease of use with the benefits of VITROS MicroSlide technology.*Under development

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Evaluation of the Efficacy of the GALAD Score for Detection of Hepatocellular Carcinoma in an Age-Stratified Clinical Population

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Background: Hepatocellular carcinoma (HCC) is a primary liver cancer with increasing incidence that most often develops in patients with chronic liver disease (CLD). Early detection of HCC is important, as treatment options become limited and survival rate decreases dramatically as the cancer progresses to advanced stages. To improve early detection of HCC in patients with CLD, a multi-analyte model (GALAD) was previously developed which incorporates gender and age along with serological biomarkers of HCC, including lectin-reactive alpha-fetoprotein (AFP-L3%), total alpha-fetoprotein (AFP), and des-carboxy-prothrombin (DCP). The GALAD model has been previously validated in different clinical cohorts with varied age and geographic distributions. Accordingly, different GALAD cutoffs (notably -0.76 and -1.18) have been applied in an effort to optimize performance. The aim of this study was to evaluate the performance of the GALAD score in a non-preselected, U.S.-based clinical population.

Methods: Patients included in this study had simultaneous measurements for AFP, AFP-L3%, and DCP measured using the uTASWako i30 between 2015 to 2020. Demographics and HCC status were obtained via chart review. Results measured after surgical or therapeutic intervention were excluded. Subsequently, GALAD score and the probability of HCC were calculated according to published formulas (PMID: 24220911). The area under the receiver operating characteristic curve (AUROC) was estimated for the GALAD score, AFP, AFP-L3%, and DCP. Sensitivity and specificity were assessed at previously published GALAD score cut-offs of -0.76 and -1.18. Performance characteristics were also assessed within interquartile age ranges (IQRs) for HCC-positive cases.

Results: A total of 183 patients without HCC and 106 patients with HCC were included in this analysis. HCC-positive interquartile age ranges were 18-60, 61-72, and 73-88 years. The AUROC of the GALAD score for HCC detection was 0.88 (95% CI 0.84-0.92), which was superior to individual components alone (0.78, 0.73, and 0.81 for AFP, AFP-L3%, and DCP, respectively). For age-stratified ranges, GALAD score AUROCs were 0.85, 0.89, and 0.82. A GALAD score cut-off of -0.76 showed an overall sensitivity of 69% and specificity of 90% for detection of HCC. Performance varied widely across age ranges with sensitivities of 59% (97% specificity), 75% (86% specificity), and 70% (76% specificity), for IQRs 1, 2, and 3, respectively. Similar trends in performance were observed at an alternate GALAD score cut-off of -1.18 for different age groups. At the -1.18 cut-off, overall sensitivity was 76% and specificity was 88%. Sensitivities were 59% (96% specificity), 83% (85% specificity), and 80% (67% specificity), for IQRs 1, 2, and 3, respectively.

Conclusion: In this non-preselected clinical population, we confirmed that the GALAD model has higher diagnostic accuracy for the detection of HCC compared to the individual biomarkers. While overall specificity was comparable to other studies, overall sensitivity failed to reach 90% as reported previously. When assessing performance characteristics in different age IQRs, the GALAD score specificity decreased significantly with age while sensitivity was best in patients above age 60. These results suggest that a universal GALAD cut-off for all age groups may not be optimal for HCC detection.

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Evaluation of Jaffé and enzymatic assays for urine creatinine on the Roche cobas 8000 platform

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Background Urine creatinine (UCr) is an important marker of kidney function and is frequently present in urine testing panels for the normalization of other measured analytes (e.g. urine albumin/creatinine ratio). Multiple methods exist for the determination of UCr including enzymatic and Jaffé assays. Enzymatic methods have greater analytical sensitivity but higher reagent costs. In selected workflows where a lower measured value may have additional clinical utility, enzymatic methods may be useful despite increased cost. The present experiments examined two automated methods for determining UCr, the Jaffé method (cobas c702) and the an enzymatic (cobas c502), to support biochemical genetic testing workflows where quantification of UCr values <5 mg/dL provide an additional clinical benefit for normalizing other measured analytes.MethodsMethod comparison for urine samples was performed on two instruments by analyzing low Cr samples (<15 mg/dL) and samples spanning the analytical measuring range (AMR) with a previously validated application. Linearity studies were performed using 5 levels of patient pools spanning the AMR. Intra-assay and inter-assay precision were evaluated using a low concentration and a high concentration urine pools. Carryover was assessed using patient pools of alternating low and high concentrations. A retrospective data analysis was performed to assess the volume of test requests with observed UCr values <5 mg/dL and evaluate enzymatic method utilization rates. Results Method comparison demonstrated a slope of 1.08 (bias -6.26%) for instrument 1 and a slope of 1.05 (bias -6.17%) for instrument 2 for low concentration samples. For samples spanning the AMR instrument 1 had a slope of 1.12 (bias 9.56%) and instrument 2 had a slope of 1.08 (bias 5.75%). Linearity was verified for the urine creatinine enzymatic assay, with percent recoveries ranging from 99.7-102.6%. Intra-assay imprecision ranged 2.0-3.1%CV for low concentration pools and 0.6-1.6%CV for high concentration pools. Inter-assay imprecision ranged 2.5-3.7%CV for low concentration pools and 0.8-1.6% CV for high concentration pools. Carryover was not exhibited by this assay. Historical data review of >200,000 UCr tests performed in our laboratory revealed <0.5% of observed measurements were <5 mg/dL. For observed results <5 mg/dL, 46% were in patients younger than 1 year old, 22% in pediatric patients 1 year to 17 years, and 32% in adult patients 18 and older. Results <5 mg/dL were more commonly observed in female patients (58%) than male patients (38%). Conclusions This validation demonstrated acceptable analytical performance for samples evaluated by an enzymatic creatinine method, specifically low concentration samples. The enzymatic UCr assay will be used to provide precise creatinine measurements for patients receiving biochemical genetic testing where the Jaffé result is below our measuring range for that assay. Selectively

targeting this workflow allows us to add additional value to the UCr result in an economically viable manner, given the relatively low frequency of observed UCr measurements at this low concentration.

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Analytical Performance Assessment of a Newly Formulated Creatinine Jaffe Assay on the ARCHITECT and Alinity c Systems.

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Introduction: Creatinine is a small molecule of 113 daltons and is a byproduct of muscle metabolism. Creatine is metabolized into creatinine as a result of muscle contraction. In a healthy individual, creatinine is cleared by the kidney and creatinine levels have been used to assess renal function. A well-functioning kidney filters creatinine from serum and excretes it in urine. Thus, an increase in serum creatinine levels indicate improper kidney function. The new formulation of creatinine Jaffe tested on ARCHITECT and Alinity c Systems is a reliable and robust test for measuring serum and urine creatinine concentrations meeting the updated CLSI guidelines.

Methods: The newly formulated creatinine Jaffe assay is a two-reagent photometric assay for the quantitative determination of creatinine in human serum and urine. In the first step of the reaction, sample is combined with alkaline reagent 1. For urine sample, a dilution of sample is performed automatically prior to combining it with reagent 1. A blank read of sample and reagent 1 is measured before reagent 2 containing picric acid is added in the second step of the reaction. Rate of formation of creatinine-picrate complex is directly proportional to the amount of creatinine present in the sample and is measured at 500 nm. The assay uses a 2-point calibration with a linear fit. The serum application is standardized to both levels I and II of the NIST SRM 967a whilst using only one physical calibrator. The urine application is standardized to NIST SRM 914.

Results: For serum application, the limit of detection and limit of quantitation were < 0.06 mg/dL and < 0.10 mg/dL, respectively. For urine application, the limit of detection and limit of quantitation were < 1.55 mg/dL and < 2.54 mg/dL, respectively. For the serum application, 20-day total imprecision, had a CV <4.0% or <0.026 mg/dL SD across the measuring range. Whereas, for the urine application, 20-day total imprecision, had CV <4% or <0.90 mg/dL SD. The serum application showed an average of -1.4% bias to NIST SRM 967a standard (0.847 mg/dL). The urine application showed an average of -3.1%, 1.3% and -0.9% bias to NIST SRM 914 standard tested at 3, 200 and 349 mg/dL respectively. Creatinine Jaffe assay demonstrates excellent linearity from 0.08 - 40.06 mg/dL for serum and 1.24 mg/dL- 871 mg/dL for urine. Reagent onboard stability of at least 10 days was observed with calibration stability of 10 days. The assay appears robust to common endogenous interferences tested at 2 mg/dL creatinine in serum. Method comparison to a current on-market creatinine assay showed a slope of 0.97 with correlation coefficient of 1.00 for the serum concentration range of 0.06 - 25.31 mg/dL. For the urine application, the slope for the concentration range of 6.65 - 727.61 mg/dL was 0.99 with a correlation coefficient of 1.00.

Conclusions: A newly formulated REACH compliant Creatinine Jaffe assay was designed for use on ARCHITECT and Alinity c Systems. This robust Creatinine assay has demonstrated analytically robust performance on the modernized CLSI protocols.

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Derivation of average analytic variation / clinical acceptability of the Radiometer ABL 90 from 3 years of ICU patient data

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Background: Excessive within- and/or between-instrument analytic variation can falsely indicate patient trends or obscure real trends. We employed a methodology that transforms sequential intra-patient results into estimates of biologic (s_b) and analytic (s_a) variation. This method was used to derive realistic s_a of blood gas (BG) analytes measured by the IL GEM 4000. We extend this methodology to derive the s_a of the Radiometer ABL90 BG analyzer.

Methods: A laboratory data repository provided safePICO syringe sampled arterial ABL90 BG, electrolyte and glucose measurements of 3 different ABL90s operated in 3 Ottawa Hospital adult ICUs between September 2016 and January2020. After eliminating biologic outliers, we tabulated all consecutive pairs of intra-patient results by the intra-test time interval. For each time interval, the between pair variations were reduced to a standard deviation of differences and then linearly regressed against the time interval with the y-intercept representing the sum of the short term analytic variation and the biologic variation: $y^2_{o\ ABL90} = s^2_{a\ ABL90} + s_b{}^2$. Assuming constant s_b among ICU patient populations, the analytical imprecision of the ABL90 is derived from $s_{aABL90} = (y^2_{o\ ABL90} - y^2_{o\ ABL800} + s^2_{a\ ABL800})^{1/2}$. **Results:** Regression graphs were derived from 1500 to 10,000 data pairs grouped into 2 h intervals from 0 to 12 h. The calculated s_{aABL90} exceed those of the Radiometer ABL800 with many ABL90 sigma ratios of s_b/s_a being close to unity. **Conclusion:** The analytic variation of the ABL90 system is inferior to that of the ABL800. Apart from iCa, the majority of the analytes are medically usable.

Test	yo_{ABL90}	s_b	s_{aABL90}	sigma ABL90	sigma ABL800
Cl, mmol/L	1.18	0.776	0.798	1.0	1.8
Glucose, mmol/L	0.87	0.515	0.503	1.0	4.7
HCO3, mmol/L	1.21	0.778	0.937	0.8	3.6
iCa, mmol/L	0.05	0.0144	0.042	0.3	3.4
K, mmol/L	0.26	0.192	0.182	1.1	7.9
Na, mmol/L	1.08	0.846	0.686	1.2	2.2
pCO2, mmHg	2.9	2.28	1.862	1.2	6.6
pH	0.028	0.0222	0.018	1.2	15.0
pO2, mmHg	12.2	13.4	4.345	3.1	9.9

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Evaluation of copeptin and psychological stress among healthcare providers during covid pandemic

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Background The (COVID-19) pandemic created a remarkable impact on healthcare providers both physically and psychologically. Perceived psychological stress (PSS) influences the homeostatic equilibrium, involving activation of the sympathetic nervous system and hypothalamus pituitary adrenal (HPA) axis. Copeptin, C-terminal portion of Vasopressin (AVP) precursor is stable. However, evidence about impact of PSS on copeptin levels is limited. Aim of this study was to estimate the influence of psychological stress on copeptin levels among healthcare providers working in intensive care units (ICU). **Methods** A total of 70 healthcare providers (HCP) served in quarantine hospitals ICU participated in this prospective study; 35 physicians (28 males and 7 females) and 35 nurses (10 males and 25 females). A control group of 40 HCP matched age, BMI and specialty in non- quarantine hospitals. Exclusion criteria included hypertension, diabetes mellitus, obesity BMI above 30, subjects with serum sodium <135 or > 145 mmol /l prior isolation or females receiving contraceptive pills. Fasting morning blood samples were withdrawn for determination of copeptin, cortisol, insulin at three points; before isolation at ICU. Second point at end of first week and third point was two weeks' post isolation. A questionnaire was conducted to all participants to assess stress (PSS). Cortisol was determined by a chemiluminescence immunoassay while insulin and Copeptin were measured by ELISA.**Results** Baseline plasma copeptin level before isolation was significantly increased 15.76±8.6 pmol/l and was positively correlated with high stress PSS score mean 66.9± 18.3. Post isolation copeptin was markedly reduced 3.98±1.28 pmol/l and mean PSS was 23.0±7.95. Also, there was positive correlation between plasma copeptin and PSS, systolic blood pressure and serum insulin. On the other hand, there was no correlation between copeptin and serum cortisol. **Conclusion** our finding suggested that copeptin may be used a potential biomarker for physiological strain during work in a stressful environment.

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Evaluation of a New Metering Algorithm to Enable Whole Blood Sampling for VITROS® A1c Chemistry Products Slides* on the Ortho VITROS® 5600/XT 7600 Integrated and 4600/XT 3400 Chemistry Systems

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Background: VITROS A1c Chemistry Products Slides* are being developed for the measurement of glycated hemoglobin A1c on the VITROS 5600/XT 7600 Integrated and VITROS 4600/XT 3400 Chemistry Systems.Fully suspended whole blood samples are required to enable accurate A1c testing on automated, routine clinical chemistry instruments. This can complicate the lab's workflow, resulting in users batch testing a limited number of samples together to ensure the red blood cells have not settled. A new metering algorithm as a part of VITROS INTELLICHECK® technology enables whole blood sampling and confirms that adequate red cells are sampled to

the VITROS A1c Slides* directly from a standard EDTA collection tube without any pre-treatment steps. The efficacy of the whole blood metering algorithm to resuspend the whole blood cells has been demonstrated for up to 20 minutes to enable high volume, random access testing, as well as testing on an automated track.Many factors can affect the efficacy of the metering algorithm to resuspend whole blood samples including, sample volume within a collection tube, dimension of the collection tube, and inherent qualities of the sample such as erythrocyte sedimentation rate (ESR) and hemoglobin concentration. The instrument protocol to test and mitigate whole blood samples for A1c must be robust to all of these factors. **Methods:** The efficacy of whole blood sample resuspension was assessed by computing the percent bias/difference between the VITROS A1c Slides measurement for samples up to 20 minutes of settling and for fully suspended samples (zero minutes settling) out of a standard 4 mL EDTA collection tube. 130 unique patient samples were cumulatively tested on four VITROS Systems (VITROS 5600/XT 7600 Integrated and VITROS 4600/XT 3400 Chemistry Systems). Samples included local draws, sourcing from various vendors, and pre-screened high ESR samples. Samples tested had hemoglobin concentrations between 8 and 17g/dL and %A1c values between 4 and 11%. A sample is considered "passing" if the bias of the partially settled sample's %A1c measurement is within ± 5% of the suspended sample's %A1c measurement. **Results:** 100% (130 samples) of sample biases were within ± 5% for the 20-minute partially settled samples in EDTA collection tubes compared to the suspended samples for all VITROS Systems tested. **Conclusion:** The data presented here demonstrates that the new metering algorithm enables whole blood sampling for VITROS A1c Slides* testing on the VITROS Integrated and Chemistry Systems and is effective for at least 20 minutes sampled out of an EDTA collection tube or sample cup. This feature combined with the use of VITROS A1c Slides* on automated routine VITROS Systems significantly enhances the ease of use for the measurement of glycated hemoglobin A1c and improves the lab's workflow by removing the need to batch test A1c samples. *Under development

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The New CDC Clinical Standardization Program for Thyroid Function Tests to Assist Laboratories and Assay Manufacturers with Improving Patient Care Through Accurate and Reliable Free Thyroxine Measurements

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Background: Thyroid function tests are the most frequently ordered tests in the U.S. Reliable FT4 measurements are critical to correctly assess thyroid function and diagnose and treat thyroid disorders. Concerns about the accuracy and comparability of FT4 measurements have been raised for many years stakeholder organizations such as The Partnership for the Accurate Testing of Hormones (PATH) have categorized FT4 as a biomarker in high need for standardization.

Methods: The goal of CDC CSP is to improve the diagnosis, treatment, and prevention of hormone-related diseases by standardizing clinical laboratory measurements. Steps taken to achieve this goal include maintaining reference measurement procedures, evaluating certification program participants, and monitoring end-users. The CDC CSP is operating a reference measurement procedure (RMP) for FT4 that is in line with RMP established by the International Federation of Clinical Chemistry and Laboratory Medicine Committee for Standardization of Thyroid Function Tests (IFCC C-STFT). The CDC FT4 RMP is based on equilibrium dialysis (ED) coupled with LC-MS/MS. The CDC RMP is part of a reference laboratory network that is being established by the IFCC C-STFT. Like other RMPs operated as part of the IFCC effort, the CDC RMP is traceable to SI and in process of being listed by the Joint Committee for Traceability in Laboratory Medicine (JCTLM).

Results: . The intra- and inter-day imprecision of the CDC RMP are 3.0% and 1.1%, respectively, and the CDC RMP absolute mean bias to 3 other C-STFT RMPs is 2.5%. The measurement range is 3.02-258 pmol/L, making the method suitable for analysis of hypo- as well as hyperthyroid patients. The performance of the CDC FT4 RMP allows for it to be used as an accuracy base to which routine methods can be traceable.

Conclusion: Using this RMP, the CDC assigns FT4 values to single-donor serum materials, where none have previously been available. These materials are then available to collaborators with the CDC CSP to evaluate the accuracy and imprecision of their methods, adjust calibration if needed, and monitor performance over time. The CDC-CSP will also monitor the performance of FT4 assays at the end-user level through partnerships with proficiency testing agencies. Based on previous studies conducted by Prof. Thienpont at the University of Ghent, it is anticipated that recalibration of existing FT4 assays to become traceable to SI can change patient results by in average 50%. This session will provide information to help communicate these changes to health care providers.

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Clinical Agreement and Method Comparison of the NOVEOS™ and ImmunoCAP™ Specific IgE Assays Using Allergens G002 (Bermuda Grass, C. dactylon), G005 (Rye Grass, L. perenne) G006 (Timothy Grass, P.pratense), G010 (Johnson Grass, S. halepense)

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Background: The NOVEOS™ Specific IgE (sIgE) assay utilizes paramagnetic microparticles as the solid phase to capture allergen IgE antibodies; the chemistry demonstrates good sensitivity and accuracy in measuring IgE in human serum. The results of NOVEOS in vitro testing serve as an aid in the diagnosis of allergy associated disease and can supplement results observed for Skin Prick Testing (SPT)1. In order to demonstrate the accuracy for NOVEOS allergen-specific IgE, comparison studies between the ImmunoCAP™ sIgE assay and NOVEOS sIgE were assessed. Four grass allergens were used for this comparative study: G002 (Bermuda Grass, C. dactylon), G005 (Rye Grass, L. perenne) G006 (Timothy Grass, P. pratense), G010 (Johnson Grass, S. halepense). Methods: The agreement of the NOVEOS sIgE assay was evaluated using clinical samples with known SPT results in accordance with CLSI EP12. In addition, a relative comparison study between the NOVEOS and ImmunoCAP specific IgE (sIgE) assays was conducted according to CLSI EP09-A3 guidelines using more than 220 samples from anonymous donors. Finally, the normal range of the assay was evaluated in accordance with CLSI EP28-A3C.

Results: Clinical sensitivity and specificity for the four grass allergens exhibited a range of 58.8% - 88.2% and 95.9% - 99.2%, respectively. The relative agreement in sensitivity to ImmunoCAP was 83.3% - 95.7%, whereas the relative agreement in specificity was 96.0% - 99.2%. The quantitative comparison between the NOVEOS and ImmunoCAP sIgE assays showed linear regression slopes ranging from 0.60 to 1.34 and correlations (r) between 0.845 and 0.955. The normal range for the NOVEOS sIgE assay is <0.35 kU/L for all four allergens assessed.

Conclusion: The NOVEOS sIgE assay demonstrates good agreement with clinical status, and comparable results to ImmunoCAP sIgE assay for the four grass allergens examined.

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Evaluation of Concordance for VITROS® A1c Chemistry Products Slides* on the Ortho VITROS Automated Solution configuration with VITROS® 5600/XT 7600 Integrated and 4600/XT 3400 Chemistry Systems

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Background: Globally, due to the rise of pre-diabetes and diabetes, expert groups recommend glycated hemoglobin A1c (A1c) for screening at-risk populations. The high demand for A1c testing requires laboratories to deliver accurate A1c results with high volume operational efficiencies. VITROS® A1c Chemistry Products Slides* are being developed for the measurement of glycated hemoglobin A1c on the automated VITROS 5600/XT 7600 Integrated and VITROS 4600/XT 3400 Chemistry Systems and are compatible for use with the VITROS Automation Solution (VAS) configuration. Compatibility with the VAS track enables customers to load and automate testing of suspended whole blood samples. The VAS modules will de-cap the primary test tube and automatically route to the VITROS Systems for sample check, testing, and analysis. There is no pre-treatment step as the red blood cells are lysed directly on the VITROS A1c Slides. Ortho verification has demonstrated acceptable sample metering within the 20-minute default time-out on VAS. User adjustment of the time-out on the VAS is possible, based on the customer's validation for red blood cell settling time with the customer's patient population. **Methods:** The result obtained with the VITROS A1c Slides* measurement on VAS compared to the VITROS 5600/XT 7600 Integrated Systems and VITROS 4600/XT 3400 Chemistry Systems was evaluated for both correlation and precision. Forty (40) unique whole blood patient samples were tested across the VITROS Systems (10 samples per VITROS System) at 10 replicates per fluid-system combination. Two (2) levels of quality control fluids were also tested across each VITROS System at thirty (30) replicates per fluid-system combination. Precision was analyzed by performing a statistical F-test across each system and quality control fluid combination and across each system and all patient sample replicates at a 95% confidence interval. VITROS System correlation to VAS was analyzed by performing a paired T-test and linear regression across each VITROS System and patient sample means at a 95% confidence interval. Data was additionally summarized by computing the grand mean, SD (SD for patient samples pooled by patient), and %CV. **Results:** The F-test conducted for precision assessment showed no statistical difference (p-values > 0.05) at a 95% confidence interval for both patient samples and

quality control fluids for each system. The paired T-test conducted across the ten (10) patient sample means showed no statistical difference at a 95% confidence interval for each system. Linear regression analysis showed excellent correlation between System and VAS: VAS = 1.036 * VITROS 4600 A1c - 0.19; (r) = 0.998, VAS = 1.019 * VITROS 5600 A1c - 0.10; (r) = 0.999, VAS = 0.975 * VITROS XT 3400 A1c + 0.138; (r) = 0.994, VAS = 0.961 * VITROS XT 7600 A1c + 0.212; (r) = 0.995. **Conclusion:** The data presented here demonstrates that VITROS A1c Slides* show excellent concordance between testing on the VITROS Automation Solution and on the VITROS Systems. VAS compatibility for VITROS A1c Slides* enables laboratories to deliver accurate A1c results with high volume operational efficiencies. *Under development

A-156

Optimization of Thawing Process for Analysis of Clinical Chemistry Analytes on Frozen Samples

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Background: Clinical studies often involve collection and shipment of serum or plasma samples in frozen conditions from collection sites and multicenter laboratories, to central labs for analysis (Cuhadar et al., 2013). These samples are thawed to room temperature for analysis but often not mixed well before testing, which is an important pre-analytical step. Mixing samples after thawing is important to maintain the homogeneity of the samples and to obtain accurate results. The purpose of this study is to evaluate how mixing of the samples after thawing effects the results of common chemistry analytes. **Methods:** Serum samples were collected from 30 participants and a three set test tube produced for each participant. Initial baseline testing for the analytes listed in the table below was performed immediately after sample collection at room temperature using the Roche Cobas8000. Tubes kept at -65 °C were thawed using two different methods by air drying without mixing, and the samples were allowed to thaw and mix on the Stuart SRT9D Roller Mixer. Both sets were analyzed immediately on Roche Cobas8000. Regression and t-test analysis were used to analyze data from the two methods against the baseline average. **Results:**

Analyte (SI-unit)	t-test between fresh vs. thaw 30 min (p value)	t-test between fresh vs. thaw by mixing (p value)	Regression between fresh vs. thaw 30 min (R-value)	Regression between fresh vs. thaw by mixing (R-value)
Calcium (mmol/L)	0.418	0.026	0.5133	0.9800
Total Bilirubin (umol/L)	0.09	0	0.9516	0.9864
Albumin (g/L)	0.019	0.005	0.4652	0.9262
Alkaline Phosphatase (U/L)	0	0.777	0.9114	0.9968
Alanine Aminotransferase (U/L)	0	0.388	0.9568	0.9948
Aspartate Aminotransferase(U/L)	0	0.835	0.9037	0.9655
Chloride (mmol/L)	0	0	-0.0964	0.9408
Bicarbonate (mmol/L)	0.658	0	0.7013	0.8732
Creatinine (umol/L)	0	0.952	0.8820	0.9339
Potassium (mmol/L)	0	0.027	0.6681	0.9539
Sodium (mmol/L)	0	0	0.1039	0.8888
Total Protein (g/L)	0.001	0.025	0.5656	0.9541
Blood Urea Nitrogen (mmol/L)	0.001	0.078	0.9421	0.9937
Glucose (mmol/L)	0.001	0.635	0.9763	0.9984

Conclusion:

The correlation coefficients (R), in the second method are much closer to 1 as compared to the first method, indicating that when samples are properly mixed after thawing, the results are much closer to the baseline results (fresh). Most of the p-values from the first method were <0.05 as compared to the second method. This demonstrates that there is much greater chance of having significant differences between means of the baseline and those of the unmixed samples. Therefore, mixing the samples before testing is very important for homogeneity of samples and to obtain accurate results.

A-157

Chemistry Assay Equivalency between the Atellica CH 930 and Atellica CI 1900* Analyzers

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Background: The Atellica® CI 1900 Analyzer* is an integrated chemistry and immunoassay analyzer that uses Atellica® CH and Atellica® IM assay reagents. The purpose of this investigation was to evaluate the analytical performance of the Atellica® CH Albumin BCP (Alb_P), Hemoglobin A1c, Enzymatic (A1c_E), Alanine Aminotransferase (ALT), Iron (Iron_2), and Vancomycin (Vanc) Assays on the Atellica CI 1900 analyzer and compare it to the performance of the same assays on the commercial Atellica® CH 930 Analyzer.

Methods: Performance testing included precision and method comparison. Precision was run over five days with five replicates per sample per day, for a total of 25 replicates per sample on the Atellica CI 1900 analyzer. Method comparison and precision were evaluated based on CLSI documents EP09-A3 and EP15-A2, respectively. Atellica CH 930 analyzer assay precision was taken from the commercial instructions for use and is based on 20-day studies.

Results: Atellica CI 1900 analyzer results were generated using Atellica CH reagents and calibrators. Atellica CH Alb_P assay within-lab precision on the Atellica CI analyzer ranged from 1.3 to 2.3% CV. Alb_P method comparison for the Atellica CI 1900 analyzer versus the Atellica CH 930 analyzer using Deming regression yielded a slope of 0.99, y-intercept of 0.0 g/dL, and r of 0.99, with 50 samples. Atellica CH A1c_E assay within-lab precision on the Atellica CI 1900 analyzer ranged from 0.5 to 1.2% CV. A1c_E method comparison for the Atellica CI 1900 analyzer versus the Atellica CH 930 analyzer using Deming regression yielded a slope of 0.98, y-intercept of 0.17%, and r of 0.99, with 48 samples. Atellica CH ALT assay within-lab precision on the Atellica CI 1900 analyzer ranged from 0.5 to 2.5% CV. ALT method comparison for the Atellica CI 1900 analyzer versus the Atellica CH 930 analyzer using Deming regression yielded a slope of 0.98, y-intercept of 2 U/L, and r of 1.00, with 52 samples. Atellica CH Iron_2 Assay within-lab precision on the Atellica CI 1900 analyzer ranged from 0.3 to 2.0% CV. Iron_2 method comparison for the Atellica CI 1900 analyzer versus the Atellica CH 930 analyzer using Deming regression yielded a slope of 1.02, y-intercept of -1 µg/dL, and r of 1.00, with 50 samples. Atellica CH Vanc assay within-lab precision on the Atellica CI 1900 analyzer ranged from 0.9 to 2.5% CV. Vanc method comparison for the Atellica CI 1900 analyzer versus the Atellica CH 930 analyzer using Deming regression yielded a slope of 0.99, y-intercept of -0.1 µg/mL, and r of 1.00, with 53 samples.

Conclusion: When evaluated on the Atellica CI 1900 Analyzer, the Alb_P, A1c_E, ALT, Iron_2 and Vanc assays demonstrated equivalent performance to that observed on the Atellica CH 930 Analyzer. These assays demonstrated acceptable precision and method comparison. Assay performance on the Atellica CI 1900 analyzer supports equivalent assay performance for laboratory settings where comparison to the Atellica CH 930 analyzer is desired.

*Product under development. Not available for commercial use or sale. Future availability cannot be guaranteed.

A-158

Analytical Performance Assessment of a Newly Formulated REACH Compliant Total Bilirubin Assay on Abbott's Alinity c Systems

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Background: Bilirubin is the degradation product of hemoglobin and other heme compounds resulting from red blood cell degradation. Total Bilirubin represents the sum of unconjugated (indirect) and conjugated (direct) bilirubin fractions. Compared to unconjugated bilirubin, conjugated bilirubin contains glucuronide moieties, offering improved aqueous solubility. Clinically, total bilirubin levels are used to assess liver, hematologic, or other metabolic disorders. In particular, for newborn infants, total bilirubin levels are commonly assessed for management of neonatal jaundice and hemolytic disease.

Methods: The newly formulated Total Bilirubin assay (LN 04T09) utilizes the diazo methodology. The new 04T09 reagent includes a commercially available 2,4-dichlorobenzenediazonium salt, which eliminates the requirement for in-process diazonium ion synthesis from precursor raw materials as employed in other total bilirubin assays. This process modification limits the potential for manufacturing-associated reagent variability. The diazo reaction is initiated by addition of serum or plasma sample to

the cuvette followed by Reagent 1, which contains surfactants and stabilizers at low pH (conditions necessary to completely solubilize indirect bilirubin). Reagent 2 is then added to the reaction mixture and the diazonium ion reacts with both direct and indirect bilirubin from the sample to form azobilirubin. The production of azobilirubin is proportional to the concentration of total bilirubin in the sample and is measured at 548 nm.

Results: The Limit of Quantitation (LOQ) was 0.07 mg/dL on c8000 and 0.10 mg/dL on Alinity c. Total within-laboratory imprecision was </= 2.2 % on c8000 and </= 2.6 % on Alinity. The assay demonstrates linearity from 0.3 - 26.4 mg/dL. Reagent on-board stability of 30 days was observed. This newly formulated assay is resistant to typical endogenous interferents at 1.2 mg/dL, (low medical decision point). Moreover, the assay demonstrated resistance to indicant interference (as indoxyl sulfate), a well-known metabolite competitor for the diazonium ion that results in positive interference, at levels up to 125 µM interferant when tested at the low MDP. Method comparison to (current) on-market Total Bilirubin assay resulted in a slope (Passing-Bablok) of 1.00 on ARCHITECT c8000 for serum samples between 0.29 - 27.90 mg/dL and a slope of 0.94 for plasma samples between 0.57 - 22.42 mg/dL. Correlation coefficient (r) was 1.00 for both matrices.

Conclusion: Abbott introduces a newly formulated, REACH compliant Total Bilirubin assay that traces directly to the Doumas reference method. This alignment strategy results in improved correlation to the traceable method across the measuring interval. The lyophilized Calibrator will offer longer shelf stability resulting in fewer lot changes improving reagent lot to lot variances. The assay is resistant to high levels of human triglycerides and hemoglobin at the low MDP of 1.2 mg/dL.

A-159

Verification study of the clinical performance of the VITROS SARS-CoV-2 Antigen assay in samples confirmed positive by RT-PCR

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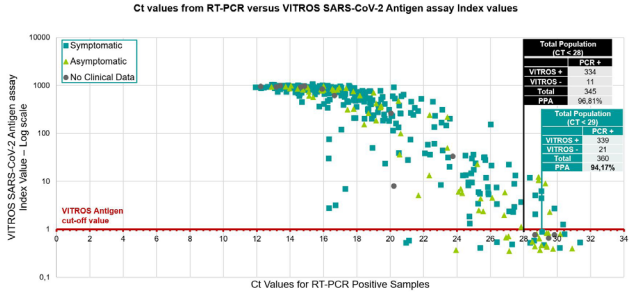
Background: The recent emergence of SARS-CoV-2 antigen detection methodologies by chemiluminescence offers an opportunity to use a cost-efficient methodology to verify the presence of the virus and with faster response times versus RT-PCR, the current "Gold Standard" method for COVID-19 diagnosis. The objective of this study was to evaluate the clinical performance of VITROS SARS-CoV-2 Antigen assay using confirmed positive RT-PCR samples for SARS-CoV-2 freshly isolated from individuals, for assessing the Positive Percentage Agreement (PPA). **Methods:** A total of 385 Positive SARS-CoV-2 samples obtained from nasopharyngeal swabs in PBS media, confirmed by a Fluorescent RT-PCR method for SARS-CoV-2 (Maccura Biotechnology (USA) LLC), were tested further using VITROS SARS-CoV-2 Antigen assay in VITROS XT7600 system, within 48 hours from collection. **Results:** We identified three groups of individuals, categorized per their clinical data: symptomatic, with 0-17 days after symptoms onset (DSO), asymptomatic and with no clinical data available. Symptomatic population (274 samples), showed overall 95.26% PPA (Fig. 1). Patients with ≤ 7 DSO showed 96.72% and 83.33% for patients with > 7 DSO. Cycle threshold (Ct) below 29, showed 97.35% PPA. Asymptomatic population (94 samples), showed 93.15% and 86.42% PPA for Ct < 28 and < 29, respectively. Population without clinical data (17 samples) showed 92.31% and 80.00% PPA for Ct < 28 and < 29, respectively. **Conclusions:** Results from the VITROS SARS-CoV-2 Antigen assay showed overall 89.6% PPA versus this RT-PCR method, demonstrating a useful diagnostic tool for expanding testing and increasing coverage for new cases. We also validated use of PBS as a common VTM for both Antigen and RT-PCR. We identified the best clinical sensitivity at Ct < 29, showing an overall 94.17% PPA. No Ct above 32 were observed using this method. This Antigen assay offers a trustable method where RT-PCR is not available or is not cost-efficient.



A-160

C-peptide Certified Reference Materials: Assessing Peptide Modifications to Avoid Bias

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Background: C-peptide assays have widely been used as a measure of insulin secretion to assess the pancreatic beta-cell function. High levels of C-peptide may be seen in insulin resistance, insulinoma, and kidney disease. A low level is usually present in patients with type 1 diabetes and, uncertain circumstances, type 2 diabetes. Worldwide collaborations have been established to fulfill the need for accurate, traceable, and standardized measurements for C-peptide. Certified reference material (CRM) from National Metrology Institute of Japan (NMIJ) has been widely used for the preparation of C-peptide calibrators and the CRM value was determined by two independent quantitative amino acid analyses using liquid phase and gas phase hydrolyses.

Methods: To assess the utility and comparability of alternative CRMs and minimize potential operational issues, we compared CRM from NMIJ (NMIJ CRM 6901-b, Lot# 058) with CRM from Cerilliant (C-161-0.1ML, Lot# FN04221901) using our C-peptide LC/MS/MS assay (a high-throughput, quantitative, multiplexed LC/MS/MS assay for intact insulin and C-peptide). CRM from NMIJ is a lyophilized power and CRM from Cerilliant is a solution in sealed ampule. The certified values from both sources include the proportion of unmodified C-peptide and total C-peptide present (mixture of unmodified, deamidated, and pyroglutamate-containing C-peptide).

Results: We compared patient C-peptide LC/MS/MS results obtained using calibrators prepared using the mass concentrations of unmodified C-versus total C-peptide and found unacceptable bias. In contrast, patient results exhibited no bias when mass concentrations of unmodified C-peptide were used to assign calibrator concentrations, regardless of manufacturer. These results reflect the ability of LC/MS/MS assays to differentiate between unmodified and modified forms of C-peptide, such as deamidated and pyroglutamylated C-peptide.

Conclusion: We conclude that CRM of C-peptide from Cerilliant and from NMIJ behave identically when the mass concentration of unmodified C-peptide is used to assign calibrator concentrations. The mass concentration of unmodified C-peptide (not the mass concentration of total C-peptide) in CRM should be used when making calibrators for mass spectrometry C-peptide assays to achieve comparable patient results as C-peptide result from mass spec assay represents only unmodified C-peptide, and the ratio of unmodified C-peptide and total C-peptide in CRM from different sources may vary from lot to lot. Special attention needs to be paid when comparing immunoassays to mass spectrometry assays as the mass concentration of total C-peptide from CRM is used in immunoassay.

A-161

Evaluation of Hemoglobin A1c Quantification by Capillary Electrophoresis Using Capillary Whole Blood

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Background: Hemoglobin A1c (HbA1c) is a glycated hemoglobin formed by the non-enzymatic addition of glucose to the amino-terminal valine residues of the hemoglobin beta chains. It reflects the average blood glucose concentration over the previous 8-12 weeks and is used clinically to diagnose diabetes, aid in diabetes management, and to identify patients at increased risk for diabetes. Coronavirus-related pandemic lockdowns have highlighted the need for patient-collected HbA1c blood samples to avoid potential exposures to the virus. The objective of this study was to validate the use of capillary whole blood for quantitation of HbA1c. Methods: HbA1c was determined by capillary electrophoresis using the CAPILLARYS 3 Tera (Sebia Inc., Norcross, GA). Capillary whole blood was obtained by fingerstick and approximately 50 µL was added to 100 µL of hemolyzing solution (Sebia, Inc.) and mixed by inversion. Accuracy was assessed by comparing HbA1c results from capillary and venous blood collected from volunteers. Between- run and within run precision were evaluated at two concentrations using patient samples tested in two replicates for twelve days. Precision samples were stored at -80°C. Sample stability was evaluated by storing samples at ambient temperature, 2-8°C, or 40°C, for up to 8 days. The quality goal was defined as a total allowable error of 4.4%. The study was approved by the WCG Institutional Review Board. Results: Deming weighted linear regression produced a slope of 1.026 (95% CI: 0.959-1.064); y-intercept of -0.127 (95% CI: -1.378-0.211); and R^2 of 0.98. Within run precision was 1.88% and 0.81% at HbA1c concentrations of 5.5% and 6.4%, respectively. Between-run precision was 1.81% and 1.53% at HbA1c concentrations of 5.5% and 6.4%, respectively. Samples were stable for at least 8 days when stored at ambient temperature and at 2-8°C and up to 12 hours at

40°C. Total error (systemic error added to twice the random error) was calculated as 4.3% at the decision level concentration of 5.6%. Conclusion: The analytical performance of capillary electrophoresis for the measurement of HbA1c in capillary blood met the quality goal for total allowable error. Capillary blood is an acceptable alternative to venous blood for HbA1c testing. Samples collected by patients can remain at ambient temperature and analyzed within 8 days of collection.

A-162

Lot to Lot Reagent Variance Quantitation for Heart Failure Diagnosis and Monitoring Assays

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Heart failure is defined as the progressive inability of the heart ventricles to pump blood out to the lungs and/or the extremities. Heart failure is a chronic condition caused by a variety of underlying disease states such as coronary artery disease, hypertension, valve disease, myocarditis, etc. Symptoms of heart failure are ranked per the New York Heart Association (NYHA) classification into increasing severity classes (NYHA I-IV). Heart failure can lead to worsening cardiovascular disease and death. Both BNP and N-terminal pro-BNP are used to guide titration of therapy. Using these biomarkers has been associated with significant reductions in all-cause mortality compared to standard of care in patients with chronic heart failure. The Galectin-3 assay is another test typically used in heart failure as a marker of fibrosis. Due to the long-term nature of heart failure and the need for monitoring and follow up the question of reagent stability and lot to lot variability becomes a real concern for laboratorians. **Objective:** The present study aims to do a look back over several years, instrument systems, reagent and control lots in our laboratory (St Vincent's Hospital) to evaluate BNP, Galectin 3 and NT-proBNP reagent lot variances. Manufacturing data on QC and human serum panels over multiple years and reagent lots is included for comparison. **Methods:** Clinical laboratory testing was performed in the Core Lab Facility at the UCD Clinical Research Centre St. Vincent's University Hospital, Dublin Ireland. Here, QC was assessed across multiple reagent lots over a 4-year period for BNP and Galectin 3 and over a 1-year period for NTpro-BNP. Abbott internal manufacturing data represent 69 lots of BNP, 12 lots of Galectin-3, and 11 lots of NT-proBNP manufactured over a 2-year period. Assays were run on the Abbott Architect ci4100 and/or i2000SR instrument. For all assays, QC and human serum panels were tested in multiple replicates and runs. Imprecision (percent coefficient of variation (%CV)) was calculated across all reagent lots on each control level mean for each assay. **Results:** The within assay reagent lot to lot variability remained less than 9% CV across all QC levels tested in the clinical laboratory setting and for all QC and serum panels tested during manufacturing. **Conclusions**: NTpro-BNP, Galectin-3 and BNP showed consistent reagent lot-to-lot performance on all control levels and human serum panels tested during manufacture and at St. Vincent's clinical laboratory.

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Beyond 25(OH)D: Using the Vitamin D Metabolite Ratio to Assess Vitamin D Status

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Background: Vitamin D is an essential hormone involved in the regulation of calcium homeostasis and bone/mineral metabolism. The vitamin D metabolic pathway involves a series of cytochrome P450-mediated reactions that tightly regulate the production of $1,25(OH)_2D$, the best-known active vitamin D metabolite. Vitamin D, once ingested or photosynthesized in skin, is metabolized to 25(OH)D, which is further metabolized to $1,25(OH)_2D$, or alternatively, is catabolized to $24,25(OH)_2D$, converted to calcitroic acid, and eliminated. Vitamin D metabolites are largely transported in circulation by vitamin D binding protein (DBP), with only ~1% unbound, or 'free'. Previous studies have noted that circulating 25(OH)D levels are strongly correlated with circulating DBP, thus potentially confounding the use of 25(OH)D as an accurate biomarker of vitamin D status. Recently, the vitamin D metabolite ratio (VMR) has been proposed as an important biomarker of vitamin D status as it was shown to be independent of DBP concentrations in a population of community-dwelling older adults. **Objectives:** To assess the relationship between VMR and DBP levels in a cohort of healthy children. In addition, we explored potential relationships between vitamin D metabolites, VMRs, and circulating PTH as to provide insight into PTH's role(s) in the regulation of active and catabolic vitamin D pathways. **Methods:** We measured 25(OH)D, $24,25(OH)_2D$, $1,25(OH)_2D$, and DBP concentrations in over 770 healthy infants and toddlers 6 to 36 months of age in a midsized northeastern U.S. urban community (New Haven, CT, USA). Serum 25(OH)D, $1,25(OH)_2D$, and $24,25(OH)_2D$ were measured by radioimmunoassay, DBP was measured by immunonephelometry, and $24,25(OH)_2D$ was measured by LC-MS/MS. VMRs of $24,25(OH)_2D$: 25(OH)D (VMR1), $1,25(OH)_2D$: 25(OH)D (VMR2), and $1,25(OH)_2D$: $24,25(OH)_2D$ (VMR3) were calculated. **Results:** Using linear regression analysis, we show that vitamin D metabolites including 25(OH)D, $1,25(OH)_2D$, and $24,25(OH)_2D$ exhibit a significant, non-linear correlation with increasing DBP concentrations. All three VMRs, however, were independent of DBP concentrations and thus represent an important biomarker to assess vitamin D status in pediatric populations where DBP levels can differ significantly. Additionally, we found significant, inverse relationships between PTH concentrations (grouped as <13, 13-24 and >24 pg/mL, representing lower 25%, middle 50% and upper 25% of the cohort) and vitamin D metabolites 25(OH)D and $24,25(OH)_2D$. Significant direct relationships were observed for VMR 2 and VMR3, whereas no significant difference was observed for VMR1. These results demonstrate that with increasing PTH concentrations, catabolic metabolites are downregulated to allow for activation of the $1,25(OH)_2D$ pathway. Increases in both VMR2 and VMR3 provide additional evidence in this regard. **Conclusion:** The utility of 25(OH)D as a biomarker for vitamin D status may be limited as it is impacted by changes in DBP concentrations. We demonstrate the utility of VMRs as a biomarker for vitamin D status in a pediatric population as they are independent of DBP concentrations. Furthermore, we utilize VMRs to demonstrate the dynamic interplay among vitamin D metabolites in the PTH-vitamin D endocrine system and how metabolic pathways are regulated in response to the changes in PTH levels.

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Development of a Novel Carrier Protein for Monoclonal and Polyclonal Antibody Production

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Background: Carrier proteins are critical in the antibody production process as they confer immunogenicity to poorly immunogenic compounds such as small molecules or peptides. Hemocyanins are copper containing proteins used for oxygen transport in arthropods and mollusks and are routinely utilized as carrier molecules due to their size, potent immunogenicity, and phylogenic distance from mammalian hosts/antigens. The most commonly used carrier protein is keyhole limpet hemocyanin (KLH). However, current immunization protocols often require alternating carrier molecules to prevent immunodominance of the carrier to the detriment of the antibody response to the hapten. Thus, there is a need for an alternative highly immunogenic carrier protein. Lobster hemocyanin (LLH), is readily available as a byproduct from the food industry. Here, we evaluate the utility of lobster hemocyanin as a carrier protein of hapten antigens in comparison with KLH.

Methods: Hemocyanin was purified from American lobster (*Homarus americanus*) serum. Peptide, protein and hormone antigens were covalently conjugated to LLH and KLH. New Zealand white rabbits and/or BALB/c mice were immunized with either the KLH-antigen conjugate or the LLH-antigen conjugate. Serum was collected at designated time points following immunizations and antigen specific antibody titers were measured by indirect ELISA. Antibody responses directed against the carrier molecule (LLH or KLH), as well as cross-reactivity of anti-LLH and anti-KLH antibodies, were also measured via indirect ELISA.

Results: Pre-existing antibodies against LLH were undetectable by ELISA in sera isolated from naïve rabbits. Immunization with both LLH and KLH conjugated antigens resulted in the production of antigen specific antibodies. There was no significant difference in the antibody titers generated against the peptide or protein antigens conjugated to LLH or KLH.

Conclusion: LLH is an immunogenic and effective carrier protein that promotes the generation of polyclonal antibodies to conjugated haptens as effectively as KLH. Anti-LLH antibodies do not cross-react with KLH, signifying that the LLH epitopes are unique from those on KLH. Thus, LLH is novel carrier protein that can be used as an alternative to or in conjunction with KLH.

A-166

Role of RNase L in Lipid Synthesis and NAFLD Development

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Background: Non-alcoholic fatty liver disease (NAFLD), a clinical manifestation with 5% or more hepatic steatosis, is mainly caused by disrupted lipid homeostasis. Currently, 30% of the US population is affected by NAFLD, and patients with NAFLD have a higher risk of developing diabetes and cardiovascular diseases. Interferons (IFNs) play a crucial role in NAFLD, particularly in the progression to non-alcoholic steatohepatitis (NASH). Interestingly, our preliminary data indicated that RNase L, an IFN-stimulated gene (ISG), could be involved in lipid homeostasis because RNase L-deficient mice showed heavier body weight and more fatty droplets in the liver. This study is to investigate the molecular mechanism by which RNase L regulates lipid metabolism and NAFLD development.

Methods: One-year old male RNase L wild-type (WT) and knockout (KO) mice (n=8/group) in the C57BL/6 background were fed with a chew-diet (CD) and a high fat-diet (HFD) containing 2% cholesterol for 10 weeks. After termination of the experiments, the mice were euthanized and the liver tissues were removed, and then fixed in 10% neutral formalin overnight. The tissues were paraffin-embedded and sectioned to 4 μm in thickness, and subsequently stained with hematoxylin and eosin for histological assessment. The expression of fatty acid synthase (FASN) and HMG-CoA reductase, a key enzyme for cholesterol synthesis, was determined by Western blot analysis.

Results: We found that after fed with HFD, the liver tissues of RNase L KO mice showed significantly more and larger lipid droplets mainly in the form of macro-vesicular steatosis, companied with some degree of liver inflammation, tissue damage and fibrosis. In addition, FASN and HMG-CoA reductase were significantly higher expressed in the livers of RNase L KO mice.

Conclusion: The results suggest that RNase L deficiency could severely disrupt the lipid homeostasis in the liver by upregulating the key enzymes that are essential for fatty acid and cholesterol synthesis. Further investigation on the specific molecular events involved in this process may provide novel therapeutic strategies for treating NAFLD/NASH based on the regulation of RNase L.

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Struture Specific Bile Acids Predict Future Cardiometabolic Outcomes

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Background: Bile acids (BAs) serve multiple biological functions, ranging from absorption of lipids and fat soluble vitamins, to serving as signaling molecules through the direct activation of dedicated cellular receptors that participate in the regulation of numerous pathways relevant to cardiometabolic diseases. Primary BAs produced by the liver are metabolized by gut microbial enzymes into secondary BAs that are reabsorbed through the enterohepatic circulation back into system. The objective of this work was to establish the relationship among circulating BAs and major adverse cardiovascular events (MACE), which is defined by one or more of the following: heart attack, stroke or death. **Methods:** An LC/MS/MS method was developed and fully validated for the quantification of sixty primary and secondary BAs in biological matrices. This method was used to examine the systemic levels of specific BAs in a clinical cohort (n=2,145) undergoing elective diagnostic cardiac evaluation with longitudinal follow up for three years. **Results:** BAs significantly associated with three-year MACE risk after adjustment for traditional risk factors (Framingham risk score) and inflammation marker C-reactive peptide (CRP) included the taurine conjugated primary BAs; taurocholic acid (HR 1.81 [95% CI 1.23-2.66] P=0.003) and taurochenodeoxycholic acids (HR 1.78 [95% CI 1.2-2.64] P=0.004). Moreover, secondary BAs derived from chenodeoxycholic acid, taurolithocholic acid (HR 1.9 [95% CI 1.29-2.8] P=0.001), tauroursodeoxycholic acid (HR 1.6 [95% CI 1.17-2.17] P=0.003) and glycohyodeoxycholic acid (HR 1.57 [95% CI 1.09-2.27] P=0.01) were also significantly associated with three-year MACE risk. Finally, we looked at the sum of different BAs categories and noticed that the sum of all taurine conjugated BAs is significantly associated with three-year MACE risk (HR 1.77 [95% CI 1.17-2.67] P=0.007). **Conclusion:** We showed that structure specific bile acids predicted future cardiometabolic outcomes. In general taurine conjugated BAs as well as the total of all taurine conjugated BAs showed positive association with three-year MACE risk. Further investigation is needed to proof a causal association and confirm the mechanistic link.

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Clinical Evaluation of a Near-Patient Diagnostic Platform for G6PD Deficiency from a Low Blood Sample Volume

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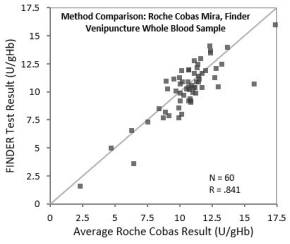
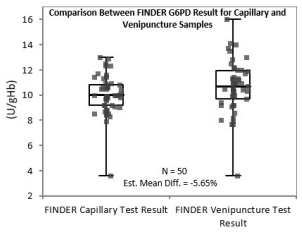
Background: Glucose-6-phosphate dehydrogenase deficiency (G6PDD) is an inherited enzymopathy that can cause antenatal and postnatal disease. Neonates with undetected G6PDD are at risk for extreme hyperbilirubinemia. Turn-around time (TAT) for send-out testing is 2-3 days and requires ≥1mL whole blood. A rapid, low blood volume, and quantitative test for G6PD will facilitate pre-discharge diagnosis.

Methods: Three evaluations were done: 1) comparison of the new platform with predicate method; 2) comparison of finger stick (FS) with venipuncture (VP) samples on the new platform, and 3) precision tests of samples across 3 clinical sites. For #1 and #2, 50 adults provided VP (Li-Heparin 2mL) and FS (100μL) whole blood samples. For #1, to cover the testing range, 1 affected venous sample (~3U/gHb) was used to make 10 contrived samples. For #1, duplicate samples were run on Pointe Scientific G6PD assay with hemoglobin normalization on Roche Cobas Mira Plus and compared with results from 50μL samples on a near-patient platform, FINDER (funded by NIH R44HD072853). For #2, using 50μL G6PD test was performed on VP and FS samples on FINDER. For #3, precision was assessed using VP blood, sent to 3 sites every day (n=3 days), and tested (n=5/day) by multiple users.

Results: TAT on FINDER was ~15min and ~1h on the comparator. For #1, FINDER assay showed a correlation R-value of 0.841 with the gold standard method (Fig 1). For #2, VP and FS samples measured on FINDER had a mean difference of 5.6% between sampling methods (Fig 2), which is within the reproducibility range of 6.2% between sites identified in evaluation #3.

Conclusion: Using 50μL samples, FINDER G6PD results were available within 15min, correlated strongly with current gold standard method, capillary and venous samples were closely related, and between-site results using FINDER on the same samples are similar.





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Evaluation of Continuous Quality Management System for Blood Gas, Electrolytes, Metabolites and CO-Oximetry

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Background: Quality management of point-of-care (POC) blood gas testing focuses on metrics for instrument accuracy and precision, in addition to performing intermittent daily quality control (QC) checks every 8 hours. At the POC and in the lab, systemic and transient sample-specific errors may negatively impact patient care.

Methods: We evaluated the performance of the GEM® Premier™ 5000 with next generation Intelligent Quality Management 2 (iQM®2) (Instrumentation Laboratory, Bedford, MA), from the analysis of approximately 84,000 patient samples across four sites. Continuous iQM2 was compared to intermittent liquid QC, either manual or automated, at two sites. Statistical characteristics of QC processes including, method sigma, and average detection time (ADT) for an error, were examined. In addition, an analysis of transient errors detected by iQM2 was included in the study.

Results: ADT was approximately 2 minutes with iQM2 and varied from hours to days with intermittent QC (Figure 1). iQM2 Process Control Solutions (PCS) precision was similar or better (>6 sigma for all analytes) than manual (sigma 3.0 for pO_2) or automated internal QC (sigma 1.3 for tHb and sigma 3.3 for pO_2). In addition, iQM2

flagged specific transient errors (such as microclots, benzalkonium chloride, optical interferences) in ~1.4% samples providing an additional safeguard against reporting erroneous results.

Conclusion: The findings in this study demonstrate excellent performance of the GEM Premier 5000 with iQM2 including >6 sigma precision for all analytes and faster error detection times. These benefits address risk in different phases of testing that are not easily detected by intermittent performance of liquid QC (manual or automated).

Figure 1. Summary data for continuous iQM2 Process Control Solutions (PCS) on GEM Premier 5000, intermittent auto-QC analyzed every 6 hours on the ABL800, and intermittent manual QC analyzed every 8 hours on the GEM Premier 5000 with iQM2.

Summary data for continuous iQM2 Process Control Solutions (PCS) Level A-E on GEM Premier 5000

		pH	PCO2 (mmHg)	PO2 (mmHg)	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{2+} (mmol/L)	Cl^- (mmol/L)	Glucose (mg/dL)	Lactate (mmol/L)	tHb (g/dL)	O2Hb (%)	COHb (%)	MetHb (%)	HHb (%)	tBili (mg/dL)
PCS A (N=45000)	Mean	6.90	64	113	107	7.1	1.84	46	144	3.3	14.6	89.4	2.4	1.6	6.6	20.1
	SD (CV%)	0.003 (0.0%)	0.5 (0.8%)	1.1 (1.0%)	0.4 (0.4%)	0.03 (0.4%)	0.015 (0.8%)	0.2 (0.5%)	2.1 (1.5%)	0.06 (1.7%)	0.03 (0.2%)	0.01 (0.0%)	0.01 (0.4%)	0.00 (0.3%)	0.00 (0.1%)	0.03 (0.2%)
PCS B (N= 53000)	Mean	7.41	33	181	156	2.0	0.79	85	0	0.0	0.0	N/A	N/A	N/A	N/A	0.0
	SD (CV%)	0.004 (0.1%)	0.5 (1.4%)	2.2 (1.2%)	0.7 (0.5%)	0.01 (0.5%)	0.007 (0.9%)	0.4 (0.4%)	1.6 (N/A)	0.03 (N/A)	0.04 (N/A)					0.04 (N/A)
PCS C (N=6000)	Mean	8.05	33	3.1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	SD (CV%)	0.007 (0.1%)	0.3 (1.0%)	1.0 (32.5%)												
PCS D (N=13000)	Mean	7.35	24	48	165	7.3	1.22	142	349	8.0	7.4	80.0	4.1	4.0	11.9	10.4
	SD (CV%)	0.004 (0.1%)	0.4 (1.5%)	3.2 (6.6%)	0.6 (0.4%)	0.04 (0.6%)	0.011 (0.9%)	1.6 (1.1%)	3.3 (1.0%)	0.24 (3.0%)	0.03 (0.4%)	0.14 (0.2%)	0.09 (2.1%)	0.10 (2.6%)	0.33 (2.8%)	0.03 (0.3%)
PCS E (N=13000)	Mean	7.22	69	92	129	4.5	0.56	101	71	1.6	16.5	50.0	10.1	8.1	31.9	20.0
	SD (CV%)	0.005 (0.1%)	1.0 (1.5%)	2.2 (2.4%)	0.5 (0.4%)	0.03 (0.6%)	0.008 (1.4%)	0.8 (0.8%)	1.0 (1.5%)	0.06 (4.1%)	0.07 (0.4%)	0.11 (0.2%)	0.05 (0.5%)	0.07 (0.9%)	0.23 (0.7%)	0.07 (0.4%)
Sigma Average		>6	>6	>6	>6	>6	>6	>6	>6	>6	>6	>6	>6	>6	>6	>6
Overall Pfr		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.152	0.152	0.152	0.152	0.152	0.167
Overall Ped		0.974	0.953	0.938	0.412	0.979	0.990	0.950	0.097	0.933	0.997	1.000	0.957	0.957	0.998	1.000
Overall ADT (min)		2	2	2	5	2	2	2	21	2	2	2	2	2	2	2

Summary data for intermittent auto-QC (Levels 1-4) analyzed every 6 hours on the ABL800

		pH	PCO2 (mmHg)	PO2 (mmHg)	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{2+} (mmol/L)	Cl^- (mmol/L)	Glucose (mg/dL)	Lactate (mmol/L)	tHb (g/dL)	O2Hb (%)	COHb (%)	MetHb (%)
S7835 (N2110)	Mean	7.10	67	143	159	1.7	1.01	121	31	4.6	8.1	44.6	5.9	4.9
	SD (CV%)	0.005 (0.1%)	1.6 (2.4%)	6.3 (4.4%)	0.7 (0.4%)	0.00 (0.1%)	0.009 (0.9%)	0.9 (0.7%)	1.0 (3.2%)	0.09 (2.0%)	0.86 (10.6%)	0.09 (0.0%)	0.18 (0.0%)	0.05 (0.0%)
S7845 (N293)	Mean	7.40	40	101	140	3.7	0.52	97	101	1.7	13.4	92.2	2.9	2.0
	SD (CV%)	0.003 (0.0%)	0.7 (1.8%)	2.4 (2.3%)	0.6 (0.4%)	0.03 (0.7%)	0.010 (1.9%)	0.3 (0.3%)	1.6 (1.6%)	0.05 (2.7%)	0.66 (4.9%)	0.08 (0.1%)	0.20 (6.8%)	0.05 (2.4%)
S7855 (N2105)	Mean	7.57	40	72	126	5.5	0.35	66	247	10.7	19.5	49.1	19.9	10.0
	SD (CV%)	0.007 (0.1%)	0.6 (1.4%)	3.2 (4.5%)	0.6 (0.5%)	0.03 (0.5%)	0.027 (7.8%)	1.0 (1.5%)	4.0 (1.6%)	0.50 (4.7%)	0.65 (3.3%)	0.09 (0.2%)	0.17 (0.8%)	0.05 (0.5%)
S7865 (N296)	Mean	6.81	94	289	125	6.3	1.63	90	-2	-0.1	2.6	3.6	9.2	19.7
	SD (CV%)	0.006 (0.1%)	1.8 (2.0%)	7.5 (2.6%)	0.4 (0.3%)	0.00 (0.0%)	0.0 (0.8%)	0.750 (0.8%)	0.6 (N/A)	0.00 (N/A)	0.39 (15.0%)	0.01 (0.3%)	0.05 (0.6%)	0.03 (0.1%)
Sigma Average		>6	5.8	3.3	>6	>6	>6	>6	>6	>6	>6	1.3	>6	>6	>6	
Overall Pfr		0.002	0.001	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.309	0.000	0.720	0.000		
Overall Ped		0.943	0.487	0.094	0.049	1.000	0.275	0.017	0.000	0.084	0.067	1.000	1.390	1.000		
Overall ADT (hours)		6	12	64	>100	6	22	>100	>100	72	89	6	8	6		

Summary data for intermittent manual QC (GEM System Evaluator Levels 1-3) analyzed every 8 hours on the GEM Premier 5000 with iQM2

		pH	PCO2 (mmHg)	PO2 (mmHg)	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{2+} (mmol/L)	Cl^- (mmol/L)	Glucose (mg/dL)	Lactate (mmol/L)	tHb (g/dL)	O2Hb (%)	COHb (%)	MetHb (%)	HHb (%)	tBili (mg/dL)
GSE 1 (N≥ 138)	Mean	7.12	89	10	123	2.3	1.58	86	373	7.3	20.8	37.3	31.7	8.3	22.7	33.8
	SD (CV%)	0.006 (0.1%)	1.7 (1.9%)	3.1 (30.4%)	0.7 (0.6%)	0.04 (1.6%)	0.019 (1.2%)	0.6 (0.7%)	5.2 (1.4%)	0.17 (2.3%)	0.16 (0.8%)	0.04 (0.1%)	0.06 (0.2%)	0.07 (0.8%)	0.05 (0.2%)	0.16 (0.5%)
GSE 2 (N≥ 138)	Mean	7.37	35	86	140	4.5	1.18	108	104	0.8	14.5	73.7	16.8	2.5	6.9	17.6
	SD (CV%)	0.005 (0.1%)	0.5 (1.6%)	3.1 (3.6%)	0.6 (0.4%)	0.05 (1.2%)	0.011 (0.9%)	0.6 (0.6%)	1.1 (1.3%)	0.03 (3.6%)	0.16 (1.1%)	0.04 (0.1%)	0.06 (0.4%)	0.09 (3.7%)	0.06 (0.9%)	0.16 (0.9%)
GSE 3 (N≥ 138)	Mean	7.56	13	342	154	7.6	0.66	141	45	2.5	7.7	93	2.2	1.4	3.4	3.2
	SD (CV%)	0.005 (0.1%)	0.5 (3.8%)	10.2 (3.0%)	0.8 (0.5%)	0.06 (0.8%)	0.009 (1.4%)	1.1 (0.8%)	1.2 (2.7%)	0.08 (3.1%)	0.13 (1.8%)	0.05 (0.1%)	0.08 (3.7%)	0.13 (9.3%)	0.09 (2.7%)	0.14 (4.2%)
Sigma Average		>6	>6	3.0	5.8	>6	>6	>6	>6	>6	5.2	>6	>6	>6	>6	>6
Overall Pfr		0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Overall Ped		0.049	0.351	0.042	0.001	0.577	0.845	0.245	0.089	0.497	0.021	1.000	0.298	0.343	0.049	0.679
Overall ADT (hours)		>100	23	>100	>100	14	9	33	90	16	>100	8	27	23	>100	12

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Improvement in Workflow and Efficiency for Measurement of Special Proteins Using the Optilite Standalone Automated Analyzer

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Background: Special protein assays (free light chains, IgA, IgG, IgM, rheumatoid factor, C3c, C4, haptoglobin and prealbumin) were analyzed on an automation/chemistry line (Roche cobas 8100/cobas c501) at our institution. However, during instrument upgrades to the c502 modules, poor analytical performance was observed for these assays. Testing was subsequently validated on the Binding Site Optilite®, a standalone automated turbidimetric platform optimized for measurement of special proteins. The objective of this study was to compare the process workflow and resources required between the Optilite and the cobas systems, as well as determine the impact on efficiency and any financial implications between an automated versus standalone platform. **Methods**: A side-by-side time and motion study was performed by an independent consulting company (Argent Global Services), who observed routine maintenance and testing activities for both systems over a period of 5 days. Historical data was also collected to verify the observation data related to maintenance (daily, weekly, and monthly), quality control, calibration, and troubleshooting events. Testing volumes and dilution rates were also assessed. Specific timing studies were also assessed for the Freelite assay, due to the higher volume of clinical testing and is representative of a special protein assay requiring a greater number of either automatic or manual dilutions. Routine specimens were analyzed daily across two shifts on both the Optilite and the cobas c501/c502. The data are presented as weighted averages. **Results**: The Optilite Freelite reagent setup and calibration required 30.5 minutes, while the cobas required 62.4 minutes. With migration of the special protein assays to the Optilite, there was a 50% reduction in both monthly instrument maintenance labor costs and consumables via avoidance of QC and calibration failures on the cobas. The difference in the average analytical times per sample was not statistically significant between the Optilite and the cobas when dilutions were not required. However, the Optilite results were 12% faster than cobas results when specimens needed an auto-dilution, and 29% faster when manual dilutions were required on the cobas. Due to the extended reportable range(s) on the Optilite there was a 100% decrease in manual dilutions and a 34% overall decrease in auto-dilutions, with significant decreased Optilite dilutions compared to the cobas for Freelite Kappa (13.1% versus 31.2%) and IgA assays (5% versus 19.5%), respectively. The average same day turnaround time from receipt to verify for the Optilite was less than one hour. Average total reagent, QC, and calibration costs were 9.0% less for the Optilite special protein assays. **Conclusions**: The Optilite required significantly less maintenance and labor to perform special protein testing, while providing faster overall analytical turnaround times and increased efficiency compared to assays analyzed on a high-throughput automation line. Fully automated special protein testing on a dedicated platform resulted in minimal or no manual intervention from technologists once specimens are loaded. Further reagent and QC cost savings were realized by utilizing integrated QC materials, compared to use of external QC.

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Verification of Thyroid Stimulating Hormone Reference Intervals Using Both Retrospective and Prospective Patient Data

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Background: In clinical practice most reference intervals (RIs) are obtained from various sources such as manufacturer package inserts, literature, RI databases, or other laboratories. However, it is best practice for clinical laboratories to verify these RIs to ensure that they are appropriate for the population served by the institution. Retrospective chart reviews and prospective sample analysis of a presumably healthy population are two methods of RI verification that can be used by clinical laboratories. Many studies have shown that serum TSH concentrations vary with age; however, few laboratories provide age specific RIs. In fact, some manufacturers do not provide age stratified RIs in their package insert. Inappropriate TSH RIs can delay accurate diagnosis or lead to misdiagnosis of hypo- or hyperthyroidism.

Methods: We utilized retrospective data gathered from patient charts and prospective analysis (direct sampling) of age-appropriate specimens to determine RI suitability for patients aged between 12-19 years for the Beckman TSH assay. The CALIPER RI for ages 12-19 years (0.68-3.35 µIU/mL) was compared with the current verified manufacturer RI for 21-88 years (0.45-5.33 µIU/mL) recommended by Beckman. 27 leftover samples from euthyroid patients seen at an outpatient clinic at the Children's Hospital of Philadelphia with no history of chronic illness were used for TSH measurements on the Beckman DXI platform. A retrospective chart review of patients 12-19 years generated 394 results over a 10-month period. Patients with an abnormal free T4 and a history of thyroid cancer and/or were experiencing TSH elevations due to pregnancy were also excluded, leaving 77 euthyroid patients.

Results:

TSH Reference Intervals, 12-19 Years						
	Beckman (21-88 years)	CALIPER	Direct Sampling (central 95%) N= 27	% Within RI (Direct Sampling)	Retrospective (central 95%) N= 77	% Within RI (Retrospective)
TSH (µIU/mL)	0.45-5.33	0.68-3.35	0.47-4.23	96% (HUP) 83% (CALIPER)	0.66-4.94	100% (HUP) 74% (CALIPER)

Conclusion: The TSH RI (0.45-5.33 µIU/mL) provided by the manufacturer was found to be appropriate for our hospital population 12-19 years. Our findings strengthen the argument for demographic-specific RI and highlight the utility of chart review and direct sampling to track the appropriateness of a RI.

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Rapid Intraoperative Measurement of Parathyroid Hormone

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Parathyroid hormone (PTH) is produced by chief cells of the parathyroid gland. Intact PTH (PTH1-84) and the truncated variant PTH1-34 are biologically active, but PTH7-84 is inactive. PTH is metabolized in the liver and excreted by the kidney. PTH is usually measured to diagnose hyperparathyroidism, and hypoparathyroidism. The half-life of intact PTH in the circulation is less than 5 min. Because of its short half-life, PTH is measured intraoperatively to monitor the success of a parathyroidectomy procedure and avoid the necessity for re-operative parathyroidectomy, which is associated with readmission and a longer postoperative hospital stay. The PTH concentration is usually measured before and after the surgical procedure, and

a 50% decrease in the PTH concentration 10-20 minutes following excision, and/or a PTH level within the reference range, indicates successful removal of the hyperplastic parathyroid gland.In our hospital, the intraoperative PTH test is currently performed in the central laboratory. In 95% of the cases, the time elapsed from specimen collection to report is within 52 min. However, specimen transportation usually accounts for over 50% of the total turn-around time (TAT). Recently, our hospital acquired a rapid intraoperative PTH platform with an analytical cycle time of 10 min. The instrument is mounted on a movable cart that can be positioned in the operating room. The PTH test can be performed immediately after the specimen is collected, significantly reducing the total TAT. We report here the method verification and its implementation in a clinical laboratory.The STAT-IO-PTH assay (Future Diagnostics, Wijchen, The Netherlands) is a two-site chemiluminescent immunometric assay that measures intact PTH in serum or EDTA plasma. Our method verification data showed that the intra-assay and inter-assay precision were within 20% and agreed with the manufacturer's specifications. The assay was linear from 10.6 - 2500 pg/mL. However, comparison to the central laboratory's Roche Diagnostics (Basel, Switzerland) assay demonstrated a proportional bias for the rapid PTH assay, and we were not able to verify the reference range provided by the manufacturer, which was similar to the reference range provided by Roche Diagnostics for their assay. The substantial bias between the assays was unexpected, as information in the product inserts indicates that both assays use antibodies against the same regions of the intact PTH molecule. The reference range of intraoperative PTH is important because it is one of the parameters used to determine whether the parathyroid gland has been completely removed. We collected over sixty serum samples with both PTH and total calcium concentration within normal ranges by Roche assays to establish the reference range for STAT-IO-PTH assay. The pros and cons of implementing the rapid intraoperative PTH assay were also considered.

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Uncommon Results For Three Common Clinical Chemistry Analytes

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Issue: A technologist in our laboratory noticed results so odd that she properly asked whether she should release them. Glucose was < 10 mg/dL, AST = 9052 U/L, and ALT = 3947 U/L. This sample was drawn at 4:45 pm. Previous results (1:25 am the same day, patient day 2) were glucose 164, AST 61, and ALT 16. We investigated. **Case Report**: An 80-yr-old woman arrived by ambulance after being rescued from a house fire. She experienced burns over 52% of her body. She was admitted to the Burn Unit in critical condition. She has a history of COPD, chronic pain, and tobacco smoking. IV vitamin C was started early on day 2. Results for the three analytes were not reported; instead, the message "Please reorder; possible interfering substance" was utilized. On day 2, the patient developed respiratory failure, renal failure, oliguria, and DIC. TPN began during day 2. Early on day 3, glucose was 155, AST 8063, and ALT 3523, so it appeared that the interfering substance was still present. Vitamin C treatment continued on day 3.**Vitamin C** is a strong antioxidant that supports immune function. Vitamin C may reduce damage from reactive oxygen species. It has recently been shown to promote wound healing and reduce fluid requirements for burn patients (Crit. Care Clin., 2016; 32: 539-46). **Analytical Details**: Glucose, AST and ALT are determined on Beckman AU 5800 platforms in our laboratory. The glucose method uses hexokinase, which converts glucose to glucose-6-phosphate (G6P). G6P is subsequently reacted to 6-phosphogluconate, with the accompanying reduction of NAD^+ to NADH. The increase in NADH, measured at 340 nm, is proportional to glucose in the sample. AST and ALT are both measured with sequential enzymatic reactions, the second of which converts NADH to NAD^+. Enzyme activity is determined by the rate of loss of NADH. **Vitamin C Interference**: Beckman's IFUs for each of these assays state "There is no interference from vitamin C up to concentrations of 20 mg/dL." Vitamin C in the patient (day 2) was 135 mg/dL (0.2-2.7). We contacted Beckman to ask whether vitamin C this high might cause the results seen. We received the following reply: "Very high doses could interfere with the glucose, AST, and ALT assays."**Action Points**: 1.This reply prompted thought about possible explanations for the results. For glucose, having that much vitamin C available inhibits the reduction to NADH, causing falsely low glucose. For AST and ALT, vitamin C stimulates the conversion to NAD+, producing falsely high results.2.We informed the clinicians about the interference and the incorrect results for these three analytes.3.

Vitamin C levels > 20 mg/dL can interfere with certain assays.4.We recommend that Beckman update their IFUs. Similar assays from other vendors should be suspected of vulnerability to the same interference.

Conclusions: This case reminds laboratorians of making right choices for the good of the patient and the importance of effectively communicating among ourselves, with the clinical team, and with our vendor representatives.

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Circulating biomarkers in triage, prognosis and risk stratification of COVID-19 patients

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Background: Coronavirus disease-19 (COVID-19) has caused numerous deaths and extensive global disruption. To date, few effective risk stratification scores have been proposed to aid in identifying those at highest risk for poor outcomes and requiring early treatment modalities such as convalescent plasma and monoclonal therapies. Circulating cardiac biomarkers such as high sensitivity troponin I (hs-cTnI), natriuretic peptides (NT-proBNP), and procalcitonin (PCT) have shown promise for this utility in COVID-19 patients. The objective of this study was to evaluate baseline characteristics of cardiac biomarkers hs-cTnI, NTproBNP, and PCT and their utility for triaging patients with COVID-19 upon diagnosis in the Emergency Department (ED).

Methods: Residual EDTA plasma specimens drawn in the ED from 144 patients with PCR-confirmed SARS-CoV-2 infection were obtained from the Barnes Jewish Hospital core laboratory. Concentrations of cardiac biomarkers hs-cTnI, NT-proBNP, and PCT were analyzed on an Abbott Architect i2000 (Abbott Diagnostics). With the use of Cox Proportional hazard models, hs-cTnI concentrations were evaluated as a continuous variable and a discrete variable using the sex-specific 99th% upper reference limit (URL, males ≥35 ng/L, females ≥17 ng/L). Continuous Cox proportional hazard models were used to assess NT-proBNP and PCT. Outcomes (mortality, intubation) were obtained from electronic health records. Statistical analysis was performed using r software.

Results. Baseline elevated hs-cTnI above the sex specific cutoff was associated with a hazard ratio (HR) of 3.76 (p<0.001) for all cause mortality. Continuous variable evaluation of mortality was associated with an HR of 1.2 (p =0.001) for hs-cTnI, 1.2 (p=0.016) for NT-proBNP, and 1.08 (p = 0.134) for PCT. For the risk of requiring ventilation, hs-cTnI was associated with a HR of 1.0 (p = 0.899), NT-proBNP with and HR of 1.0 (p=0.797) and PCT with a HR of 1.1 (0.056). Area under the ROC curve (AUC) for hs-cTnI, NT-proBNP and PCT were 0.78 (0.70-0.87), 0.80, (0.73-0.89), and 0.77 (0.67-0.86) for predicting mortality and 0.55 (0.44-0.65), 0.56 (0.46-0.67) and 0.59 (0.48-0.7) for predicting ventilation. Kaplan-Meier curves revealed a higher likelihood of all-cause mortality (p<0.001) and in-hospital mortality (p = 0.006) in patients with baseline hs-cTnI above the 99th% URL.

Conclusion: Elevated baseline hs-cTnI, NT-proBNP, and PCT measured at diagnosis in SARS-CoV-2 infected patients in the ED are associated with elevated risk of mortality. Cardiac biomarkers measured in the ED may provide clinical utility for risk stratification and require further examination.

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Prediction of Antibody Response Based on Clinical and Demographic Variables Following SARS-CoV-2 Vaccine Administration

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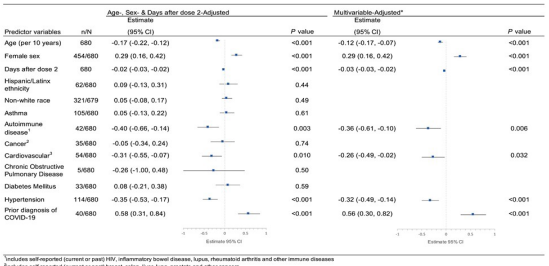
Background: Our previous work demonstrating antibody response to one vs. two doses of SARS-CoV-2 mRNA vaccine (Nature Med, April 2021, Ebinger et al.) showed that persons with prior SARS-CoV-2 infection received the same benefit after one dose of vaccine as infection naive individuals after two doses. We now present a prediction model for antibody response to vaccine based on demographic and clinical factors.

Methods: We studied 680 participants who received 2-doses of Pfizer-BioNTech mRNA vaccine (median age: 41.1 years, sex: 66.8% female, prior SARS-CoV-2 infection: 5.9%). Participants completed questionnaires and "predictor variables" correlated to antibody levels are shown in Figure 1. EDTA plasma was obtained ≥7 days after 2 doses of vaccine for measurement of IgG antibody against viral spike receptor binding domain [IgG(S-RBD)] via an Abbott Architect method. We performed age, sex, and time from last dose adjusted linear regression models on log-transformed IgG(S-RBD) levels for each of the clinical/demographic characteristics. Variables with p-values <0.10 were then included in a final multivariable linear regression model. **Results:** In age, sex and time from last dose adjusted models we observed female sex (95%CI 0.29, 0.16-0.42) and prior SARS-CoV-2 infection (95%CI 0.58, 0.31-0.84) were associated with higher IgG(S-RBD). Increasing age, number of days

since last dose, autoimmune conditions, cardiovascular disease and hypertension were all associated with lower IgG(S-RBD) (Figure 1). These variables all remained significant in the multivariable model.

Conclusion: These findings indicate that quantifiable patient characteristics are associated with variations in immune response following mRNA vaccination. Specifically, female sex and prior SARS-CoV-2 infection are associated with higher IgG(S-RBD), while autoimmune and cardiovascular disease are associated with lower IgG(S-RBD). Future studies examining long term differences in both antibody levels, prior SARS-CoV-2 infection, and clinical outcomes among patients with these important features may be helpful in understanding if "booster" vaccine doses offer benefit.



Predictor variables	n/N	Age-, Sex- & Days after dose 2-Adjusted Estimate (95% CI)	*P* value	Multivariable-Adjusted* Estimate (95% CI)	*P* value
Age (per 10 years)	680	-0.17 (-0.22, -0.12)	<0.001	-0.12 (-0.17, -0.07)	<0.001
Female sex	454/680	0.29 (0.16, 0.42)	<0.001	0.29 (0.16, 0.42)	<0.001
Days after dose 2	680	-0.02 (-0.03, -0.02)	<0.001	-0.03 (-0.03, -0.02)	<0.001
Hispanic/Latinx ethnicity	62/680	0.09 (-0.13, 0.31)	0.44		
Non-white race	321/679	0.05 (-0.08, 0.17)	0.49		
Asthma	105/680	0.05 (-0.13, 0.22)	0.61		
Autoimmune disease[1]	42/680	-0.40 (-0.66, -0.14)	0.003	-0.36 (-0.61, -0.10)	0.006
Cancer[2]	35/680	-0.05 (-0.34, 0.24)	0.74		
Cardiovascular[3]	54/680	-0.31 (-0.55, -0.07)	0.010	-0.26 (-0.49, -0.02)	0.032
Chronic Obstructive Pulmonary Disease	5/680	-0.26 (-1.00, 0.48)	0.50		
Diabetes Mellitus	33/680	0.08 (-0.21, 0.38)	0.59		
Hypertension	114/680	-0.35 (-0.53, -0.17)	<0.001	-0.32 (-0.49, -0.14)	<0.001
Prior diagnosis of COVID-19	40/680	0.58 (0.31, 0.84)	<0.001	0.56 (0.30, 0.82)	<0.001

[1]Includes self-reported (current or past) HIV, inflammatory bowel disease, lupus, rheumatoid arthritis and other immune diseases
[2]Includes self-reported (current or past) breast, colon, liver, lung, prostate and other cancers
[3]Includes self-reported (current or past) atrial fibrillation or flutter, coronary artery disease, heart failure and other cardiac diseases
*To avoid model overfitting given the sample size, covariates included in the multivariable model were selected from age-, sex- & days after vaccine dose 2-adjusted models based on significance with P<0.05.

Figure 1: Predictors of antibody response to SARS-CoV-2 mRNA vaccine after single and multivariable linear regression analysis.

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Investigation of transient monoclonal protein production due to SARS-COV2 infection via serum protein electrophoresis

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Background: Serum protein electrophoresis and immunofixation are routinely used clinically to screen and characterize protein abnormalities in samples. The presence of monoclonal proteins can be determined through interpretation of serum protein immunofixation by trained personnel, and frequently aids in the diagnosis and management of multiple myeloma or other plasma cell disorders. During routine clinical testing, we have recently observed an increase in protein abnormalities, including transient monoclonal proteins, in select patient samples. The aim of this study is to determine if the abnormalities and transient monoclonal proteins observed during routine testing could result from immune response to SARS-CoV-2 vaccination, or COVID-19 disease progression in naturally infected patients. **Methods:** 10 residual serum samples confirmed positive for SARS-CoV-2 by PCR, as well as 5 samples from subjects post-SARS-CoV-2 vaccination were selected for this study. All residual samples selected were positive for SARS-CoV-2 IgG. Serum samples were screened for protein abnormalities using capillary electrophoresis (Sebia Capillarys-2). Serum samples interpreted as having significant protein abnormalities (n = 4) were further characterized by protein immunofixation (Sebia Hydrasys 2). For subjects determined to have monoclonal proteins by immunofixation, additional serial samples (n = 3-6/subject), ranging from 9-51 days post symptom onset, were analyzed in an effort to track changes during COVID-19 disease progression.**Results:** Of the samples investigated, 9 of 15 were interpreted as having protein abnormalities when screened by a trained laboratory professional using protein electrophoresis. Abnormalities observed included shoulders or distinct peaks in the gamma region (6 of 15) for naturally infected subjects, and non-Gaussian gamma distribution of proteins (3 of 15) for vaccinated and naturally infected subjects. The most pronounced abnormalities were selected for further characterization by immunofixation, which revealed the presence of IgG-Kappa, or IgM-kappa monoclonal proteins in naturally infected COVID-19 subjects. For 2 subjects, monoclonal proteins were present at the earliest available time point, 9 days after COVID-19 symptom onset, with the most striking result having a peak monoclonal protein magnitude (1.6 g/dL) as late as day 40 with decreasing quantitation thereafter. Vaccinated subjects were largely unremarkable, with a protein abnormality in 1 subject showing no monoclonal protein by immunofixation. **Conclusions:** These data suggest SARS-CoV-2 infection could result in the appearance of transient monoclonal proteins during COVID-19 disease progression. Laboratory professionals and clinicians should take COVID-19 diagnosis into consideration when interpreting serum protein electrophoresis and immunofixation results, as COVID-19 related monoclonal proteins may confound assessment of multiple myeloma and other plasma cell disorders. For the limited vaccinated subjects investigated, serum protein electrophoresis was largely unremarkable suggesting immune response to SARS-CoV-2 vaccines is not a significant source of observed protein abnormalities.

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Two novel oral estrogen receptor degraders against ER-positive breast cancer

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Background: Endocrine therapy is recommended for ER positive breast cancer, and there has been significant reduction in mortality in women with ER-positive breast cancer. Endocrine therapy has remained the backbone of treatment for patients with early and metastatic ER-positive breast cancer for over the past forty years. Tamoxifen, and third generation aromatase inhibitors remain frontline interventions in the management of ERα positive breast cancer. However, Tamoxifen and aromatase inhibitors treatments resistance was occurred in most patients. Recently study suggest fulvestrant (ER degrader) shows potent activity for ER positive breast cancer patients, and fulvestrant has been approved by FDA in 2017 as mono therapy for naive ER positive breast cancer patients. Due to the poor solubility of fulvestrant, it is only used via muscle injection drug administration route, is not convenient for patients. **Methods:** Two Estrogen degraders are synthesized. The water solubility was measured by "slow-stirring" method. ER gene and protein level will be detected via RT-PCR and Western Blot method.**Results:** Compound 172 and 183 synthesized by our lab have been identified that could significantly down regulate ERα at 1 μM in wild and mutant type ER-positive breast cancer cells via proteasomal pathway, which indicating its potential activity of the potential activity of the compounds in endocrine resistant breast cancer. **Conclusion:** In summary, this result provides potential new drug candidates to treat ER positive breast cancers.

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Multi-Site Validation of Automated HEp-2 ANA Interpretation Using A Newly Developed Fluorescent Microscope System

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Background: Detection of anti-nuclear antibodies (ANA) by indirect fluorescent antibody (IFA) assays is a mainstay in the field of autoimmune diagnostics. ZEUS Scientific has manufactured an FDA-cleared IFA ANA HEp-2 Test System for over 30 years, which relies upon manual interpretation of results using a fluorescent microscope. This same HEp-2 IFA Test System was recently utilized to train software, in conjunction with a specialized fluorescent microscope, towards automating interpretation of qualitative (Q) and ANA pattern (P) results (collectively termed dIFine). The objective of this study was to validate the performance of the newly developed dIFine at three independent laboratories.**Methods:** Multi-site Reproducibility–Low-positive, mid-positive and high-positive serum samples were selected for each of the following 8 patterns: Homogeneous, Speckled, Centromere, Nucleolar, Nuclear Dots, Nuclear Membrane, Cytoplasmic (Mitochondrial), Cytoplasmic (Ribosomal), as well as one ANA-negative sample. The 25 samples were assayed at a 1:40 dilution, in triplicate, twice per day, on 5 independent days (90 total slide wells per pattern, 30 total slide wells for the negative sample), using the ZEUS HEp-2 Test System, then scanned and interpreted by dIFine. Clinically Characterized Samples–350 serum samples derived from patients with various Rheumatic diseases and Control diseases were assayed at a 1:40 dilution using the ZEUS HEp-2 Test System, then scanned and interpreted by dIFine. **Results:** Reproducibility (Actual/Expected)-Homogeneous [Site 1,Q (90/90),P (90/90)], [Site 2,Q (90/90),P (90/90)], [Site 3,Q (90/90),P (89/90)]; Speckled [Site 1,Q (90/90),P (90/90)], [Site 2,Q (90/90),P (90/90)], [Site 3,Q (90/90),P (90/90)]; Centromere [Site 1,Q (90/90),P (90/90)], [Site 2,Q (90/90),P (90/90)], [Site 3,Q (90/90),P (90/90)]; Nucleolar [Site 1,Q (90/90),P (90/90)], [Site 2,Q (90/90),P (90/90)], [Site 3,Q (90/90),P (90/90)]; Nuclear Dots [Site 1,Q (90/90),P (90/90)], [Site 2,Q (90/90),P (90/90)], [Site 3,Q (90/90),P (90/90)]; Nuclear Membrane [Site 1,Q (90/90),P (89/90)], [Site 2,Q (90/90),P (88/90)], [Site 3,Q (90/90),P (90/90)] Cytoplasmic (Mitochondrial) [Site 1,Q (90/90),P (90/90)], [Site 2,Q (90/90),P (90/90)], [Site 3,Q (90/90),P (90/90)]; Cytoplasmic (Ribosomal) [Site 1,Q (90/90),P (90/90)], [Site 2,Q (90/90),P (90/90)], [Site 3,Q (90/90),P (90/90)]; Negative [Site 1,Q (30/30)], [Site 2,Q (30/30)], [Site 3,Q (30/30)]. Clinically Characterized Samples - Percent positivity reported by 3 different dIFine systems: (n = 190 Rheumatic disease samples, Site 1 = 57.37%, Site 2 = 55.79%, Site 3 = 54.21%); (n = 160 Control disease samples, Site 1 = 20.00%, Site 2 = 18.75%, Site 3 = 18.75%).

Conclusions: These multi-site validation studies demonstrate that the newly developed ZEUS dIFine yields highly reproducible qualitative and pattern interpretation results for ANA HEp-2 IFA.

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Evaluation of the Sampson equation to estimate Low-density lipoprotein-cholesterol (LDL-C): comparison with the Friedewald equation and a direct homogenous LDL assay from Roche

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Background: Cardiovascular diseases are the number one cause of death worldwide. Low-density lipoprotein cholesterol (LDL-C) is a key contributor to atherosclerosis pathogenesis. It serves as a major modifiable factor for atherosclerotic cardiovascular disease risk assessment, and as the primary treatment target for patient management. Therefore, accurate determination of LDL-C is of utmost importance in clinical practice for appropriate prevention and treatment. In clinical laboratories, LDL-C is often estimated using the Friedewald calculation and/or it is directly measured by homogenous enzymatic colorimetric assay when triglyceride (TG) concentrations are above 400 mg/dL. However, each of these methods has limitations. These become especially problematic when considering that with modern effective lipid reducing therapies, much lower LDL-C treatment targets are now emphasized by current guidelines. Recently, several novel equations for LDL-C estimation have been introduced, one of which is the Sampson equation. **Objective:** To evaluate the utility of the Sampson equation for LDL-C estimation in a heterogeneous patient population as compared to the Friedewald equation and a direct LDL-C measurement. **Methods:** A prospective study was conducted over a period of two weeks in 2021 in the core laboratory at Chandler hospital at the University of Kentucky Medical Center. All patient specimens sent for routine lipid panels were automatically reflexed to perform direct LDL-C measurements. The routine lipid panel includes measured total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL) and TG and calculated LDL-C (Friedewald Calculation). All lipid testing was performed on the Roche Cobas 701. LDL- C calculations via the Friedewald and Sampson equations were compared to Direct LDL measurements. Linear regression and bias analyses were performed using EP evaluator and R software. **Results:** During the two-week study period, a total of 952 patients' specimens (525 females, 426 males and 1 unknown; age (50±37)) were collected. Mean concentrations of CHOL, HDL and TG were 178±89 mg/dL, 50±32 mg/dL and 150±220 mg/dL respectively. In patients with TG <400 mg/dL (n=919), the Sampson equation correlated more closely with Roche dLDL-C than the Friedewald calculation *Slope 0.965,R^2=0.97* vs *Slope 0.960, R^2=0.95* . In patients with TG < 400 mg/dL and LDL < 100 mg/dL (n=410), the correlation between the Roche dLDL-C and Sampson equation calculation was superior to the Friedewald equation (*Slope 0.981,R^2=0.91)* vs (*Slope 0.981,R^2=0.82)* . For specimens with TG 0-800 mg/dL (n=948), the Sampson equation correlates well with Roche dLDL-C *Slope=0.954,R^2=0.96* . On average, the Sampson equation displayed a 7.6% negative bias compared with Roche dLDL-C. **Conclusion**: Our data shows that LDL-C estimated by the Sampson equation is more reliable than when calculated by the Friedewald equation, especially for specimens with low LDL-C. In specimens with TG up to 800 mg/dL, the Sampson equation displayed a good correlation with Roche dLDL-C measurements. The Sampson equation can be implemented to calculate LDL-C in patients with TG up to 800 mg/dL and replace current, less accurate calculation strategies.

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Did your laboratory select the appropriate constant-factor for calculating TIBC from transferrin?

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Background: Transferrin (TF), an iron binding protein, transports iron to various tissues. TF is routinely measured using immunoassays, and its concentration is used for calculation of Total Iron Binding Capacity (TIBC) by multiplying a theoretical constant-factor 1.41, based on the two iron to one transferrin molar ratio, or 1.27 as recommended by some manufacturers. The objective of the study was to investigate variation of constant-factors used by clinical laboratories. **Methods:** An internet-based search was performed to obtain reference intervals for TF and TIBC published by 14 clinical laboratories. The constant-factors were back-calculated by dividing the lower-limit of TIBC by that of TF, and the same for the upper limits. **Results:** The constant-factors ranged 0.74-1.44 for the lower limits and 1.07-1.42 for the upper limits (Table 1). All 14 laboratories did not use the same constant-factor in calculating both the lower-limit and the upper-limit of the TIBC reference interval, with the absolute difference values ranging from 0.01 to 0.67. **Discussion & Conclusion:** TF results of different manufacturers' assays showed good agreement according to CAP surveys suggesting a similar constant-factor be used for calculating TIBC. Both TF and TIBC are important biomarkers for diagnosis and management of iron-associated disorders. We observed a wide variation of the constant-factors used by laboratories, many significantly deviated from the theoretical constant-factor value (1.41) and the manufacturer recommended value (1.27), exceeding the practical limits after the consideration of the uncertainty of the TF molecular mass (90 KDa or 79.57 KDa), and existence of iron nonspecific binding proteins. Further, different constant-factors were used within a laboratory for calculating lower-limit and upper-limit of TIBC reference interval. This may cause disconcordance between reference intervals for TF and TIBC, resulting in unnecessary repeat measurements and even confusion in diagnosis. Laboratories should re-examine and assure that a constant-factor is appropriately selected.

Table 1.

Laboratory (Gender for reference)	TIBC Adult Ref Interval (ug/dL)		Transferrin Ref Interval (mg/dL)		Constant-Factor Used (TIBC/TF)		Absolute Difference[1]
	LL[2]	UL[3]	LL	UL	LL	UL	
UC San Diego (male/female)	148	506	200	360	0.74	1.41	0.67
Vanderbilt (male/female)	250	450	174	382	1.44	1.18	0.26
Mayo Clinic (male/female)	250	400	200	360	1.25	1.11	0.14
Johns Hopkins (male/female)	250	450	200	400	1.25	1.13	0.12
Cleveland Hospital (male/female)	232	386	200	360	1.16	1.07	0.09
ARUP (male/female)	240	450	200	400	1.20	1.13	0.07
U Iowa (male/female)	250	425	200	360	1.25	1.18	0.07
UCSF (male/female)	240	450	182	360	1.32	1.25	0.07
U Chicago (male/female)	230	430	168	302	1.37	1.42	0.05
LabCorp (male/female)	250	450	177	329	1.41	1.37	0.04
Emory (male/female)	250	450	201	352	1.24	1.28	0.04
Mass General (male/female)	230	404	200	360	1.15	1.12	0.03
Quest (male)	250	450	188	341	1.33	1.32	0.01
U Washington (male)	250	460	180	329	1.39	1.40	0.01

Footnotes:

1. Absolute difference = |constant-factor for lower-limit – constant-factor for upper-limit|.
2. LL refers to lower-limit.
3. UL refers to upper-limit.

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Pediatric Ionized Calcium Reference Intervals from Background Radiometer Data

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Background: Calcium homeostasis is disturbed in a number of disorders in children. In this context, the measurement of ionized calcium (iCa) is preferable to total calcium because it is a better representation of biologically available stores, requires less blood volume per test, and has a rapid turnaround time. A challenge for pediatric laboratories wishing to promote iCa use, however, is ensuring reference intervals (RI) accurately reflect concentrations from a healthy population throughout childhood. **Methods**: Thirty three months of lab results internally archived on 4 Radiometer ABL800 instruments were extracted to a spreadsheet. A total of 107,569 result sets, including data for all measurable analytes, were organized by patient age at specimen collection date. Data was filtered to remove results from non-patient specimens, those missing demographics, duplicate entries, orders originating from critical care locations, results with an abnormal pH, and results from individuals with more than 1 order in 24 hours or more than 2 orders in 7 days (n=5,177). iCa results were plotted from 0 to 7000

days of life and reviewed for inflection points. Age bins were generated by visual inspection and subjected two rounds of Tukey outlier removal (n= 5,012). Histograms and normality were evaluated and the Harris-Boyd test was applied to assess statistical significance between age bins. The central 95th percentile RI were determined by nonparametric rank order calculation. An internal validation of the exclusion criteria was performed by applying the same approach to creatinine and sodium results in the data set and comparing newly calculated RI to established in-house ranges. **Results:** Table 1. **Conclusions:** The unique data acquisition strategy employed on Radiometer instruments provides a rich source of data for indirect pediatric RI determinations, such as iCa. When coupled to stringent exclusion criteria, and validated internally, this approach may improve the accuracy of pediatric RI.

Table 1: Proposed Pediatric iCa RI and Exclusion Criteria Validation (Sodium not shown)

Analyte	n=	Age (d)	Sex	Calculated (New) RI				Current RI	
				Lower	90% CI	Upper	90% CI	Lower	Upper
iCa (mg/dL)	137	0-0.99	All	4.50	4.40-4.64	5.75	5.67-5.81	3.90	5.20
	137	1.00-3.99	All	4.15	4.06-4.25	5.37	5.31-5.46	3.90	5.20
	596	4.00-179.99	All	4.85	4.81-4.90	5.81	5.78-5.88	3.90	5.20
	307	180.00-364.99	All	4.72	4.61-4.77	5.58	5.55-5.65	3.90	5.20
	3835	365.00-6574.5	All	4.48	4.47-4.50	5.32	5.30-5.34	3.90	5.20
Cr (mg/dL)	1052	<2191.5	F	0.14	0.13-0.14	0.48	0.46-0.49	0.10	0.60
	1200	<2191.5	M	0.14	0.13-0.15	0.47	0.46-0.48	0.10	0.60
	538	2195.5-4748.3	F	0.24	0.22-0.26	0.71	0.67-0.71	0.20	0.80
	585	2195.5-4748.3	M	0.22	0.19-0.23	0.70	0.67-0.74	0.20	0.80
	714	4748.3-6939.8	F	0.36	0.33-0.38	0.95	0.91-0.98	0.40	1.00
	617	4748.3-6939.8	M	0.28	0.23-0.34	1.19	1.15-1.22	0.40	1.20

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SARS-CoV-2 Serological Response Among COVID-19 Patients After Positive RT-PCR Result

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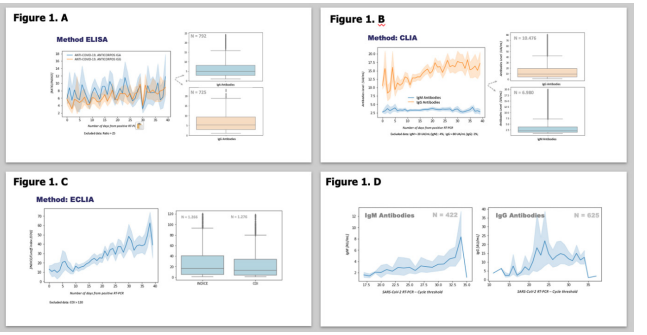
BACKGROUND: The COVID-19 diagnosis is based on molecular testing detecting genetic material of the virus. Serological assays include a variety of technologies and measure different classes of immunoglobulins against different viral antigens. The aim of this study is to evaluate immunological response of COVID-19 patients after confirmed diagnosis by positive SARS-CoV-2 reverse transcription (RT)-polymerase chain reaction (PCR) in the largest private laboratory in Latin America.

METHODS: The study included SARS-CoV-2 testing performed from February until October 2020. Among 1,275,401 patients who performed SARS-CoV-2 RTPCR, 26% resulted positive. From those with positive RT-PCR, 8% also performed at least one SARS-CoV-2 serology testing within 40 days from positive RT-PCR, and 58% of them (16,324 patients) had a positive serology. RT-PCR was performed by different platforms. At the period of the present study the available SARS-CoV-2 serology testing at our laboratory were chemiluminescent immunoassay (CLIA) method based on SARS-CoV-2 nucleocapsid and spike proteins(IgM and IgG), enzyme-linked immunosorbent assay (ELISA) based on spike protein (IgA and IgG) and total antibodies detected by enhanced CLIA (ECLIA) based on nucleocapsid protein.

RESULTS: The results are showed in the figure 1: (A, B and C), Time kinetic results for antibodies at different days from the positive RT-PCR, according to different serological testing; (D), Immunological response for IgM and IgG at different Cycle-threshold RT-PCR.

CONCLUSION: We demonstrated that the levels of SARS-CoV-2antibodies vary between COVID-19 patients along the course of the infection.

Figure 1. Immunological response of COVID-19 patients after confirmed diagnosis by positive SARS-CoV-2 reverse transcription (RT)-polymerase chain reaction (PCR) (A, B and C). Time kinetic results for antibodies at different days from the positive RT-PCR, according to different serological testing. A, ELISA; B, CLIA; C, ECLIA; (D), Immunological response for IgM and IgG at different Cycle-threshold SARS-CoV-2 RT-PCR.



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Cervical Cytology and High-Risk Human Papillomavirus (hrHPV) Testing Rates and Trends in Privately Insured Women, 2013-2019: Implications for Practice Interventions

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Background: In 2012, professional organizations recommended cervical cytology every 3 years for women age 21-65 or cytology and high-risk human papillomavirus (hrHPV) testing (co-testing) every 5 years for women age 30-65. In 2018, the U.S. Preventive Services Task Force (USPSTF) added hrHPV testing alone every 5 years for women age 30-65 as an additional screening strategy. The purpose of this study was to examine utilization rates and trends for cervical cytology, hrHPV testing, and co-testing in different age groups from 2013 through 2019 using claims data from a large sample of privately insured U.S. women.

Methods: We used commercial and Medicare supplemental claims from employer-sponsored health plans obtained from IBM MarketScan® claims database from January 2013 through April 2020 accessed using Treatment Pathways.® This encompassed 7.92-10.42 million women with continuous enrollment for each calendar year in 2013-2019. We excluded all claims with diagnoses of cervical dysplasia, cervical cancer, or treatment procedures of precancer to exclude all testing events that may have been for purposes other than screening. We identified cervical cytology and hrHPV testing using relevant medical codes to determine the number of women in different age groups in a calendar year who had ≥1 cervical cytology but no hrHPV tests, ≥1 hrHPV tests alone, or co-testing, which we defined as hrHPV testing from 3 days before to 30 days after cervical cytology. We used a 2-sided Poisson regression, adjusted for potential overdispersion, to examine cervical cancer screening trends over time. Trends were considered significant at $P < 0.05$.

Results: In 2013-2019, utilization rates, expressed as minimum-maximum annual rates, were: 7.5%-11.6% (age 18-20), 26.4%-34.2% (age 21-29), 8.6%-18.9% (age 30-64), and 2.2%-3.6% (age ≥65) for cervical cytology alone; and 1.4%-2.0% (age 18-20), 5.6%-6.1% (age 21-29), 14.9%-19.3% (age 30-64), and 0.7%-1.2% (age ≥65) for co-testing. From 2013 to 2019, cervical cytology alone decreased by 35%, 23% and 54%, respectively, in women age 18-20, 21-29 and 30-64 ($P < 0.0001$), and by 22% in women age ≥65 ($P = 0.005$). During this period, co-testing increased by 29% and 70%, respectively, in women age 30-64 ($P < 0.0001$) and age ≥65 ($P = 0.002$); and it decreased by 30% and 5%, respectively, in women age 18-20 ($P < 0.0001$) and age 21-29 ($P = 0.90$). The rate for hrHPV testing alone was <0.5% for women in all age groups. This rate increased only for women age 30-64, from 0.2% in 2013 to 0.4% in 2019, but remained flat or decreased for women in other age groups.

Conclusion: These data show decreasing trends in utilization rates of cervical cytology alone from 2013 to 2019, an increase in co-testing consistent with changes in screening recommendations made in 2012, and very low but increasing hrHPV testing alone in women age 30-64 per the USPSTF recommendation in 2018. Despite guidelines discouraging screening before age 21, significant proportions of individuals age 18-20 appear to be screened. This has implications for screening-practice interventions for dissuading screening for cervical cancer in this age group.

A-191

Clinical manifestations of Liver and Renal impairments in asymptomatic, moderate and severe COVID-19 patients of Madinah city of Saudi Arabia a retrospective analysis

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Background. Coronavirus disease (COVID-19) is a budding infectious disease that has affected various countries globally. However, most of the research on COVID-19 has mainly focused on lungs as a key organ involved in the disease, whileas, very little data is available regarding the involvement of other organs including liver and kidneys, which are also reported to be severely affected by the disease. Therefore, the objective of this study was to analyze the effect of COVID-19 disease on liver and kidney functions and to determine their association with the severity and mortality of disease. **Methods.** A total of 100 confirmed COVID-19 adult patients from Madinah city of Saudi Arabia hospitalized between April 28, and June 30, 2020 were included, and categorized into asymptomatic, mild to moderate and severely ill patients. We analyzed the clinical status of liver and renal functioning in all of the three groups. **Results.** The majority of patients (51%) were diagnosed with mild to moderate disease, 27% of patients were severely ill and 22% of patients were asymptomatic. The liver and renal functional analysis showed that the severity of the COVID-19 patients were significantly associated with the kidney and renal impairments exhibiting higher levels of ALT, AST, Creatinine, Urea levels ($P < 0.05$). Furthermore, in this study, a novel association is found between high Na and Cl levels with the severely ill COVID-19 patients.**Conclusion.** We concluded from the present study that a significant percentage of COVID-19 patients continued to have a normal liver and renal function during the course of their disease. Nevertheless, severely ill COVID-19 patients were more prone to have abnormal liver and renal functions. During the course of treatment, the patients had a gradual normalization of their liver and kidney parameters and subsequently achieved a complete normal liver and renal functions upon discharge with no mortality. The present findings, however, further demands to study the association between liver and kidney impairments with Covid-19 infection for better clinical management.

A-193

Vitamin D Testing Rates and Trends in Privately Insured Individuals in the United States, 2012-2018

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Background: Vitamin D testing is primarily used to screen for its deficiency and to assist in diagnosis or monitoring treatments for bone disorders. The U.S. Preventive Services Task concluded in 2014 that current evidence was insufficient to assess relative benefits and harms of screening for vitamin D deficiency in asymptomatic individuals age ≥18. Our objective was to determine the rates and trends for vitamin D testing in 2012-2018 for those with or without a filled prescription for this vitamin.

Methods: Claims data from employer plans for individuals age ≤65 were from IBM Watson® Health's MarketScan® databases for commercial claims and encounters in 2012-2018 encompassing 13.5-16.0 million individuals. To assess the impact of age, the studied population was broken into 5 age groups. We identified those with filled prescription for vitamin D by searching for all national drug codes and generics that included this vitamin. Annual testing rates were the proportion of individuals with 12 months of continuous enrollment having ≥1 vitamin D test(s) during each calendar year. We used a 2-sided Poisson regression, adjusted for potential overdispersion, to examine testing trends over time. Trends were considered significant at $P < 0.05$.

Results: In 2012-2018, 97.8%-98.2% of enrollees did not have a filled prescription for vitamin D. Within this group, annual vitamin D testing rates were: 1.4%-2.6%, age ≤18; 4.4%-6.1%, age 18-29; 8.7%-10.9%, age 30-39; 12.4%-14.8%, age 40-49; and 16.5%-18.6%, age 50-64. Testing rate increased from 2012 to 2018 (P for trend < 0.0001) by 95%, 37%, 23%, 17% and 10%, respectively, for those age ≤18, 18-29, 30-39; 40-49 and 50-64. For those with filled vitamin D prescriptions (1.8%-2.2% of all enrollees), testing rates were: 36.6%-55.4%, age ≤18; 77.7%-80.8%, age 18-29; 78.9%-82.0%, age 30-39; 77.8%-80.7%, age 40-49; and 75.1%-77.8%, age 50-64. For these groups, testing rate from 2012 to 2018 increased by 6% for individuals age ≤18 (P for trend = 0.96), and it decreased by 3% for the other 4 age groups (P for trend = 0.0040-0.034). Filled vitamin D prescription increased from 2012 to 2018 for all 5 age groups (P for trend < 0.0001-0.0005).

Conclusion: Substantial proportion of individuals in this large database are increasingly tested for vitamin D. Testing rate increased with age, particularly for those without a filled prescription for vitamin D. The vast majority (98%) of enrollees did not have a filled prescription for vitamin D during each year during the study time frame of 2012-2018. This may suggest that most tests are primarily for screening purposes. Further research is needed to understand the reasons for increased vitamin D testing, especially in age groups with typically low prevalence of bone diseases. These results should be considered in view of several limitations. Claims data underrepresent actual testing events since some performed tests may not be reimbursed. Similarly, MarketScan data do not reflect over-the-counter vitamin D usage. Also, MarketScan databases are not expected to be representative of the entire insured U.S. population.

A-194

Analytical Validity of Multi-Analyte Assays with Algorithmic Analysis for Endoscopic Disease Activity Assessment in Crohn's Disease

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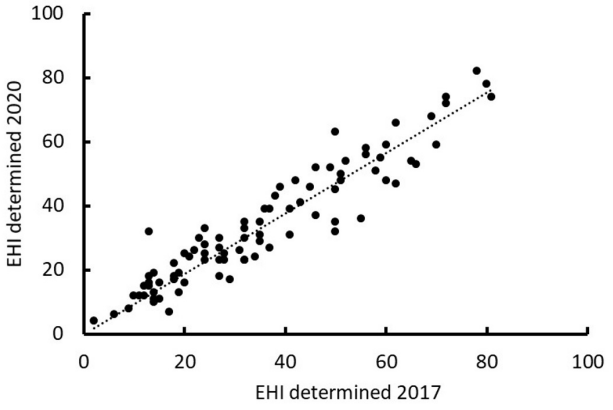
Background: A blood-based biomarker panel combining 13 markers to generate an Endoscopic Healing Index (EHI) score is a novel instrument to assess endoscopic healing in patients with Crohn's disease (CD) (Gastroenterology 2020;158:515–526). We describe the analytical performance of the EHI score in a clinical laboratory setting. **Methods:** The EHI score is tested using nephelometry (n=1 analyte) and magnetic bead based multiplex assays (n=12 analytes), which are combined to generate EHI (0 to 100). Pre-analytical variability was evaluated using serum collected in SST tubes (n=22 normals) for 72hrs. Analytical performance was evaluated using individual and pooled serum samples and consisted of Intra-day (n=8 specimens) and Inter-day precision (n=12 specimens processed 6 times). EHI consistency across a 3yr testing period was established using 87 specimens paired with endoscopy (47/87 with active disease). AUROC curve was used for differentiating endoscopic Active Disease from Remission. **Results:** The EHI is stable in the SST tube for up to 72hrs at ambient temperature (R^2= 0.963, Deming's Slope= 0.950, Intercept: 0.486). Intra-day and Inter-day %CVs ranged from 2.9 to 8.1% (median: 4.1%), and 1.0 to 9.6% (median: 4.8%), respectively. As presented in Fig.1, there was no difference in EHI score determined in 87 specimens tested over a 3yr period (2017 median EHI: 32, IQR: 19 - 50 and 2020 median EHI: 30, IQR: 18 - 48) (R^2= 0.888, Deming's Slope= 0.952, Intercept: 0.049) with similar AUROC achieved between testing periods (AUC=0.637 (95% CI, 0.520- 0.753) vs 0.631 (95% CI, 0.514- 0.749) in distinguishing endoscopic disease states. **Conclusion:** The analytical validity of the EHI score has been established and it performs acceptably in a clinical laboratory setting.

Fig.1 EHI Comparison between Testing Periods in a Clinical Laboratory



A-195

Development of Liquid-stable Calibration Verification Control Kits for Diabetic Markers to Characterize Method Linearity and to Verify the Reportable Range

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Introduction: Diabetes is a health condition that impacts how the body processes glucose. When blood glucose (GLU) levels rise, the body signals the pancreas to release the hormone, insulin. Insulin (INS) aids in allowing glucose to enter the cells and be used as an energy source. Patients with diabetes either do not make enough insulin or cannot use the insulin produced by their bodies. C-Peptide (CPEP) is a connecting peptide produced at a 1:1 ratio with insulin and can be used as a biomarker for insulin production in type 1 diabetics and it can distinguish between native and injected insu-

lins. Depending on the disease state of an individual, a variety of tests can be used to help diagnose and monitor diabetes. Fructosamine (FRA) is a fairly new marker for early diagnosis of diabetic risk, results indicate average blood glucose over a 1-4 week period and can be tested in the presence of abnormal hemoglobin variants that could interfere with HbA1c results. Beta-Hydroxybutyric Acid (BHBA) is a ketone body produced when glucose cannot be used as an energy source and is used to monitor diabetic ketoacidosis. There are numerous assays cleared by the FDA that are used to monitor and diagnose diabetes. To support the use of new biomarkers for diabetic testing and monitoring, LGC's objective was to develop a five-level, liquid-stable, human serum based, diabetes control kit for the following analytes: FRA, BHBA, INS, CPEP and GLU. Methods: VALIDATE® Diabetes (204RO) was formulated as a multi-constituent kit, in a human serum matrix according to CLSI EP06-A into five equal-delta concentrations to cover the reportable ranges of the Roche cobas®. Reported recoveries were evaluated for mean, SD, and linearity using MSDRx®, LGC's proprietary linearity software. Limits were applied as a percentage of the total allowable error (TE_a), specific for each analyte. Product stability was determined by a combination of stress and real-time stabilities. Results: For VALIDATE® Diabetes (204RO), all levels were stable, recovering within ±10% of the recovered values on the date of manufacture in stability studies for each analyte: FRA, BHBA, INS, CPEP and GLU. Linear regression analysis of theoretical versus mean recovered values demonstrated a linear performance of each assay through the manufacturer's reportable range and within the applied TE_a limits. Stability studies are ongoing. Conclusion: The VALIDATE® Diabetes, 204RO, is a liquid stable, ready to use product able to be stored at freezer temperatures. 204RO is fit-for-purpose as a calibration verification test kit that covers the full reportable ranges for all claimed analytes on the Roche cobas®. VALIDATE® 204RO provides a reproducible and dependable product for laboratories to meet regulatory requirements for some of the most commonly tested assays for diabetes diagnosis and treatment monitoring. This product conforms to CLSI EP06-A guidelines to linearity testing and is FDA listed.

A-196

Evaluation of anti-PR3 by Chemiluminescence Immunoassay and Atypical p-ANCA by Digital Imaging in Inflammatory Bowel Diseases

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Background: Both anti-PR3 (Proteinase 3) and atypical p-ANCA (perinuclear antineutrophil cytoplasmic antibodies) can be important in facilitating inflammatory bowel disease (IBD) diagnosis and stratification (ulcerative colitis [UC] versus Crohn's disease [CD]). We evaluated the performances of anti-PR3 by chemiluminescence and atypical p-ANCA by digital imaging in distinguishing IBD. **Methods:** Specimens were collected from consented subjects enrolled in a prospective biobank in autoimmune gastrointestinal diseases (Prometheus Laboratories, San Diego, CA). All specimens were tested in the diagnostic immunology laboratory. A total of 60 healthy, 91 CD, and 96 UC patient specimens were analyzed using Inova Diagnostics QUANTA Flash® PR3 (Cat. # 701138) and NOVA Lite® DAPI ANCA Ethanol (Cat. # 704338) and Formalin (Cat. # 704337) Kits. The ethanol and formalin ANCA slides were read using the Inova Diagnostics NOVA View® digital microscope. Atypical p-ANCA results were identified when a fluorescent perinuclear pattern was observed on the ethanol slide in combination with absence of fluorescence on the formalin slide. Manufacturer cutoffs were used for PR3 (> 2.3 CU) and atypical p-ANCA (≥ 1:20). Diagnostic performance in distinguishing IBD from normal healthy and UC from CD was established using sensitivity, specificity, likelihood ratio (LR) diagnostic odds ratio and predictive value. **Results:** Atypical p-ANCA yielded 49.7% sensitivity and 98.3% specificity in distinguishing IBD from normal healthy (positive LR=29.8 CI95%: 5.59-169.3; negative LR=0.51 CI95%: 0.44-0.59; OR=58.2 CI95%: 9.9-340.1) with 61% positive predictive value (pre-test=5%). Anti-PR3 was 100% specific for IBD (17% sensitive). As presented in Table 1, anti-PR3 was highly specific for UC (96%). Patients with anti-PR3 were 8.96-fold more likely to have UC than CD. Atypical P-ANCA yielded 66% sensitivity and 67% specificity, in distinguishing UC from CD, respectively. **Conclusion:** The data suggests that PR3 and atypical p-ANCA on NOVA View have value in the differential diagnosis of IBD and stratification.

Table 1. Diagnostic Performances of anti-PR3 and Atypical p-ANCA in CD vs. UC

	anti-PR3	Atypical p-ANCA
TP (Sensitivity)	0.29 [0.21 to 0.39]	0.66 [0.56 to 0.74]
TN (Specificity)	0.96 [0.89 to 0.98]	0.67 [0.57 to 0.76]
Positive LR	6.63 [2.57 to 17.67]	1.99 [1.45 to 2.79]
Negative LR	0.74 [0.64 to 0.84]	0.51 [0.37 to 0.69]
Diagnostic OR	8.96 [3.11 to 25.64]	3.88 [2.11 to 7.12]
PPV (0.50 Pretest)	0.87 [0.71 to 0.95]	0.67 [0.59 to 0.73]
NPV (0.50 Pretest)	0.57 [0.54 to 0.61]	0.66 [0.59 to 0.73]

A-198

Establishment of Reference Intervals of Thyroid Function Tests (TFT) in Chinese Population on Abbott Alinity i System and Exploring the Possible Effect from Thyroid Autoantibodies

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Objective: To establish reference intervals for TFT assays in Chinese population using the Abbott Alinity i System and to explore the possible effect from thyroid autoantibodies

Method: Serum samples were collected from 216 healthy Chinese adults (98 males and 118 females, aged 22 to 78 years old) in accordance with the inclusion and exclusion criteria defined based on the National Academy of Clinical Biochemistry (NACB) guidelines for the establishment of reference intervals of thyroid tests. Measurements of TSH, Free T4, Total T4, Free T3, Total T3 as well as both thyroid peroxidase autoantibodies (TPO-Ab) and thyroglobulin autoantibodies (Tg-Ab) were done. Nonparametric methods were used to establish the various reference intervals. Due to a relatively high prevalence of thyroid autoantibodies (19%) in our apparently healthy population we also investigated the effect of the autoantibodies on these intervals.

Results: Reference intervals of the thyroid function tests based on the NACB criteria for subjects without thyroid autoimmunity were determined as follows: TSH, 0.41 - 3.88 μIU/mL; Free T4, 0.76 - 1.32 ng/dL; Total T4, 5.34 - 9.94 μg/dL; Free T3, 1.79 - 3.26 pg/mL; and Total T3, 60.84 – 122.02 ng/dL.

Since there was a high prevalence of thyroid autoantibodies in our apparently healthy individuals (19%), for interest, we re-evaluated the entire cohort to see if there was any effect on the new reference intervals. Results were as follows: TSH, 0.41 – 3.87 μIU/mL; Free T4, 0.76 - 1.31 ng/dL; Total T4, 5.34 - 9.64 μg/dL; Free T3, 1.82 - 3.23 pg/mL; and Total T3, 60.74 – 115.67ng/dL. Most of the reference intervals were not affected by the thyroid autoantibodies except Total T3 and Total T4 assays. However, statistical analysis shows that the median Free T3 value in TPO-Ab or Tg-Ab positive individuals was significantly lower than that in TPO-Ab and Tg-Ab negative individuals (2.44 pg/mL vs 2.62 pg/mL, P=0.013) whereas the median values of TSH, Free T4, Total T4 and Total T3 were not significantly affected.

Furthermore, the new reference intervals determined for our population were found to be narrower than those provided by the manufacturer which could be due to ethnicity and/or dietary differences of the population being studied.

Conclusion: The Abbott Alinity i analyser was used to establish reference intervals of thyroid function tests in apparently healthy Chinese population. Reference intervals and median values were found to be different in some assays in individuals with thyroid autoimmunity. Since thyroid autoantibody tests have some effects on reference intervals and are not commonly ordered as screening tests, it is hoped that our observations might be useful for clinicians in their interpretation of thyroid function test results.

A-199

Weak Precipitin Ring from A Fecal Specimen with Markedly Elevated Alpha-1 Antitrypsin Level Measured by Radial Immunodiffusion

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Background: Radial immunodiffusion (RID) is a classic methodology for antigen quantification that relies on the development of distinct precipitin rings from precipitated antigen-antibody complex. Our lab uses RID to measure fecal alpha-1 antitrypsin (A1AT) levels to aid in the diagnosis and monitoring of Protein Losing Enteropathy (PLE).

Methods: Extracted stool specimens were deposited on RID plates prepared with anti-human alpha-1-antitrypsin. After incubating at room temperature for 48 hours, diameter of each precipitin ring was measured with a Transidyne General Biological Diffusion Plate Calibrating Viewer.

Results: A patient did not produce the typically expected precipitin ring, but an unexpected very large ring that was barely visible. Dilution studies confirmed a markedly elevated fecal A1AT level of 67 mg/g dry stool. The unexpected weak and large precipitin ring was reproduced with a spiked specimen with similar A1AT level prepared by spiking a blank patient specimen with commercial A1AT.

Conclusion: This case report shows a rare case of an unexpected very weak and large precipitin ring in RID that could have been easily misinterpreted as undetected despite of a markedly elevated analyte level of 67 mg/g dry stool. Meticulous attention and caution must be used when reading and interpreting gel-based immunoassays. In cases where gel-based immunoassays show results without the expected precipitation, we recommend repeat testing with diluted specimens.

A-200

Serial quantitative anti-SARS-CoV-2 antibody measurements in healthy subjects receiving the COVID vaccine

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Introduction: The recent SARS-CoV-2 pandemic remains a global problem. Reports of waning antibody titers and re-infection have raised considerable concern regarding the protective capacity of humoral immunity. Recently, mRNA-based vaccines with good efficacy have been made available. Quantitative anti-SARS-CoV-2 antibody measurements of serial samples from healthy volunteers in a prospective study are key to better defining humoral immune response to the vaccine. **Objective**: To define extent and temporal evolution of humoral immunity in healthy volunteers after Moderna or Pfizer vaccine using a quantitative anti-SARS-CoV-2 IgG assay. **Methods**: An in-house quantitative lab developed test, Cov2Quant, with a reportable range of 1.0 - 128,000 µg/mL was used. Cov2Quant is specific for IgG against the receptor binding domain of SARS-CoV-2 spike protein. Sixty-one volunteers participated. After receiving either Moderna or Pfizer vaccine, sera from subjects were collected at the following time points: week 1, week 2, and week 3 after the first dose; week 1, week 2, month 1 after the second dose. **Results**: At week 1 post first dose, 59/61 subjects had no detectable antibodies. Two subjects had very high titers and were excluded from Figure 1 after confirming their COVID exposure prior to vaccination. For 59/61 subjects, antibody titers ranging from <1.0µg/mL to 51 µg/mL were first detected at week 2 post-first dose and further increased by week 3 (Figure 1). After the second dose, a spike in antibody concentrations with great heterogeneity was observed and sustained from week 1 to week 2. In the following 2 weeks, antibody concentrations declined (Figure 1). **Conclusion**: This data indicate that vaccination successfully results in IgG against the SARS-CoV-2 spike receptor binding domain by week 2 post-first dose. Second dose of vaccine resulted in a spike in concentrations, ranging on average from 100 to 400 µg/mL.

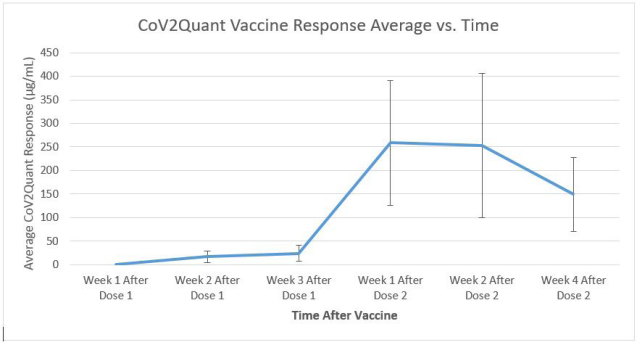

Figure 1: Anti-IgG RBD temporal response to SARS-Cov-2 vaccine regimens. Data plotted as mean ± S.D.

A-201

Detection of High-Dose Hook Effect in Urine Myoglobin Specimens Using the Roche cobas e602 Analyzer

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Background: Myoglobin is an oxygen-binding protein found in cardiac and skeletal muscles. High concentrations of myoglobin in urine may indicate acute muscle injury and increased risk of renal failure. For this reason, there is clinical value in measuring myoglobin in urine, although the Roche Myoglobin assay on the cobas e602 analyzer is currently only approved for use in serum and plasma. As high concentrations of myoglobin in urine has the potential to cause a high-dose hook effect, a predilution of specimens may be necessary to obtain accurate results for these specimens. However, dilutions of specimens with relatively low concentrations of myoglobin may show falsely elevated myoglobin concentrations. The present study was conducted to a) determine an appropriate predilution factor that would detect a high-dose hook effect in urine specimens with severely elevated myoglobin concentrations, while also avoiding falsely elevated results in specimens with low concentrations of myoglobin, and b) evaluate the onboard dilution capability of the cobas e602 analyzer for the Roche Myoglobin assay. **Methods:** Residual human urine specimens previously tested for urine myoglobin (n=35) were obtained from storage (-20°C) and de-identified according to an IRB-approved protocol. All specimens had previously reported urine myoglobin concentrations >1000 ng/mL (normal <1000 ng/mL) using an onboard 1:50 predilution. Myoglobin was measured in all specimens with no dilution, a 1:10 and a 1:50 onboard dilution. Specimens that had results above the linear range after a 1:50 dilution (>150,000 ng/mL) were tested further by using an onboard 1:400 dilution to obtain a result. Results for 1:10 and 1:50 dilutions were used to determine if a high-dose hook effect was detected using either dilution. Specimens that had results <3000 ng/mL for no dilution, 1:10 and 1:50 dilution were categorized as not exhibiting a high-dose hook effect. Specimens that had a reportable value for the onboard 1:10 dilution (n=10) were also selected and tested using a manual 1:10 dilution. Percent differences between onboard and manual dilutions were compared. **Results:** The comparison between no dilution, a 1:10 and a 1:50 onboard dilution showed that 5 of the 35 specimens did not exhibit a high-dose hook effect. There was a 100% agreement between 1:10 and 1:50 onboard dilution in the detection of a hook effect with urine myoglobin concentrations up to 556,527 ng/mL. Of these 30 specimens that had 1:10 and 1:50 results that were >3000 ng/mL, 27 specimens had results that were <3000 ng/mL when they were tested without a dilution. Results between an onboard and manual 1:10 dilution had an average percent difference of -4.23%. **Conclusion:** A predilution of urine myoglobin specimens using the Roche Myoglobin assay on the e602 is necessary to detect specimens with severely elevated concentrations of myoglobin that might exhibit falsely low results, due to high-dose hook effect. Both 1:10 and 1:50 dilutions are capable of detecting a high-dose hook effect in urine specimens. An initial predilution of 1:10 appears to avoid false elevation of results for specimens with lower concentrations of myoglobin. Both manual and onboard 1:10 dilutions are acceptable for use.

A-202

HbA1c as a marker of glycemic status in patients posted for Liver Transplantation

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Abstract

Background : Liver is one of the most commonly transplanted organ As with any other surgery the presurgical profile of liver transplantation includes the assessment of the glycemic status of the patient. In our study we evaluated the utility of HbA1c as a marker of glycemic status in those patients posted for liver transplantation**Materials and Methods:** A retrospective analysis of the HbA1c values were done in 50 patients who were posted for Liver Transplant during the time period November 2015 to November 2018The estimated average glucose (eAG) was calculated from the HbA1c values. The comparison was done between the measured glucose and estimated average glucose. The comparison was done with Random blood sugar (RBS) in 21 patients and with Fasting Blood Sugar (FBS) in 29 patients

Results: The 'r' value was 0.1881 for FBS versus eAG. Though a positive correlation, the relationship is weak as the nearer the value is to zero, the weaker the relationship. The value of R2, the coefficient of determination, is 0.0354. The *t*-value is 5.1241. The *p*-value is < .00001. The result is significant at $p < .05$. The 'r' value was 0.3439 for RBS versus eAG. Though a positive correlation, the relationship is weak as the nearer

the value is to zero, the weaker the relationship. The value of R2, the coefficient of determination, is 0.1183. The *t*-value is 6.0225. The *p*-value is < .00001. The result is significant at $p < .05$. **Conclusion**: We conclude that HbA1c is not a reliable marker in patients posted for Liver transplant surgeries and should be interpreted with caution in such patients taking into account liver function tests and other glycemic markers.

A-203

Evaluation of Hook Effect on Prolactin Assay in Patients with Prolactin Levels higher than 10,000 ng/mL.

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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 < 200 ng/mL. Generally, the serum PRL values above which the hook effect may be induced are given in the instruction sheets of the immunoassay kits, it depends on each particular assay. The Siemens Advia Centaur PRL sheet says that serum PRL concentrations up to 30,000 ng/mL will give results > 200 ng/mL, without experiencing the hook effect. The objective of this study was to evaluate the hook effect in PRL measurements in Siemens Advia Centaur in patients with PRL values above 10,000 ng/mL.

Methods: We analyzed blood samples requesting serum PRL levels from patients admitted to a private reference clinical laboratory in Brazil, from 01/01/2001 to 12/31/2020. Anonymized data on laboratory tests were available from a database of the local Laboratory Information System. All the patients included in our study had PRL levels above 10,000 ng/mL (ICMA, Advia Centaur, Siemens). We evaluated the first result obtained by the equipment for each patient and verified if they were above 200 ng/mL, indicating dilution, not experiencing hook effect.

Results: Forty-one patients were evaluated, 34 (83%) men, 7 (17%) women; mean age 42 (17 to 73) yrs. They were divided in three groups: 26 (10,000 to 19,999 ng/mL), 6 (20,000 to 30,000 ng/mL) and 9 patients with PRL levels higher than 30,000 ng/mL. In this last group, 7 (78%) men and 2 (22%) women, mean age 40 (30 to 64) years, mean PRL levels 46,426 (31, 810 to 85,614) ng/mL. All the patients had the first result above 200 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 between 10,000 and 30,000 ng/mL, as reported in the package insert; and also for values above 30,000 ng/mL, indicating that this effect can vary according to the platform and that the PRL assays have been improved in the last decades.

A-204

Method Evaluation of the Siemens IgG Subclass Assay on Siemens BNII Nephelometer

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Background: Quantification of IgG subgroups is commonly used to help evaluate patients with immunodeficiency or IgG4-related disease. This test is currently performed using Binding Site reagent on a Siemens BNII nephelometer at our laboratory. Recently, Binding Site had issued a notice to discontinue supplying the reagent on BNII. The aim of this study is to perform a full method evaluation on the candidate Siemens IgG subclass assay for this replacement.

Methods: Method evaluation was carried out in Special Chemistry laboratory at the University of Alberta hospital. Quantification of IgG subgroups in patient serum samples was performed using the Siemens BNII® nephelometer and the Siemens IgG subgroup assay. Following the CLSI guidelines for method validation, we evaluated precision, linearity, method comparison, limit of quantitation, sample stability, and reference intervals.

Results: Our data demonstrated acceptable precision, linearity, and sensitivity of the Siemens IgG subgroup assay. The between-day and within-run precision were within 5%CV and 7%CV, respectively, for all subgroups. In the method comparison study against Binding Site assay on BNII nephelometer, the Siemens assay had a positive bias for IgG1 (+18%) and IgG4 (+40%) but negative bias for IgG2 (-3%) and IgG3 (-66%). Comparison with Binding Site assay on Optilite instrument, positive bias was observed in all IgG subgroups (IgG1: +15%; IgG2: +4%; IgG3: +18%; IgG4: +14%). While frozen samples showed good stability for up to 28 days (<10%), repeated freeze-thaw cycles demonstrated up to 15% increase in values for all subgroups. The manufacturer's adult reference interval was verified for IgG 2 to 4 using 60 healthy adult samples. IgG1 reference interval will need to be established.

Conclusion: The Siemens assay exhibited good reliability for quantification of IgG subclasses. However, results obtained from this assay is not interchangeable with the current IgG subclass assay. Careful review of the reference intervals must be accomplished before switching of the assays.

A-206

Study of Soluble fms-Like Tyrosine Kinase-1 and Placental Growth Factor Ratio in Algerian Women With Suspected Preeclampsia

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Background: Preeclampsia (PE) is a pathology specific to pregnancy, which is associated with significant perinatal and maternal morbidity and mortality. The angiogenic markers PlGF, sFlt-1 implicated in the pathogenesis of PE could provide valuable help in the diagnosis of this pathology. The main objective of this work is to assess the accuracy of the angiogenic biomarkers (sFlt1 and PlGF) in the early diagnosis of PE. It also analysed the prognostic value of these biomarkers in the occurrence of maternal-fetal adverse outcome (MFAO). **Methods:** It is a nested case-control study, conducted at the Biochemistry Laboratory of Annaba University Hospital, on 405 pregnant women of at least 20 weeks of gestation (WG) (197 at risk and 208 controls), recruited in two hospital gynecology-obstetrics departments in the region of Annaba and Guelma, between October 2017 and October 2018. The initial biological assessment carried out at the time of inclusion mainly included measurement of sFlt-1/PlGF ratio (Elecsys Roche Kit) and conventional parameters with diagnostic or prognostic value in PE. **Results:** Our study showed that the sFlt-1/PlGF ratio has the best discriminating value, compared to the angiogenic markers used separately and to conventional markers too, used in the diagnosis of PE before 34 WG in a population at risk. Thus, a sFlt -1/PlGF ratio > 85 before 34 WG, and > 110 from 34 WG, allows the diagnosis of PE with a sensitivity of 82.67% and a specificity of 96.67%. Optimal diagnostic and prognostic cut-off with the occurrence of PE or IMFAO, respectively have been determined based on the analysis of the ROC curves. Thus, at the cut-off of 36.97, the ratio is more efficient in the diagnosing of PE with a sensitivity of 97.00% and a specificity of 92.70%. In addition, the sFlt-1/PlGF ratio at the level of 173, best classified the preeclampsia at risk of MFAO with a sensitivity of 82.64%, a specificity of 64.52%. **Conclusion** : The sFlt-1/PLGF ratio measured between 20 WG and 34 WG appears to be a promising biomarker to predict short-term PE and assess maternal-fetal prognosis, particularly in early and severe forms of the disease.

A-207

A semi-qualitative assay for detection of antiSARSCoV2 antibodies in human serum and plasma: an evaluation of the Roche Diagnostics Elecsys® Anti-SARS-CoV-2 application

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Background: The objective of this study was to evaluate the analytical performance of the Elecsys® Anti-SARS-CoV-2 on the Roche Diagnostics cobas® e 602. To date, ~130 million cases of Coronavirus 2019 (covid19) have been confirmed worldwide. However, this number underestimate total cases since not all individuals with COVID19 are tested. Nucleic acid testing (NAT) remains the gold standard for confirming acute COVID19 infections; however, these tests cannot detect previous exposure. Epidemiological information regarding cumulative incidence of infections is important for understanding the proportion of deaths among infected individuals, infection rates among different populations, and the number of mild or asymptomatic cases that were not detected by routine disease surveillance. Serological assays detect the presence of anti-SARS-CoV2 immunoglobins, identify subjects who can provide plasma for convalescent plasma therapy, and guidance on vaccinations.

Methods: The Elecsys® Anti-SARS-CoV-2 electrochemiluminescence sandwich immunoassay was evaluated on the Roche Diagnostics cobas® e 602 (Laval, QC). This assay detects total antibodies (IgG, IgM, and IgA) against the nucleocapsid protein of SARS-CoV-2 and can be performed in 18 min. Control materials from Roche and Bio-Rad Laboratories (Hercules, CA) as well as 40 patient plasma specimens (20 positive

and negative for COVID19) taken from the a COVID19 BioBank were used in this study. The evaluation was performed according to CLSI guidelines. Within run and between run precision were evaluated using quality control materials from Roche and BioRad. Accuracy was assessed by measuring samples with anti-SARS-CoV-2 antibody titers assigned by the College of American Pathologists (CAP). The assay cutoff value [cutoff index (COI): 1.0] was validated by measuring Roche calibrator material. The comparisons of 40 specimens (20 positive and 20 negative) were analyzed on two cobas e 602 modules at two different laboratories. Carryover was assessed by measuring three negative control sample, followed by a high positive patient specimen (COI = 73.2) and one negative control.

Results: Within run precision using Roche control material was 2.6%CV and 0.9%CV for the negative (COI = 0.08) and positive (COI = 2.8) materials, respectively. Between run precision using the same Roche control material was 4.9%CV and 3.8%CV for the negative and positive QC materials, respectively. Between run precision using BioRad control material was 7.5%CV and 5.0%CV for the negative (COI = 0.08) and positive (COI = 20.3) materials, respectively. Analytical accuracy determined using stored proficiency survey samples demonstrated that results fell within 3 standard deviation indices of target means. Patient sample comparisons between two e 602 modules produced a strong correlation (coefficient = 0.999, slope = 0.98, *y*-intercept 0.49). There was no carryover. The assay has a clinical sensitivity of 99.5% (CI: 97-100%) and a specificity of 99.8% (95% CI: 99.69-99.88) for plasma specimens obtained ≥14 days post PCR confirmation.

Conclusion: The Roche Diagnostics Elecsys® Anti-SARS-CoV-2 is a rapid, robust, accurate and precise semi-qualitative test for detecting antibodies against the SARS-CoV-2 virus.

A-210

Evaluation of Electrode Maintenance and Quality Control for Ionized Calcium to Support Post-Filter Testing During Continuous Renal Replacement Therapy (CRRT) With Regional Citrate Anticoagulation

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Background: Conflicting results were published on the stability of ionized calcium electrodes used to measure ionized calcium at 0.3 mmol/L during a 12 week maintenance interval the manufacturer recommends for the Radiometer ABL 837. Analytic accuracy is important at the low iCa level of 0.3 mmol/L because this is the target concentration of iCa achieved in patient post-filter whole blood specimens when citrate is added for regional anticoagulation during CRRT. The objective of this study was to assess evidence of iCa electrode stability in quality control (QC) data for a six month interval (2 cycles of electrode membrane changes at 12 week intervals per ABL 837 instrument).

Methods: QC data for six months for 2 Radiometer ABL 837 instruments was extracted. Maintenance logs were used to determine dates of iCa electrode membrane changes. OLS linear regression was used to assess the relationship between the age of iCa electrode membranes in days and the change in iCa QC results (delta QC) from the initial QC result on the date of membrane change.

Results: Levy-Jennings plots of QC level 1 (approx. 0.3 mmol/L) for two ABL 837 instruments were synchronized to the initial date of iCa electrode replacement and change in iCa values were determined daily. Delta QC results demonstrated a positive slope of +0.001 mmol/day and there was no systematic bias or slope change for level 2 and 3 QC results. The time dependent slope for level 1 delta QC achieved a bias of +0.08 mmol in 12 weeks. Note that this QC bias occurred even though the instruments were re-calibrated four or more times per 24 h.

Conclusion: Based on this analysis of level 1 QC data, the iCa electrode membranes were not stable for 12 weeks. The iCa electrode membrane replacement at 4 week intervals was implemented to avoid systematic positive bias of iCa measurements for QC and patient results and to enable re-assessment of day-to-day Level 1 QC standard deviation and determination of new QC rule limits. Future studies will assess the impact of iCa electrode membrane change intervals on CRRT patient outcomes.

A-211

Morning serum cortisol: A good screening tool to exclude the diagnosis of adrenal insufficiency, but not to confirm it.

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Background The diagnosis of adrenal insufficiency (AI) still have many challenges: the lack of specific criteria for clinical suspicion and the difficulties of laboratory investigation. Currently, when there is a clinical suspicion, consensus statements recommends, in the majority of cases, a cortisol stimulus test to exclude or confirm AI. The tests considered gold standard are: the standard dose (250mg) cosyntropin stimulation test (CST) and the insulin tolerance test (ITT). In this context, the use of morning serum cortisol as an AI screening tool has not a well-defined role or uniform cutoff values in the literature.The objective of this study was to assess the accuracy of the cutoff values for morning serum cortisol proposed in the literature to exclude or confirm AI in a suspected population of Rio de Janeiro, in order to propose a better use of this tool in the AI diagnostic flowchart in our population.**Methods** Retrospective study with analysis of Laboratory information system (LIS) database. Inclusion criteria were (1) >=18 years, (2) submitted to CST or ITT between 2014 and 2019. We excluded patients with conditions that could interfere in cortisol levels (pregnancy, use of OCP or MHT, cirrhosis or hyperproteinemia). Cortisol measurements were performed with chemiluminescent immunoassay by Beckman Coulter.**Results** We evaluated 516 patients (255 CST and 261 ITT), 384(74,4%) women, aged 18 to 89 years. Overall, 235/516 (45.5%) were classified as AI and 281/516 (54.5%) as not AI by the cortisol stimulus test performed. The morning serum cortisol levels were evaluated for accuracy according to the cutoff values proposed in the literature. The 2 cutoff values proposed to confirm AI showed that: (1) cortisol <3µg/dL (n=26/516) had specificity of 97.5% and positive predictive value (PPV) of 73%, (2) cortisol <5µg/dL (n = 69/516) had specificity of 89.5% and PPV of 68%. While, the 3 cutoff values proposed to exclude AI: (1) cortisol>= 10µg/dL (n = 166/516) had sensitivity of 82% and negative predictive value (NPV) of 75%, (2) cortisol >= 15µg /dL (n = 45/516) had sensitivity of 98% and NPV of 90% and (3) cortisol> = 18µg/dl (n = 19/516) had sensitivity of 99.5% and NPV of 95%.**Conclusion** The levels of morning serum cortisol proposed in the literature to confirm AI had low accuracy in our population, therefore, we are still unable to recommend the use of morning serum cortisol as a tool to confirm AI. The morning serum cortisol levels >= 15µg/dL demonstrated high accuracy to exclude AI, allowing us to recommend this cutoff value as a way to exclude AI, reducing the need for cortisol stimulus tests in our population. These data can help to guide doctors in our population on the best use of morning serum cortisol as a screening test in the diagnosis of AI, excluding this diagnosis in some cases, safely omitting the stimulus test and reducing expenses, but not yet being able to confirm it.

A-212

Ensuring the Accuracy and Reliability of Testosterone and Estradiol Measurements Through Standardization and Analytical Performance Monitoring

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Background: Elevated or deficient levels of steroid hormones can contribute to a wide range of diseases, such as hypogonadism, polycystic ovary syndrome, and certain cancers. Correct diagnosis, treatment, and prevention of diseases depend on accurate and reliable laboratory measurements and are critical for patient care and public health decisions. Currently, overall global accuracy and reliability levels of laboratory measurements impede the effectiveness of these vital clinical actions. Standardization ensures that laboratory tests meet these accuracy and reliability requirements, regardless of method, time, and location.

Methods: The CDC Clinical Standardization Program (CSP) offers the Hormone Standardization (HoSt) Program. The goal of the program is to standardize clinical measurements of hormones, by assisting manufacturers of clinical assays and developers of lab-developed tests (LDTs), using a comprehensive range of activities and programs for total testosterone (TT) and estradiol (E2). The program includes calibration procedures and/or quarterly blinded challenges to ensure that accuracy, precision, and other analytical performance parameters of a test are improved, verified, and maintained. The HoSt uses single-donor samples that are value assigned by reference measurement procedures (RMPs). Laboratories successfully meeting established evaluation criteria are certified by the HoSt. Another program, the CDC

Accuracy-based Monitoring Program (AMP) for TT is intended for end-users of clinical assays. The AMP monitors end-user performance by assessing bias and precision over time using single-donor/pooled serum materials with reference values assigned by the CDC RMPs. Currently, the AMP participants include commercial, clinical and research laboratories.**Results:** In 2020, HoSt TT participants included 52% clinical laboratories, 32% research laboratories, and 16% manufacturers. HoSt E2 participants included 50% clinical laboratories, 40% research laboratories and 10% manufacturers. Enrollment of assay manufacturers and developers of LDTs in the HoSt have resulted in improvements of calibration accuracy. The among laboratory bias has decreased from 16.5% in 2007 to 2.03% in 2020 for TT and 54.8% in 2012 to 0.19% in 2020 for E2. The HoSt also highlights sample-to-sample variabilities that are often observed in clinical settings. In 2020, mean bias ranges for male and female TT samples were -31.6% to 33.1% and -41.4% to 75.9%, respectively. Depending on the participant, 10% to 98% of individual samples analyzed met program bias criteria. For E2, mean bias ranges for samples < 20 pg/mL and > 20 pg/mL were -43.3% to 105.9% and -24.4% to 17.4%, respectively. 68% to 100% of individual samples analyzed met program bias criteria among participants. The AMP TT results indicate that end user data are comparable to the HoSt manufacturer data for assays used in clinical settings, and variabilities in sample bias are also observed at the end user level. **Conclusion:** The CDC CSP provides comprehensive standardization programs for manufacturers, research, and clinical laboratories. The HoSt provides unique information that is not offered through other EQA or PT programs. Programs show improvements in calibration, but problems with individual samples, especially at low concentrations, still exist.

A-213

Parathormone Reference Interval Should be Defined by the Age

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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. **Objective:** To define RI for PTH in the Brazilian population and assess if different RI should be defined by age. **Methods:** Data of PTH measurements in Roche platform were collected through a retrospective big data laboratory approach. Subjects age over 20 years (y) were inlcuded. Data collection was from 11/04/2019 to 10/15/2020. 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, and vitamin D <30 ng/mL. The RI provided by the manufacturer is from 15 to 65 pg/mL. The central 95% PTH levels were considered for determination of RI. The level of statistical significance was p <0.05. **Results:** Thousand seven hundred and ninety nine subjects were selected, 1068 (59,3%) female (F). PTH RI was statistically different according to age and gender. PTH RI results in pg/mL are in the teble. **Conclusion:** Although the serum PTH IR in the whole group agree with those provided by the manufacturer, lower results are observed in young subjects and higher values in older subbjects. These findings justify that the PTH laboratory reports contain specific IRs for age and gender.

PTH Data for Reference Interval by age

		Age range (years)			
	Gender	20 - 39	40 - 49	50 - 59	>=60
Mean age	F	32.5	44.6	54.5	71.0
	M	33.3	43.9	54.7	69.9
Mean PTH	F	30.1	33.2	36.7	39.1
	M	26.9	32.0	31.3	35.9
p value between gender		0.02	0.12	<0.01	0.02
Minimum PTH	F	10.4	11.9	12.8	12.7
	M	8.4		6.4	11.8
Maximum PTH	F	76.2	77.1	85.7	92.4
	M	65.8		58.9	82.8
25th percentile PTH	F	22.4	24.0	25.7	28.0
	M	19.4		21.6	25.7
75th percentile PTH	F	37.0	38.4	45.2	47.1
	M	31.9		38.4	42.9
Lower Limit 95% CI	F	27.1	29.6	32.4	34.9
	M	24.0		29.3	31.5
Upper limit 95% CI	F	29.5	31.7	35.9	37.7
	M	26.3		33.3	35.4
PTH reference interval	**F**	**14.0 – 57.1**	**15.5 – 60.7**	**16.0 – 72.9**	**17.3 – 76.0**
	M	**12.2 – 52.0**		**10.4 – 52.3**	**15.8 – 70.5**

A-214

Evaluation of Sensitive Analytes to Hemolysis Interference on the Beckman Coulter DxC 700 AU Chemistry Analyzer

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Background: Hemolysis is a common reason for specimen rejection in the chemistry lab. Most automated chemistry platforms define hemolysis (H) flag limits in assay package inserts. Our experience suggested that these flags are too strict for some analytes leading to unnecessary specimen rejections. This study summarizes hemolysis flags for commonly rejected analytes based on patient specimens on the Beckman Coulter DxC 700 AU chemistry analyzer. **Methods:** We evaluated analytes with low limit H flags and high rejection rates. These included: AST, ALT, iron (IRN), potassium (K), direct bilirubin (DBIL), magnesium (Mg), amylase (AMY), sodium (Na), GGT, phosphorus (PO4), albumin (ALB), alkaline phosphatase (ALKP), and LD. Five patient plasma pools were made from 50 patient specimens. Neat pools were analyzed to establish baseline analyte concentrations. A hemolysate was created by diluting whole blood with distilled water. Each analyte was tested after spiking with the hemolysate to specific hemoglobin concentrations corresponding to H flags. Percent differences were calculated between baseline pool means and each flag's pool mean. Acceptance limits were based upon the average of the 2019 CLIA and method precision limits as a conservative approach. Percent differences greater than the acceptance limits were considered significant. These were compared to the manufacturer's defined limits.

Results: This study proved that the manufacturer's defined hemolysis flags can be updated to greater than 1+ for Na, K, and AST, greater than 3+ for ALKP, and greater than 4+ for AMY and Mg. No changes were noted for the remaining analytes. These data are shown in the table.

Conclusion: Based upon this study, the hemolysis criteria set for ALKP, AMY, AST, Mg, K, and Na were updated in the Remisol Advance middleware data management software that lead to a 45% reduction in rejected hemolyzed specimens.

% Differences by Flag and Manufacturer's Defined Compared to Adjusted Flags									
	Flag 1	Flag 2	Flag 3	Flag 4	Acceptance Limit**	Manufacturer's Defined Hemoglobin (mg/dL)/Flags		Adjusted Hemoglobin (mg/dL)/ Flags	
Albumin	-1.9%	-2.5%	-0.9%	-1.8%	5.5%	450	≥4+	450	≥4+
ALKP	-3.9%	-4.1%	-2.8%	-7.9%	6.0%	450	≥4+	300	>3+
ALT	1.1%	-3.3%	-2.3%	0.0%	9.5%	500	≥4+	500	≥4+
Amylase	-5.4%	-4.7%	-4.4%	-4.0%	6.5%	250	≥3+	400	>4+
AST	9.3%	11.0%	6.9%	11.4%	10.0%	No H	≥1+	100	>1+
Direct Bilirubin	-43.5%	-61.5%	-10.0%	22.2%	6.9%	10	≥1+	10	≥1+
GGT	-4.8%	-3.1%	-3.2%	-0.7%	9.0%	350	≥4+	400	>4+
Iron	-7.1%	-16.1%	-5.9%	-25.4%	6.0%	No H	≥1+	No H	≥1+
LD	22.4%	23.9%	10.6%	249.2%	9.0%	No H	≥1+	No H	≥1+
Magnesium	0.0%	2.0%	-1.0%	0.0%	8.8%	150	≥2+	400	>4+
Phosphorus	-1.1%	-1.1%	0.6%	-2.7%	3.0%	350	≥4+	350	≥4+
Potassium	5.0%	4.0%	2.0%	6.0%	6.0%	70	≥1+	100	>1+
Sodium	-1.0%	-2.0%	-2.0%	-3.0%	6.0%	250	≥3+	100	>1+

**Acceptance limits are based on the average of method precision and CLIA limits

A-215

Evaluation of a screening test for urine myoglobin

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Background: Detection of urine myoglobin may help in the differential diagnosis of myopathies such as rhabdomyolysis. We evaluated a procedure in our laboratories that was historically used to screen for urine myoglobin. This test involved visual macroscopic urinalysis for blood pre- and post- centrifugation of a urine sample suspected of containing myoglobin. If urinalysis for blood pre-centrifugation was positive (≥ trace), but post-centrifugation was negative, the test result was negative for myoglobin as intact red blood cells were likely responsible for the pre-centrifugation positive result. If myoglobin was present, positivity would persist post-centrifugation. However this could also occur if free hemoglobin or any other soluble pigment with peroxidase activity was present pre- and post- centrifugation. We therefore hypothesized that this test was no better than a simple instrument-read macroscopic urinalysis for blood. Our objective was to determine the diagnostic performance of this test for a positive macroscopic urinalysis for blood, and to compare diagnostic performance of each test for elevated plasma creatine kinase (CK) - an indicator of rhabdomyolysis. **Methods:** We retrieved urine myoglobin screen test results over one year (n=645 patients) from the laboratory information system. Among these, 464 patients (72%) had a macroscopic urinalysis on the same sample. To this data we linked plasma creatine kinase results that were verified +/- 4 days from myoglobin screen/urinalysis test date.

We calculated the sensitivity, specificity, and diagnostic efficiency of the myoglobin screen for a positive macroscopic urinalysis result for blood (≥trace). We also contrasted the same parameters of the myoglobin screen vs macroscopic urinalysis for blood predicting a plasma CK > 1000 U/L and > 10 000 U/L, which are suggestive of rhabdomyolysis. **Results:** The sensitivity of the myoglobin screen for a positive macroscopic urinalysis for blood was 90% whereas specificity was 88%. Diagnostic efficiency was 89%. The sensitivity, specificity and diagnostic efficiency of the myoglobin screen for a plasma CK > 1000 were 70%, 50% and 58% whereas for macroscopic urinalysis for blood they were 80%, 43% and 59%. For a CK > 10 000, the sensitivity, specificity and diagnostic efficiency of the myoglobin screen were 89%, 46%, and 50% whereas for macroscopic urinalysis for blood they were 89%, 36%, and 42%. **Conclusions:** Given the high sensitivity and specificity of the screening test for a positive macroscopic urinalysis for blood, and that each test had similar diagnostic performance for elevated CK, the urine myoglobin screening test was discontinued in favor of macroscopic urinalysis for blood.

A-216

Vitamin D metabolite concentrations in blood-cerebrospinal fluid and serum in relation to bloodcerebrospinal fluid barrier function

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Background: Vitamin D, an established regulator of calcium and phosphate metabolism, has important functions in the central nervous system (CNS). The impact of blood-cerebrospinal fluid (CSF) barrier (BCB) integrity on vitamin D metabolite concentrations in CSF was not investigated until now. This study analyzed vitamin D metabolite concentrations in serum and CSF in relation to BCB function.**Methods:** In 292 pairs of serum and CSF samples the concentrations of 25(OH)D and $24,25(OH)_2D_3$ were measured with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). CSF/serum ratios ($Q_{25(OH)D}$, $Q_{24,25(OH)2D3}$) were calculated. BCB function was assessed by the CSF/serum ratio of albumin (Q_{ALB}). The efficacy of the vitamin D metabolite transport across the BCB was studied by correlating the serum concentrations of both metabolites with the respective CSF/serum ratio. **Results:** Median (IQR) serum concentrations of 25(OH)D and $24,25(OH)_2D_3$ were 63.8 (43.4-83.9) nmol/L and 4.2 (2.2-6.2) nmol/L, respectively. The CSF concentrations of both metabolites were 3.7 and 3.3 % of the serum concentrations. Low serum 25(OH)D concentrations were associated with higher $Q_{25(OH)D}$ and $Q_{24,25(OH)2D3}$. In 117 patients with BCB dysfunction, the CSF concentrations of all vitamin D metabolites were higher than in 175 individuals with intact BCB. Furthermore, these patients showed stronger associations between $Q_{25(OH)D}$ and Q_{ALB} as well as $Q_{24,25(OH)2D3}$ and Q_{ALB} (β-coefficients 0.847 and 0.866; p-values < 0.001). **Conclusion:** The CSF concentrations of 25(OH)D and $24,25(OH)_2D_3$ depend on BCB function and the respective serum concentrations of both metabolites. With decreasing 25(OH)D serum concentrations the transport across the BCB becomes more efficient. Higher vitamin D metabolite concentrations in CSF of patients with impaired BCB function may be due to passive diffusion across the BCB.

A-217

Voxelotor does not Interfere with Hemoglobin S Quantitative Measurement on the Tosoh G8 HPLC Analyzer in Hemoglobin A1c Mode

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Background: In 2019, voxelotor (Oxbryta, Global Blood Therapeutics) was approved in the United States for the treatment of sickle cell disease in adults and pediatric patients aged ≥ 12 years. Several studies have shown voxelotor interfered with quantitative and qualitative hemoglobin variant analysis on the BioRad Variant II HPLC system in β-thalassemia short program and on the Sebia Capillarys 2 Flex-piercing Hemoglobin program. At our hospital, the Tosoh G8 HPLC analyzer in A1c mode is used to monitor Hemoglobin S (HbS) percentage as a laboratory developed test for patients who need urgent red blood cell exchange apheresis. The objective of this study is to examine whether voxelotor interferes with HbS measurement on the Tosoh G8 HPLC analyzer.

Methods: Six residual whole blood specimens collected in lavender top tubes containing EDTA anticoagulant were used for this study. These six samples were from patients with either sickle cell trait or patients with sickle cell disease before and after transfusion. The HbS percentage of these samples ranged from 24.6% to 85.5%. Each specimen was diluted with normal saline to make two 1-ml aliquots of dilutions with final hematocrit of 20%. One 1-ml aliquot was mixed with 20 µl of dimethyl sulfoxide (DMSO) as a control sample, and the other 1-ml aliquot was mixed with 20 µl of 50 mM voxelotor dissolved in DMSO. Both aliquots were incubated at 37°C for 2 hrs and then analyzed by the Tosoh G8 HPLC analyzer in A1c mode.

Results: Chromatographically, HbA_0 and HbS peaks showed no remarkable change, whereas HbF peak was flattened or mis-shaped in samples treated with voxelotor. The HbS results of samples incubated with voxelotor (y) showed an excellent correlation with the results of control samples (x) in Deming regression analysis: y (voxelotor) = 0.983x (control) + 1.03%, R=0.9996, SEE=0.63%. The biases ranged from -0.5% to 1.3%.

Conclusion: Voxelotor does not interfere with HbS quantitative measurement on the Tosoh G8 HPLC analyzer in A1c mode. HbF peak is mis-shaped after voxelotor treatment. This method may be used for HbS quantitation for sickle cell disease patients treated with voxelotor if other platforms are unable to perform the test due to voxelotor interference.

A-218

Assessment of seroconversion after SARS-CoV-2 vaccination in elderly: evaluation of four different immunoassays.

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Background: Vaccination against SARS-CoV-2 is potentially the most effective approach to curb the advancement of COVID-19, however, data on the appearance of specific antibodies after vaccination are still scarce. The aim of the present study was to evaluate seroconversion after administration of the Sinovac vaccine, using 4 different immunoassays to assess the presence of antibodies against SARS-CoV-2, in a group of institutionalized elderly in South Brazil.

Methods: We included 51 institutionalized elderly who underwent complete vaccination regimen with two doses of COVID-19 Adsorptive Vaccine (Inactivated) CoronaVac, imported by the Butantan Institute (São Paulo, Brazil), manufactured by SINOVAC LIFE SCIENCES CO., LTD. (Beijing, China). The application interval between each dose was 23 days, administered intramuscularly. None of the elderly had ever been diagnosed with COVID-19 prior to vaccination.

Blood samples were collected 47 days after the second dose and analyzed by the following assays: SARS-CoV-2 Total antibodies (IgM/IgG) (electrochemiluminescent immunoassay, Nucleocapsid (N) target protein, Cobas-Roche, Mannhein, Germany); SARS-CoV-2 isolated IgG antibody (chemiluminescent immunoassay, Nucleocapsid N and Spike S1+S2 target proteins, Snibe, Maglumi, Shenzhen, China); anti-S-RBD IgG antibody (electrochemical chemiluminescent immunoassay, Snibe, Maglumi, Shenzhen, China); and SARS-CoV-2 neutralizing antibodies (functional competitive enzyme immunoassay, target protein of the Spike S1 RBD termination of the original conjugated Wuhan strain, Genscript, Nanjing, China) - all according to manufacturer instructions. The 20% signal inhibition cuttoff was used for neutralizing antibodies according to the manufacturer's instructions approved by the Brazilian Health Regulatory Agency (Anvisa). The observed agreement criterion between assays was used for statistical analysis.

Results: In the group of study, there were 26 male (51%), the mean (±SD) age was 76.4 (±9.95) years. Total antibodies (IgM/IgG), isolated IgG antibody, specific anti-S-RBD IgG antibody and neutralizing antibodies against SARS-CoV-2 were detected in 7 (13.7%), 16 (31.4%), 45 (88.2%) and 43 (84.3%) individuals, respectively. The mean signal inhibition for positive results in neutralizing antibodies assay was 44%. The best agreement between different immunoassays was observed for IgG anti-S-RBD antibodies and neutralizing antibodies (84.3% general agreement). The 3 patients that were positive for neutralizing antibodies but negative for anti-S-RBD antibodies were all between 20-30% signal inhibition. There were also 5 patients that were positive for anti-S-RBD IgG, but negative for neutralizing antibodies. When adopting the criteria of 30% signal inhibition (as used in USA), the seroconversion for neutralizing antibodies fell to 35 (68.6%) and general agreement between this assay and anti-S-RBD IgG fell to 39 (76.5%).

Conclusion: Conventional assays (mainly based in N-protein or N+S protein) showed a low rate of seroconversion after CoronaVac vaccination, in concordance with previous publications. Both neutralizing antibodies and anti-S-RBD assays showed increased rates of seroconversion and the best agreement between assays.

A-219

Sex-specific absolute delta thresholds as potential sex-specific cutoff for high-sensitivity cardiac troponin T assay

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Background: Universal definition of acute myocardial infarction (AMI) requires the detection of a rise and/or fall of a cardiac biomarker, preferably high-sensitivity cardiac troponin (hs-cTn). Open questions regarding the hs-cTn algorithms include the optimal delta changes of the rise and/or fall pattern and whether sex-specific cut-offs improve the diagnosis of patients with possible AMI. Current sex-specific cut-offs of hs-cTn assays focus on the use of sex-specific 99th percentiles. This study tested the hypothesis of using sex-specific delta thresholds to improve the diagnostic performance and determined the optimal delta thresholds of hs-cTn T assay for males and females to rule in AMI in patients presenting to the emergency department (ED) with acute symptoms. **Methods:** This retrospective cohort study included 18,056 ED cases (male 9524, female 8523) who had serial hs-cTn T measured using a 0h/3h algorithm at a US medical center. The primary outcome was AMI diagnosis at discharge. Baseline and delta hs-cTn T levels were compared between male and female patients. Receiver operating characteristic (ROC) analysis was performed in males and females and assay performance was quantified at different delta thresholds. Threshold analysis was also performed in an external acute coronary syndrome (ACS) cohort as a validation dataset. Statistical analysis was performed using the R computing environment. **Results:** 42% of all cases with serial hs-cTn T testing presented with baseline hs-cTn T above the 99th percentile, which highlighted the importance of dynamic changes to predict AMI. Males had significantly higher baseline (male mean 52.4 ng/L, female mean 34.1 ng/L, p<0.0001) and absolute delta hs-cTn T (male mean 12.8 ng/L, female mean 10.0 ng/L, p<0.0001) than females in non-AMI patients, but not in AMI patients. This difference warrants a sex-specific delta threshold. ROC analysis showed that absolute delta thresholds (male AUC 0.9480, female AUC 0.9526) is superior to percentage delta thresholds (male AUC 0.8765, female AUC 0.8749) in ruling in AMI. Sex-specific absolute delta thresholds of 10 ng/L for males and 7 ng/L for females performed best based on Youden's J index (male 0.7915, female 0.7923). This resulted in a sensitivity and specificity of 91.7% and 87.4% in males, and 92.4% and 86.8% in females respectively. When a higher specificity is desired, delta thresholds of 14 ng/L for males and 11 ng/L for females reached a minimum of 90% specificity (male 90.7%, female 91%), with a sensitivity of 87% for males and 85.4% for females. The sex-specific delta thresholds was confirmed in the external ACS validation dataset. **Conclusion:** With a significant number of ED patients presented with baseline hs-cTn T above the 99th percentile, delta changes become important in ruling in AMI. Sex-specific absolute delta thresholds improve classification performance and are robust across different study populations. Further study is needed to assess whether these sex-specific thresholds impact outcomes in male and female AMI patients.

A-220

Re-assessing the pituitary thyrotropin-free thyroxine relationshipusing the Abbott Architect immuno-chemiluminometric assays.

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Background: Current concepts of thyroid testing based on thyrotropin (TSH) first is contingent on the notion that pituitary thyrotrophs are exquisitely sensitive to small changes in free thyroxine (fT4). The 3rd generation immunochemiluminometric assay (ICMA) for TSH introduced in 1990 demonstrated that a 2-fold increase in fT4 (reflected by fT4 index) is accompanied by a 160-fold reduction in TSH. This inverse log/linear relationship has been confirmed with several different assays. We have re-evaluated this TSH-fT4 relationship using a contemporary ICMA (Architect, Abbott) capable of reliably measuring low TSH (0.004mU/L) and low fT4 (5.5pmol/L).**Methods**: All initial outpatient samples with requests for both TSH and fT4 received by the immunoassay section were recruited from the Laboratory Information System (LIS). Where a patient had more than one result the earliest values were selected (n=437) to ensure that the values were probably at their steady state. Samples with results below the detection limit of TSH (n=61) and fT4 (n=16) were excluded. Our reference interval for TSH is 0.40-4.0mU/L and for fT4 is 10.0-20.0pmol/L. The relationship of TSH and fT4 was examined by regression analysis using MedCalc Statistical Software v19.8 (Ostend, Belgium). **Results**: There were 360 pairs of TSH and fT4 results comprising 276 euthyroid (TSH 0.42-3.96mU/L, fT4 10.1-19.0pmol/L), 11 hyperthyroid (TSH 0.005-0.36mU/L, fT4 11.6-28.2pmol/L) and 73 hypothyroid (TSH 10.6-139mU/L, fT4 5.6-9.9pmol/L) values. A significant inverse log/linear relationship between serum TSH and fT4 (log TSH = 2.559 - 0.182 fT4; r = -0.78) was apparent (see figure). From this derived statistical relationship a 2-fold increase in fT4 from 14 to 28pmol/L would be expected to be accompanied by an approximate 350-fold reduction in TSH from 1.03 to 0.0029mU/L. **Conclusion**: Our study reaffirms the sensitivity of pituitary thyrotroph to minor changes in circulating fT4 and underscores the utility of TSH in the evaluation of thyroid disorders.

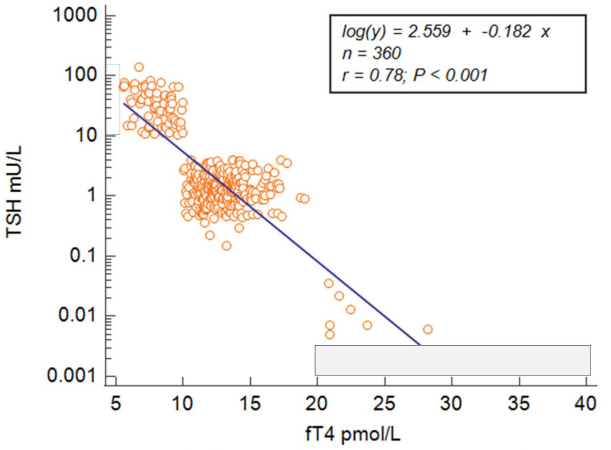

Figure. Relationship between Abbott TSH & fT4

Hematology/Coagulation

A-222

Thromboelastometry as guidance for blood management in patients undergoing cardiac surgery: study of postoperative complications.

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Background: Perioperative coagulopathy and postoperative bleeding are the most common complications in patients undergoing cardiac surgery, especially when the cardiovascular surgery is associated with cardiopulmonary bypass (CPB).In this context, some studies suggest that implementation of viscoelastic point-of-care tests (POCT), such as rotational thromboelastometry (ROTEM®), in conjunction with a specific algorithm for coagulation management, allow for better control of hemostatic pathology. **Methods:** Retrospective cohort study including 675 patients who underwent cardiac surgery with cardiopulmonary bypass. The incidence of clinical postoperative complications were analyzed before and after ROTEM® implementation. **Results:** Following viscoelastic testing and the implementation of a specific algorithm for coagulation management, the incidence of any allogeneic blood transfusion decreased (41.4% vs 31.9%, p=0.026) during the perioperative and postoperative period (26.5% vs 19.2%, p=0.061).In addition, significant reductions were detected in the incidence of heart disease (57.7% vs 55.8%, p=0.275; statistically significant reductions were detected in the incidence of postoperative pericarditis (3.6% vs 1.2%, p=0.043), postoperative renal failure (1.6% vs 3.2%, p=0.435), postoperative sepsis (1.2% vs 0.9%), p=0.337) and postoperative hematologic complications (postoperative bleeding (9.5% vs 5.3%, p=0.037), surgical reexploration (6.0% vs 2.9%, p=0.035).and length of Intensive Care Unit (ICU) stay (6.0 days vs 5.3 days, p=0.026). Finally, we observed a statistically significant decrease in the lengths of Intensive Care Unit (ICU) stay (6.0 days vs 5.1 days, p=0.026), after implementation of the POCT system and the specific algorithm for coagulation management. There were no statistically significant group differences with respect to total hospital stay (16.7 days vs 13.5 days, p=0.076). In-hospital mortality associated with cardiac surgery also did not change (4.5% vs 2.4%, p=0.132). **Conclusion:** The monitoring of hemostasis by ROTEM® in cardiac surgery, was associated with decreased incidence of allogeneic blood transfusion, clinical postoperative complications and lengths of hospital and ICU stay.

A-223

Validation of Hematology Parameters in NHP, Dog, Rat and Mouse Blood Samples on the ADVIA® 2120i

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Objective: As part of the GLP regulation, it is essential to do the validation of the methods or techniques to ensure the reliability and accuracy of the laboratory analysis before being used in the laboratory environment. Therefore, in compliance with GLP regulations, we went through the validation of hematology parameters to prove the reliability of method and to verify the obtained results before performing the hematology test of species of interest in a new hematology instrument. In this study we have validated the hematology parameters from four species including NHP, dog, rat and mouse blood samples in the Advia 2120i.

Methodology: A total of 18 hematological parameters (RBC, HGB, MCV, HDW, CHr, CHDW, CH, CHCM, WBC, PLT, MPV, RETIC, NEUT, LYMPH, MONO, EOS, BASO and LUC) were validated in animal species NHP, Dog, Rat and Mouse. In order to carry out the validation, qualification testing including intra-assay, inter-assay, accuracy, linearity and reference interval were performed. For intra-assay, blood samples collected in K2EDTA and three levels of control (Abnormal 1, Normal, Abnormal 2) were analyzed 10 consecutive times (one replicate) within a single run for each parameter. For inter-assay, three levels of control were assayed on at least six different runs (one replicate per run) over a minimum of 10 days. Accuracy was calculated using the data obtained from Inter-assay. Linearity was performed for RBC, HGB, WBC and PLT making 7 different concentrations of samples from 0% to 100% for each of these parameters. Finally, Reference Interval for each parameter was calculated by analyzing at least 21 different samples for each species.

Results: Results show that the hematology parameters met the acceptance criteria for intra-assay, inter assay and accuracy for all four species. For intra-assay, all hematology parameters had %CV 1/3 to 1/2 of the Total allowable Error (TEa) or less than or equal to 15%. For inter-assay and accuracy all hematology parameters had Total observable Error (TEobs) less than TEa (TEobs<TEa) or %CV was less than or equal to 15%. Linearity was accepted since % bias for RBC and HGB was < 10%, WBC was < 25% and PLT was < 25%. Therefore, RBC, HGB, WBC and PLT were all linear for NHP, dog, rat and mouse. Additionally, reference interval for all parameters were measured.

Conclusion: All hematology parameters met the acceptance criteria for each qualification testing for all four species. Since all these parameters were validated for NHP, dog, rat and mouse species, the new Advia 2120i can be used for preclinical studies.

A-224

Assessment of the analytical performance of the Abbott Alinity hq by Sigma metrics

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Background Sigma metrics estimates the quality of laboratory tests based on the allowable total error (TEa) goal for a measurand, and the imprecision and bias of the process. Sigma metrics allows for visualization of performance as well as planning and optimization of QC rules and number of control measurements. The goal of this study was to assess the analytical performance of the Abbott Alinity hq hematology analyzer on the Sigma scale. **Methods**Total imprecision was obtained by calculating the average weighted %CV over a 5-month period, across three control lots at three levels (low, normal and high) according to the method described in CLSI H26-A2. Bias was obtained by comparing results for 991 patient samples between the Abbott Alinity hq and Abbott CELL-DYN Sapphire. Sigma statistics were calculated for 13 CBC and WBC differential parameters at three levels (39 assessments), using clinically relevant TEa targets derived from either biological variation data or from proficiency testing (CLIA) goals.**Results** All 29 reportable parameters demonstrated state-of-the-art total imprecision. Sigma values ranged from 3.2 (monocytes, normal level) to 12.4 (platelets, high level) for 32 out of the 39 measurands/levels. A Sigma value of < 3 was obtained for monocytes, eosinophils, hematocrit and mean platelet volume for the low control only, and for basophils at all three levels. Low cell count and associated higher %CV was the likely reason behind < 3 Sigma performance for monocytes (0.32 x 10^9/L), eosinophils (0.07 x 10^9/L) and basophils (0.03-0.14 x 10^9/L). Technological differences (optical vs impedance volume measurement) resulting in between-analyzer bias might have impacted mean platelet volume and hematocrit results.

Measurand	Average weighted %CV(Low-Normal-High Control)	TEa	TEa source	Sigma value (Low-Normal-High)
WBC	2.7% - 1.8% - 1.3%	15%	CLIA	5.3 - 7.8 - 11.2
RBC	0.9% - 0.8% - 1.3%	6%	CLIA	5.2 - 7.3 - 4.6
Hemoglobin	0.8% - 0.6% - 0.5%	7%	CLIA	5.0 - 9.5 - 12.3
HCT	1.1% - 1.1% - 1.6%	6%	CLIA	1.0 – 3.6 – 4.1
MCV	0.6% - 0.6% - 0.7%	2.4%	Des BV*	3.7 – 3.8 - 3.3
PLT	1.8% - 2.2% - 3.4%	25%	CLIA	8.3 - 11.4 - 12.4
MPV	2.3% - 1.2% - 0.7%	5.8%	Des BV*	1.6 – 3.4 – 5.3
NEU	4.1% - 2.8% - 2.1%	22.4%	Des BV*	5.8 – 8.3 – 11.2
LYM	4.2% - 3.1% - 2.4%	16.0%	Des BV*	4.2 – 5.8 – 7.4
MONO	8.3% - 5.5% - 4.5%	27.9%	Des BV*	2.1 – 3.2 – 4.0
EOS	13.7% - 8.7% - 6.2%	37.1%	Des BV*	2.7 - 4.3 – 5.9
BASO	37.2% - 17.9% - 13.4%	38.5%	Des BV*	0.5 – 1.0 – 1.3
Reticulocytes	2.8% - 2.2% - 2.0%	16.5%	Des BV*	6.7 - 5.8 - 3.6
* Desirable total error based on Ricos biological variation database (2014)				

ConclusionSatisfactory to excellent Sigma metrics performance was obtained for most reportable parameters with the Alinity hq hematology analyzer, meeting the quality needs of today's laboratories.

A-225

Equivalent comparative performance by Abbott Alinity hq, CELL-DYN Ruby and CELL-DYN Emerald 22 AL

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Background: Automated hematology analyzers utilize a range of technologies, including impedance, optical and advanced optical with fluorescence flow cytometry. It is important to correlate complete blood count (CBC) results in laboratories using multiple analyzers and healthcare networks with multiple testing locations. We performed a side-by-side comparison of CBC parameters by three hematology analyzers from Abbott, which are used in low to high volume laboratories

Methods: Six hundred thirty-one blood samples were tested in the laboratory of the General Hospital of Bjelovar (Croatia). CELL-DYN Ruby utilizes Multi-Angle Polarized Scatter Separation (MAPSS™) technology, based on optical flow cytometry and four light detectors; Alinity hq uses an advanced version of the MAPSS technology with seven optical detectors plus fluorescent flow cytometry; and CELL-DYN Emerald 22 Autoloader (E22 AL) uses UNI-FLOW optical technology for white blood cell (WBC) differential along with electrical impedance for WBC, red blood cell (RBC), and platelet (PLT) counts. Results were analyzed with Pearson correlation, Passing Bablok, or Deming regression. Alinity hq WBC differential was compared to microscopic differential as well. **Results:** Excellent correlation and agreement were demonstrated for all RBC, WBC, and PLT parameters, with few exceptions (Table-01). Measurands that showed lower correlation were red cell distribution width (RDW) and mean platelet volume (MPV), which are known to be technology dependent, and %basophils, which are known to vary between analyzers. Comparison of Alinity hq WBC differential to manual differential demonstrated excellent correlation for %neutrophils and %eosinophils (r=0.96), %lymphocytes (r=0.97), moderate correlation for %monocytes (r=0.81) and weak (but reasonable) correlation for %basophils (r=0.40). **Conclusion:** All three analyzers demonstrated good concordance for CBC and WBC differential parameters and are well suited to be used in network laboratories where equivalent results across sites are required. MPV and %basophils showed the best correlation between Alinity hq and Cell-DYN Ruby, which use similar technology.

Table 1. Inter-Instrument Method Comparison Results

Measurand	Range tested by Alinity hq		Alinity hq vs CELL-DYN Ruby	Alinity hq vs E22 AL	CELL-DYN Ruby vs E22 AL	Alinity hq vs CELL-DYN Ruby	Alinity hq vs E22 AL	CELL-DYN Ruby vs E22 AL
	Min	Max	Pearson's r			Slope		
RBC (x 10^{12}/L)	2.40	7.90	0.99	0.99	0.99	1.00	0.98	0.97
MCV (fL)	48.60	110.00	0.98	0.97	0.98	0.96	1.03	1.06
RDW-CV (%)	9.40	33.20	0.92	0.93	0.93	0.97	0.74	0.77
MCH (pg)	17.10	37.90	0.97	0.98	0.98	1.11	1.09	0.98
HCT%	21.35	60.40	0.99	0.99	0.99	0.97	0.90	0.92
HGB (g/dL)	7.15	18.50	0.99	0.99	1.00	1.04	1.00	0.96
PLT (x 10^9/L)	27.30	1483.00	0.99	0.99	0.99	0.91	0.97	1.06
MPV (fL)	5.63	12.10	0.93	0.87	0.86	1.40	0.86	0.60
WBC (x 10^9/L)	1.52	61.00	0.99	0.99	0.99	1.04	1.04	1.01
%NEU	0.74	97.17	0.99	0.99	0.99	1.01	0.99	0.97
%LYM	1.18	97.40	0.99	0.98	0.99	1.01	1.01	1.00
%MONO	0.16	39.90	0.94	0.96	0.92	0.91	0.91	0.99
%EOS	0.00	67.00	0.99	0.98	0.98	0.98	0.91	0.93
%BASO*	0.00	1.98	0.58	0.01	-0.10	1.55	0.02	0.14
*Deming Fit								

A-226

Machine Learning Based Decipherment of Cell Population Data; a Promising Hospital Front-Door Screening Tool for COVID-19

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Background: Key challenges against early diagnosis of COVID-19 are its symptoms sharing nature and prolong SARS-CoV-2 PCR turnaround time. Hither machine learning (ML) tools experienced by routinely generated clinical data; potentially grant early prediction.**Methods:** Routine and earlier diagnostic data along demographic information were extracted for total of 21,672 subsequent presentations. Along conventional statistics, multilayer perceptron (MLP) and radial basis function (RBF) were applied to predict COVID-19 from pre-pandemic control. Three feature sets were prepared, and performance evaluated through stratified 10-fold cross validation. With differing predominance of COVID-19, multiple test sets were created and predictive efficiency was evaluated to simulate real-fashion performance against fluctuating course of pandemic. Models validation was also inducted in prospective manner on independent dataset, equating framework forecasting to conclusions from PCR.**Results:** RBF model attained superior cross entropy error 20.761(7.883) and 20.782(3.991) for Q-Flags and Routine Items respectively while MLP outperformed for cell population data (CPD) parameters with value of 6.968(1.259) for 'training(testing)'. Our CPD driven MLP framework in challenge of lower (<5%) COVID-19 predominance affords greater negative predictive values (NPV >99%). Higher accuracy (%correct 92.5) was offered during prospective validation using independent dataset. Sensitivity analysis advances illusive accuracy (%correct 94.1) and NPV (96.9%). LY-WZ, Blasts/Abn Lympho?, 'HGB Interf?', and 'RBC Agglutination?' are among novel enlightening study attributes.**Conclusion:** CPD driven ML tools offer efficient screening of COVID-19 patients at presentation to hospital to backing early expulsion and directing patients' flow-from amid the initial presentation to hospital.

A-227

Iron Status Testing in Ottawa, Canada, 2014-2019

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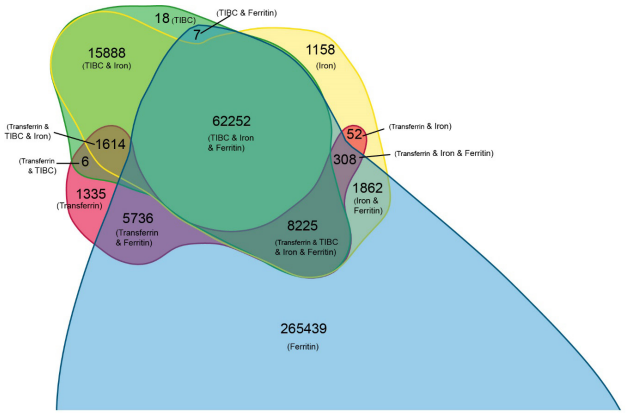
Background: The laboratory diagnosis of reduced or increased iron stores is problematic as iron markers are influenced by chronic disease and inflammatory disorders. Ontario Health has recommended ferritin testing alone for iron deficiency. We examined 5 years of Ottawa Hospital serum/plasma iron, total iron binding capacity (TIBC), ferritin, and transferrin utilization data. **Methods:** We obtained de-identified Ottawa Hospital inpatient and outpatient ferritin, iron, TIBC, transferrin, date and time of their sampling and patient age. Same day tests were identified and grouped into discrete test sets and presented as a Venn diagram to compare and evaluate iron test utilization. Linear regression was used to demonstrate the equivalence of transferrin and TIBC. We tested our hypothesis that transferrin and TIBC were used interchangeably when ordered with ferritin by comparing the relative cumulative frequency distributions if ferritin levels when ordered 1) either separately or either with 2) transferrin and iron or 3) TIBC and iron or 4) TIBC and iron and transferrin. **Results:** The Venn diagram (Figure) shows the 15 test combinations used to investigate iron status. 76.7% of ferritins were ordered alone, 18% were ordered in combination with TIBC and iron. The ferritin frequency distribution of ordered alone, or in combination with other iron markers were very similar. Moreover, linear regression demonstrated that transferrin level can be determined from TIBC with high accuracy. **Conclusions:** TIBC should be substituted for transferrin, saving estimate $31K (USD) per year. TIBC and iron are recommended for patients with high ferritin, eliminating these tests when ferritin is ≤ 100 µg/L can save estimate $89K (USD) per year. In patients without inflammatory disorders or with low to moderate inflammation, ferritin can be used to assess iron status. To influence more efficient testing for iron status, Venn diagrams of iron utilization can be shared with the appropriate care groups.



Venn diagram showing utilization of various iron test combinations at Ottawa Hospital, 2014-2019

A-228

Peripartum Reference Intervals for Coagulation Parameters Derived in a Healthy, Multicultural Cohort of Mothers as part of the Pregnancy Reference Intervals for Safe Medicine (PRISM) Study

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Background: Reference intervals (RIs) are derived from testing a representative healthy population and should be established by individual labs. However, this undertaking is not routinely carried out due to its resource intensiveness. It is well known that there are changes to coagulation parameters throughout pregnancy and using non-pregnant adult RIs may impede clinical decision making during maternity care-especially around the time of parturition. Accurate RIs applicable to modern methodologies and ethnically diverse Canadian pregnant women are lacking. Therefore, we aimed to recruit healthy, term, peripartum women to donate blood samples for establishing RIs for commonly ordered lab investigations, including coagulation tests.

Methods: Healthy mothers with singleton pregnancies delivering at BC Women's Hospital were recruited antenatally in the community or at the time of admission for induction, scheduled Caesarean section or labour, as part of the Pregnancy Reference Intervals for Safe Medicine (PRISM) study. Blood was collected primarily by nurses during IV start and included a citrate tube. Plasma was frozen and batch tested for aPTT, PT, fibrinogen, D-dimer, Factor VIII, von Willebrand factor antigen and activity, and Protein S on a Siemens BCS XP analyzer. Eligibility was assessed by participant ante- and post-partum health questionnaires and chart reviews. RIs were calculated using Microsoft Excel and MedCalc (v19.2) software in alignment with CLSI EP28-A3c guidelines. Partitioning between labouring and non-labouring stages was assessed by the Harris and Boyd method. When data did not have a normal distribution and could not be transformed, a modified Freedman-Diaconis (mFD) method was used for outlier detection, otherwise, the Tukey method was used. Non-parametric lower 2.5% and 97.5%tile limits with associated confidence intervals were calculated for partitions with more than 120 data points, otherwise, the robust method of Horne and Pesce was used.

Results: A total of 430 consented participants donated blood over 13 months and 424 (99%) self-reported ethnicity which showed a distribution (34% Caucasian, 24% Chinese, 8% Filipino, 8% South Asian, 5% South East Asian) similar to the 2016 Metro Vancouver census data. Only 196 (46%) participant samples remained eligible for inclusion in RI calculations. Reasons for exclusion included maternal health, delivery complications, or missing or hemolyzed samples. No tests required partitioning into labour versus non-labouring cohorts. Our peripartum cohort had higher fibrinogen, factor VIII, von Willebrand factor antigen and activity, and D-dimer levels, and

shorter aPTT, PTs, and Protein S antigen levels compared to non-pregnant adult RIs. Additionally, RIs were similar for tests when both Tukey and mFD outlier detection methods were used.

Conclusion: Peripartum coagulation RIs differ from those of non-pregnant women, but do not appear to differ between women who are in active labour versus those not yet in labour. The present findings also illustrate the need for recruitment of larger pregnancy cohorts as only half of participants had samples that remained eligible for RI inclusion with additional exclusions after outlier detection. We determined RIs for various coagulation tests in the peripartum period in an ethnically diverse population that may aid other laboratories in providing care to similar maternal populations.

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Heparin Monitoring using Finger or Heel stick Blood Samples in Pediatric Patients on a Near-Patient Platform

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Background: aPTT and anti-FXa are the most common tests to adjust dosage of heparin in pediatric patients requiring anticoagulation. However, the current anticoagulation practice requires frequent blood draws (at least 1ml) that can lead to iatrogenic anemia and potentially further blood transfusions. Here, we test the feasibility of utilizing ~60µl finger/heel stick sample (FS) to perform aPTT, functional FXa and Antithrombin-III (ATIII) tests on a digital microfluidic (DMF) system that can be used for near-patient monitoring of heparin in children.

Methods: In a prospective research study, whole blood samples (33 for FXa and ATIII, 6 for aPTT) were collected by both venipuncture and FS from consented pediatric patients who were on heparin therapy post-cardiac surgery. Functional assays for FXa and ATIII, and an aPTT assay were performed on the DMF cartridge by loading one droplet of whole blood (venipuncture or FS) which is mixed with a droplet of specific activator, incubated for approximately 2 min at 37°C, followed by addition of specific substrate and measurement of fluorescence or absorbance. Results were compared between the two collection methods and to clinical laboratory reference method (obtained from hospital records).

Results: There was a strong correlation between venipuncture and FS samples tested on DMF platform for all the three assays as shown in the figure. Bland-Altman plots show good limits of agreement for both tests. A bias was observed between capillary and venous samples for aPTT. There was also a moderate correlation between DMF method vs. the clinical laboratory reference method for these assays (data not shown).

Conclusion: Automated and rapid testing for heparin monitoring using low FS volumes on a DMF platform is feasible and will enable frequent monitoring in neonates and pediatric patients. The use of this near-patient technology in a clinical setting needs further investigation.

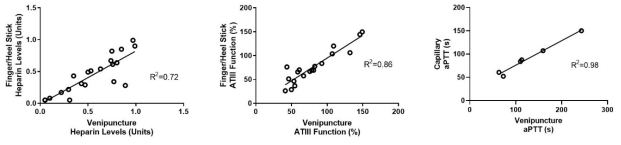

Figure. Venipuncture vs. FS for FXa activity (left), ATIII function (center), and aPTT assays (right) on the DMF platform

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Development and validation of a glucose-6-phosphate dehydrogenase activity assay with on-board sample lysis on the Roche cobas 501 automated analyzer

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Background: Glucose-6-Phosphate Dehydrogenase (G6PD) activity is an important pharmacogenetic marker. Individuals with decreased G6PD activity (<60%) are at risk of acute hemolytic anemia upon administration of certain drugs i.e. hydroxychloroquine, sulfonamides, and doxorubicin. Despite the clinical utility, few laboratories have the resources to devote to manual assays. G6PD assays can be run as laboratory developed tests in automated chemistry analyzers after a time consuming manual lysis step or by using automated liquid handlers which are not commonplace across hospital laboratories. Here, we evaluated the performance characteristics and feasibility of the MedTestDx Pointe G6PD assay with on-board lysis and hemoglobin assay in the Roche cobas c501 analyzer. **Methods:** We used residual EDTA whole blood (WB) samples. G6PD was measured using the enzymatic MedTestDx assay, normalized to the colorimetric hemoglobin (Hb) assay using the MedTestDx Pointe Hemoglobin reagents, both set up as open channel assays in the Roche cobas c501 (Roche Diagnostics). We compared linearity, precision (intra-assay), and accuracy in samples prepared following the manual lysis protocol (100 µL WB+900 µL lysis buffer for 5 minutes) with samples directly loaded onto the analyzer for on-boards lysis. Subsequently, the G6PD with on board lysis and hemoglobin assays were validated by performing the following experiments: AMR/linearity, inter and intra-assay precision, interferences, and accuracy by method comparison with a reference laboratory using the same G6PD and Hemoglobin assays on the same analyzer with automated lysis in a liquid handler. We established reference intervals for G6PD/Hb in 246 healthy individuals. The integrity of results when using an automated workflow for sample lysis was evaluated. **Results:** The %CV intra-assay for on-board and manual lysis preparations were 2.1% and 3.0%, respectively, for a deficient sample (<7.0 U/gHb); 1.8% and 2.3%, respectively for a sample near 7.0 U/gHb; and 0.6% and 3%, respectively, for a sample with activity >7.0 U/gHb. Samples tested after manual lysis had a bias of -2.26 U/gHb (-16.32%) while the bias for the on-board automated lysis was -0.20 U/gHb (-1.44%). Both on board and manual lysis assays were linear. On subsequent validation of the on-board lysis assay, the total %CVs were 9.5%, 11.5% and 8.1% using samples with G6PD activities 1.8, 8.3 and 21.8 U/gHb, respectively. The assay was linear in the range of 0.7 to 16.5 U/gHb within allowable error of 0.4 U/gHb or 20% and acceptable fit of polynomial data (Standard estimate of error 0.8, CLSI EP6). Compared to the method in a reference laboratory (n=39), we observed mean bias of 13.1% for G6PD activity, 0.2% for hemoglobin, and 9.6% for G6PD/hemoglobin in samples with G6PD activity 1.6-15.9 U/gHb. The established reference interval was 7.9-15.0 U/gHb with no statistical difference between genders. There were no observed interferences from icteria and lipemia. Delays in testing of more than 5 minutes impacted result integrity due to cells in WB settling. **Conclusion:** Overall, G6PD assay performance with onboard lysis is acceptable. Testing delays effected result integrity therefore samples should be assayed without delay. This method results in an improved workflow and reduced opportunity for human error.

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Rapid Diagnosis of Thrombotic Thrombocytopenic Purpura (TTP) using a New Fully-Automated Chemiluminescent Assay for ADAMTS13 Activity

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare thrombotic microangiopathy (TMA) that can be caused by congenital or acquired severe deficiency of a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), an enzyme that cleaves the von Willebrand factor (VWF). ADAMTS13 deficiency leads to the accumulation of ultra-large VWF multimers, which causes platelet aggregation and the formation of microthrombi. So beside clinical symptoms, diagnosis of TTP laboratory usually include thrombocytopenia, hemolytic anemia, the presence of schistocytes and the severe reduced activity of ADAMTS13 (<10%). As TTP is a life-threatening condition characterized by microvascular thrombosis, it is of great importance to shorten the time to the treatment. In that condition, a rapid ADAMTS13 testing is critical for an early diagnosis and optimal management of acute TTP. **Objectives:** The aim of the study was to evaluate the performance of a new fully-automated assay for ADAMTS13 activity and to compare test results to those obtained using the FRETS-VWF73 assay. **Material and Methods:** The HemosIL AcuStar ADAMTS13 assay (Werfen, Bedford, MA, USA) is a quantitative activity assay for ADAMTS13 that is fully-automated on the ACL AcuStar chemiluminescent analyzer from the same manufacturer. It is ready-to-use, cartridge-based and available on-demand with a turn-around time of 33 min, 24/7. We evaluated 46 samples (number to be extended) obtained from patients with acute TMA. Diagnosis of TTP was confirmed in 5 cases. Agreement between methods was assessed using the cut-off value of 10%, which is the commonly used threshold allowing the diagnosis of TTP in case of activity below that level. **Results:** ADAMTS13 activity was not significantly different when evaluated using the HemosIL AcuStar ADAMTS13 and the FRETS-VWF73 assays (median=72.6% (range: 0.2 - 126) vs. 79% (range: 5 - 150); p=0.08). Moreover the concordance of test results as whether the ADAMTS13 activity was below (n=5) or above 10% was found to be excellent (kappa=1.00) in the present series, allowing an accurate TTP diagnosis in the patients. **Conclusions:** Together with its short turnaround time (33 min) and its full automation, our results suggest that the HemosIL AcuStar ADAMTS13 activity assay could be an assay of choice to rapidly measure ADAMTS13 activity in plasma and thus to establish the diagnosis of acute TTP in emergency settings.

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Case Report Gamma Heavy Chain Disease with Massive Splenomegaly and Questionable Presence of Lymphoma

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Background: Heavy chain disease is a rare B-cell proliferative disorder characterized by the production of immunoglobulin heavy chains, IgG, IgM or IgA without an associated light chain. Fewer than 200 gamma (IgG) heavy chain disease (GHD) cases have been reported in the literature to date. Presentation of GHD has heterogenous clinical and pathological presentation that often includes splenomegaly, associated autoimmune disease, normocytic anemia and thrombocytopenia and a low concentration (<10 g/L) monoclonal IgG heavy chain in serum and/or urine. Histologically, they appear as a pleomorphic lymphoplasmacytic non-Hodgkin's lymphoma negative for CD5 and CD10. Up to 66% of patients present with a disseminated lymphoma, 25% with localized lymphoproliferative disease contained to the bone marrow and up to 17% have no evidence of proliferative disease.**Case:** Our patient is a 73-year-old male transferred from a peripheral hospital to our acute care facility with a 1-year history of cytopenia and 2-month history of massive splenomegaly with subacute pancytopenia and no evidence of lymphadenopathy. Laboratory results at presentation showed anemia (hemoglobin of 102 g/L), thrombocytopenia (62 x10^9/L), neutropenia without abnormal morphology (0.63 x10^9/L), hypercalcemia (2.86 mmol/L), acute kidney injury (creatinine of 250 umol/L), hyperbilirubinemia (total bilibrubin of 28 umol/L), and elevated ALP (121 U/L) & LD (274 U/L). Protein electrophoresis showed a small monoclonal protein typed as monoclonal IgG heavy chain present in serum (1.6 g/L) and urine. Serum free light chains showed a small elevation of free kappa with a normal kappa:lambda ratio. Bone marrow analysis revealed normal cellularity with good trilineage hematopoiesis. Bone marrow aspirate demonstrated a small B-cell clone CD19, CD20 positive with a kappa light chain restriction and CD5 & CD10 negative. Flow immunophenotyping failed to show any abnormal hematopoietic cell population. He was diagnosed with B-cell lymphoma and referred for symptomatic splenectomy. Thrombocytopenia resolved following splenectomy and the patient was discharged. Clinical, laboratory and histological findings will be discussed in detail and compared to common findings in gamma heavy chain disease.

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Using a novel complete blood cell count based parameter as a non-invasive tool to predict the development of MDS in patients diagnosed with prior malignancies

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Background: Diagnosing myelodysplastic syndrome (MDS) can be challenging especially in patients previously treated or currently being treated for malignancy. Diagnosis involves evaluation of peripheral blood as well as bone marrow biopsy. Boutault *et al*. combined parameters found in the complete blood cell (CBC) count differential with a novel advanced hematologic parameter, NE-WX, to produce a score (MDS-CBC) that predicts patients who are likely to have MDS. The NE-WX parameter is measured by automated hematology analyzers and provides information on the median neutrophil complexity and dispersion width. We planned to apply the MDS-CBC score to patients with (1) newly diagnosed MDS, (2) MDS and concurrent multiple myeloma (MM), and (3) previous diagnosis of lymphoma/solid tumor malignancies (L/STM) and newly diagnosed MDS to elucidate if this score is predictive for a diagnosis of MDS. **Methods:** We performed a database search to identify patients with MDS, a concurrent diagnosis of MDS and MM, and those previously diagnosed with L/STM and new diagnosis of MDS from 2018 to 2020. Chart reviews were performed by investigators (N=80). Parameters from the CBC collected included mean corpuscular volume (MCV), absolute neutrophil count (ANC), and the NE-WX at the time of MDS diagnosis. The MDS-CBC score was calculated using a formula combining these values. A cutoff score of 0.2 was used; scores above this cutoff was proposed to predict a diagnosis of MDS. **Results:** We found the true positive rate (confirmed MDS diagnosis) to be 78% (95% CI: 64-88) for patients with MDS only, 80% (95% CI: 44-97) for patients with concurrent MDS and MM, and 85% (95% CI: 62-97) for patients with pre-existing L/STM and MDS (Table 1). **Conclusion:** The MDS-CBC score can be used as a noninvasive tool to assist in determining patients with preexisting MM, L/STM that are at high risk for MDS.

MDS-CBC Score Contingency Table

Cohort	N	True Positives, N	False Negative, N	Sensitivity, (%)
Newly diagnosed MDS	50	39	11	78%
Concurrent MM and new MDS	10	8	2	80%
Previously diagnosed Lymphoma/Solid Tumor Malignancy and new MDS	20	17	3	85%

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Continuous reference curves for common hematology markers in the CALIPER cohort of healthy children and adolescents on the Sysmex XN-3000 system.

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Background: Clinicians and healthcare professionals rely heavily on health-associated standards, such as reference intervals, for appropriate laboratory test result interpretation. Indeed, the detection of several hematological conditions, including anemia, malignancy, and bacterial infection are contingent on the accuracy of reported test results to guide clinical decision-making. Although reference intervals are traditionally partitioned into discrete age/sex bins based on statistical and/or clinical significance, this method often oversimplifies the complex variation in analyte concentration throughout pediatric growth and development. Continuous reference intervals are suggested to better approximate this dynamic relationship in pediatrics. Thus, the objective of the current study was to establish continuous reference intervals for clinical hematology parameters in the healthy pediatric Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) cohort.

Methods: Data from healthy children and adolescents (6 months to <19 years) from the CALIPER cohort were used to generate continuous reference intervals (i.e. 2.5th and 97.5th quantiles) for 19 hematological parameters. Continuous curves were statistically established by applying non-parametric quantile regressions using penalized splines with non-crossing constraints. Flagging rate analysis (i.e. the percentage of reference data falling outside the upper and lower reference limits) was completed for the established continuous reference intervals and subsequently compared to previously published discrete CALIPER reference intervals for all parameters.

Results: Continuous reference intervals were established for 19 hematology parameters, where seven required sex-specific reference curves. Based on flagging rate assessment and visual detection, continuous reference intervals appear to more accurately estimate hematological analyte concentration over the pediatric age range. This is particularly true for analytes with complex age- and sex-specific reference value patterns, such as red blood cell count and hemoglobin.

Conclusion: This is the first study to generate continuous reference intervals for a breadth of hematological markers in a healthy pediatric Canadian population. The increased power of continuous reference intervals to accurately estimate the complex relationship between hematological analyte concentration and age during a time of extensive growth and development is expected to improve laboratory test result interpretation, and subsequently, pediatric clinical decision-making.

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Ferritin, Iron, TIBC, and Iron Saturation: Big Data Diurnal Variation Graphs Promote Ferritin's Marginal Intraday Variation and Diagnostic Superiority

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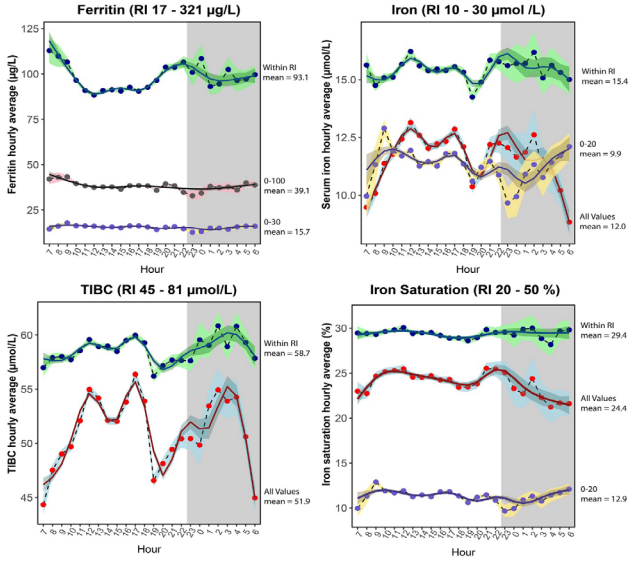
Background: Over the last decade, various groups have attempted to define diurnal variation in humans by simulating day and night patterns of eating, activity, and sleep with frequent sampling of multiple analytes. We propose an alternate big data approach in which hourly hospital and/or outpatient data are averaged to provide 24 hour variation profiles. As the big data approach is based on patient data, it is immediately applicable to the interpretation of laboratory data of patients requiring diagnosis or monitoring. We present our experience in deriving and evaluating the relative usefulness of markers of iron deficiency. **Methods:** We obtained de-identified

Ottawa Hospital inpatient and outpatient ferritin, iron, TIBC, iron saturation, date and time of their sampling from 2014 to 2019. We isolated only weekday data, rounded and grouped the sample collection time to the nearest hour. For each hour group, we calculated and plotted the average of each iron parameter on a 24 hour time scale to visualize the diurnal variation. **Results:** Totals of 329016 ferritins, 85433 irons, 82481 TIBC, and 82468 iron saturations were graphed. Ferritin level is stable throughout the day at lower range (≤ 100 µg/L). TIBC and iron show significant oscillations with amplitudes of 12.0 and 4.3 µmol/L, and gradually increasing mean levels from 7 am to 12 pm by 24% and 38.7%, respectively. **Conclusions:** Low level ferritin shows the most stable intraday level. The diurnal variations of TIBC and iron are severe, especially between 7 am and 10 am. While clinicians and laboratorians should be educated about these variations, it may be prudent to de-emphasize and restrict the sampling of nonferritin iron tests to 10 am to 7 pm.



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Combined effects of gene modifiers on sickle cell nephropathy

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Backround: Sickle cell disease (SCD) substantially causes renal structural damage and kidney dysfunction. Nephropathy, like other chronic complications associated with SCD, may be influenced by the presence of gene modifiers. The objective of the study was to evaluate the effect of some gene modifiers on sickle cell nephropathy.**Methods:**After patients with SCD recruitment, molecular analysis of the *HBB* gene and blood glucose levels were performed. The alpha-thalassemia (3.7kb *HBA1/HBA2* deletion) was screened using gap PCR multiplex and Senegal haplotype (*XmnI*-rs7412844), *BCL11A*-rs4671393, *NPRL3*-rs11248850 were genotyped using Mass Array. The effect of the variants on sickle cell nephropathy was then evaluated using STATA software, with the significance level set at $p < 0.05$.**Results:**The number of confirmed patients with included in this study was 162 with a median age of 20 years [4 - 57] and a sex ratio of 1.14. The Senegal haplotype (*XmnI*-rs7412844), the *BCL11A*-rs4671393 variant were protective factors against microalbuminuria with *odds ratios* (*OR*) of 0.*21* (95% CI 0.05 - 0.90) and *0.25* (95% CI 0.07 - 0.94) respectively. The combination *NPRL3*-rs11248850 variant - 3.7kb *HBA1/HBA2* deletion was a protective factor against microalbuminuria (*OR = 0.10,* 95% CI 0.01 - 0.87) but it was a risk factor for glomerular hyperfiltration (*OR = 14.15,* 95% CI 1.51 - 132.36). **Conclusion:**All four variants have displayed protect against microalbuminuria. The combination alpha-thalassemia - *NPRL3*-rs11248850 variant is, in addition, a risk factor for glomerular hyperfiltration. These variants could thus constitute relevant genotypic biomarkers to assess the risk of microalbuminuria and glomerular hyperfiltration in Senegalese living with SCD.**Keywords**: Sickle Cell Disease, Kidney Disease, Molecular Diagnostics

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Earlier Multiple Myeloma Diagnosis through a laboratory Result Management intervention

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Background: Test request, and the action taken after receiving test result, are the steps of the laboratory cycle where more errors have been reported, making necessary the design and establishment of demand and result management (RM) interventions. Identifying Multiple myeloma (MM), in its early stages often allows for more treatment options. We hypothesized that a RM intervention after high levels of serum total protein (sTP) in a patient for the first time, could shorten the period of MM diagnosis. Our objective was to shorten the period of diagnosis of MM patients through a RM intervention based on algorithms after elevated sTP values

Methods: The study was conducted from October 1st 2019 to January 31st 2021 at a 370-bed Public University Hospital, that serves 234,551 inhabitants. The laboratory receives samples from hospitalized patients, outpatients and primary care patients. Patient's requests are ordered electronically and reports sent out from the Laboratory Management Information System (LIMS) to the patient's electronic medical record (PEMR). sTP is not included in any laboratory panel and is requested individually. LIMS stores and manages the laboratory data of every request, in a patient's data base. Currently we have at our disposal 14 years of laboratory data of the population of our area.

The intervention was designed in a meeting between laboratory professionals, hematologists and members of the hospital board. The LIMS would automatically register serum immunoglobulins (Igs: IgA, IgG, IgM) when a patient with a sTP value above 8mg/dL for the first time. When concomitantly one Ig presented a value above and another below the laboratory reference range, the LIMS would automatically register a serum protein electrophoresis (sPEP). A serum immunofixation (sIFX) test would be additionally registered when a monoclonal spike in sPEP, for the detection of oligoclonal bands. When positivity, a comment in the laboratory report would explain the intervention, suggesting sending the patient to hematology for evaluation. In a pre-intervention period (year 2015), through a search in LIMS were identified the patients with sTP>8 mg/dL. In both periods, in pre-intervention and in the intervention period, and through a revision in PEMR, was identified the time between this first elevated sTP value and MM diagnosis, in patients finally diagnosed as presenting MM.

Results: In the intervention period 542 patients presented sTP values above 8 mg/dL for the first time, and Igs were measured. Eighty-two fulfilled criteria for sPEP, and 27 had a monoclonal spike, that was confirmed in 22 through the sIFX. Thirteen were new findings. Six were new MM diagnosis and 4 monoclonal gammopathy of undetermined significance. 16.6 days was the period between the sTP elevated value and MM final diagnosis by the hematologist. In pre-intervention period, out of the 400 cases of sTP elevated values, 13 were finally diagnosed as MM and the period until MM final diagnosis was 119 days. Both the pre and intervention periods, time to diagnosis, were significantly different (P=< 0.000).

Conclusion: RM Interventions designed from the laboratory in agreement with clinicians, shorten the period for MM new diagnosis.

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D-Dimer reported according age-adjusted cut-off point in Emergency Department patients with suspected deep venous thrombosis reduces imaging request

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Background: Acute venous thromboembolism (VTE) represents the third cause of cardiovascular death after myocardial infarction and stroke and is considered one of the leading causes of death in hospital. The diagnosis is made through clinical symptoms, radiology and D-dimer, that can be reported through a conventional cut-off point (500g/ml) or adjusted for the patient`s age (Douma index).

Objective: To compare the diagnostic performance of reporting D-dimer through conventional cut-off versus age adjusted, in a hospital emergency department (ED) for the management of VTE patients. Also to explore how ED physicians requested Eco-Doppler and Angio-Tac in patients suspected to have VTE, before and after reporting D-dimer adjusted per age.

Methods: A cross-sectional study was performed to compare the diagnostic performance of reporting D-dimer through conventional cut-off versus adjusted per age, in ED patients between 50 and 100 years, with consecutive requests for D-dimer. An intervention was agreed with ED clinicians, to additionally report D-dimer adjusted per age and count the number of requests for radiology tests in patients with divergent results (positive through conventional and negative when adjusted per age).

Results: Through the study in 392 patients, 25 with VTE and 367 with other pathologies, the same sensitivity was obtained, using the conventional cut-off point and the age adjusted, however the specificity was higher when age adjusted D-dimer cut off, and lower the negative like hood ratio, but without statistical significance (Table). 93 patients with divergent results, showed a decrease in both tests ordering, Angiotac 13 (14%) versus 5 (5.3%), and Eco-Doppler 21 (22.5) versus 12 (12.9%), being the difference statistically significant in Angiotac.

Conclusion: The study shows good diagnostic efficiency of the D-dimer reported through the age adjusted cut-off in the diagnosis of VTE, and a decrease in the request for radiology tests after its implantation.

Diagnostic accuracy of Conventional cut-off and Douma cut-off

	Conventional cut-off	**Douma cut-off**
Sensitivity	0.96	0.96
Specificity	0.42	0.51
PPV (%)	10%	12%
NPV (%)	99%	99%
LR+	1.59	1.96
LR-	0.19	0.08

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COVID19 Associated Thrombotic Angiopathy Improved After Plasma Exchange

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Background: Thrombotic angiopathy represents a dire consequence of COVID-19 infection and such endothelial cell dysfunction and microvascular thrombosis are thought to contribute to resultant multi-organ complications. Treatments have included anti-viral/anti-bacterial agents, immunoglobulins, thrombolytics, and other immune-modulators with variable results. Here we present therapeutic plasma exchange (TPE) in a such a patient with presumptive thrombotic thrombocytopenic purpura (TTP).

Methods: A 44 year old female patient received dexamethasone as standard of care for severe COVID-19 infection and also received empiric TPE for presumptive TTP (polychromasia, basophilic stippling, schistocytes, pending ADAMTS13)

Results: After plasma exchange, patient demonstrated significant improvement including decreases in lactate dehydrogenase [3802 to 945]/total bilirubin [4.0 to 2.9]/schistocytes, increase in platelet count [18,000 to 68,000] and declining vasopressor requirements. However, ADAMTS13 levels drawn prior to TPE was 50.7% decreasing probability of TTP diagnosis

Conclusion: COVID-19-associated thrombotic microangiopathy exhibited a dramatic response to TPE. It is conceivable that plasma exchange accounts for cytokine removal of inflammatory mediators in the plasma and reinstitution of immune homeostasis. Plasma exchange used on a limited scale, particularly for patients with COVID-19 microangiopathy, may represent a useful treatment for a particularly devastating manifestation of COVID-19.

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Comparison of different incubation temperatures in mixing studies: Preliminary study

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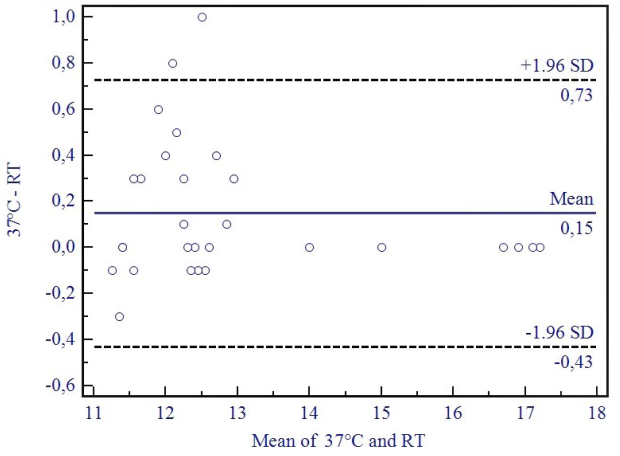
Background: Mixing tests are used as a guide in the diagnosis of prolonged PT and aPTT.Although they provide limited contribution in terms of sensitivity and specificity, they are preferred as the first step because they are easily applicable to understand whether prolongation is due to factor deficiency or the presence of inhibitor.During the test, the patient's plasma is mixed with the normal plasma pool in a ratio of 1:1. PT/aPTT analysis is performed periodically from the mixture that is kept in an oven at 37°C for up to 2 hours.With this preliminary study, it was aimed to evaluate the effect of performing the incubation temperature at room temperature instead of 37°C on the results.**Methods:** 6 patient samples for which mixing test was requested due to prolonged PT were divided into 2 portions and both portions were mixed with normal plasma pool at a ratio of 1:1. One of them was kept at 37°C and the other was kept at room temperature(23±2).PT analyzes were performed by photometric method at the 5th, 30th, 60th and 120th minutes of both(ACL TOP 300,USA).SPSS23.0 and MedCalc programs were used for statistical evaluation.**Results:** The median values of PT results obtained at 37°C and room temperature were 12.4 seconds(11.2-17.2) and 12.3 seconds(11.3-17.2),respectively. r^2 was 0.975(Slope:0.96)Bland-Altman plot was shown in the graph.**Discussion:** In the preliminary study conducted with a limited number of samples,it was observed that the results were very close at both incubation temperatures.The limitations of the study were the number of samples and the evaluation of PT prolongation only. This preliminary study has revealed a possibility that mixing studies can be performed at room temperature instead of an oven. A comprehensive study is planned in which both prolonged PT and aPTT are evaluated and designed in accordance with the CLSI EP09 guideline.



Precision Medicine

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Precision IGT Predicts Elevated 1-Hour Glucose Levels in Normoglycemic Patients

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While the oral glucose tolerance test (OGTT) has been a standard in the prediction of diabetes risk, it is not typically used due to the logistical difficulty of the procedure. Recent studies show that elevated levels of the serum metabolite alpha-hydroxybutyrate (AHB) are predictive of impaired glucose tolerance (IGT). Furthermore, it has been suggested that the 1-hour glucose (OGTT) should be considered as the preferred marker of IGT.

We tested the hypothesis that AHB predicts abnormal 1-hour glucose levels and beta-cell dysfunction inferred from plasma insulin kinetics during a 75 g OGTT. This cross-sectional study included 217 patients at increased risk for diabetes. 75 g OGTTs were performed with multiple postload glucose and insulin measurements over a 30-120 minute period.

Mean±SD age was 51±15 years. Fasting glucose (FPG) and insulin levels, but not age or BMI, were significantly higher in the second/third AHB tertiles (>3.9 µug/mL) than in the first tertile. Patients in the in the second/third AHB tertiles had a higher glucose area under the receiver operating characteristics curve (AUC) and reduced initial slope of insulin response during OGTT.

The AUC for predicting 1-hour glucose ≥ 155 mg/dL was 0.82 for a base model that included age, gender, BMI, fasting glucoseFPG, hemoglobinA1cC (A1C), and insulin, and increased to 0.86 with AHB added (p=0.015). AHB also independently predictive of 1-hour glucose in normoglycemic patients with an AUC of 0.74, compared to AUC values of 0.65 and 0.65 for A1C and FPG, respectively.

In summary, serum AHB levels predicted elevated 1-hour glucose during OGTT, potentially due to impaired insulin secretion kinetics. This association persisted in

patients with otherwise normal insulin-glucose homeostasis. Based on these findings a commercial assay, Precision *IGT*, is being developed which incorporates AHB and other clinical variables to predict 1-hour glucose levels during OGTT.

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Analysis of CPTAC Proteomic Data for Stage II Colon Adenocarcinoma Reveals Overexpression of Eosinophile Peroxidase in Tumors with Lymphovascular Invasion

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Background: Colon cancer is the third leading cause of cancer-related deaths and while potentially curable by surgery, prognosis varies widely according to the disease stage, extent of lymph node and vascular invasion, and distant metastasis. Over a quarter of patients diagnosed with colon cancer have stage II disease with reoccurrence rates at this stage approximating 20%. Among these patients, lymphovascular invasion (LVI) is a significant factor shown to convey a higher risk of relapse. Routine use of adjuvant chemotherapy for stage II patients is not recommended, nevertheless, a subset of stage II patients may greatly benefit from its use. Therefore, there is a great need to uncover prognostic markers that identify individuals at risk of relapse to better direct the clinical course of treatment. Data sharing in mass spectrometry-based proteomics is becoming common practice through public repositories creating a largely untapped resource for the reuse and orthogonal analysis of largescale proteome data and potential to extract new information. In this investigation we have re-interrogated publicly available data on colon cancer to reveal proteome changes in tumors with respect to LVI in stage II colon adenocarcinoma patients. **Methods:** Proteomics data used in this analysis were generated by The Clinical Proteomic Tumor Analysis Consortium on an Orbitrap Velos mass spectrometer using a label free quantitative approach. Data were filtered to include 11 stage II colon adenocarcinoma samples. Mass spectrometry data were then searched against the human reference proteome database with reverse sequence decoys by MSFragger considering y- and b-ions in scoring, allowing fully tryptic peptides, up to 1 missed cleavage, with cysteine carbamidomethylation specified as a fixed modification and methionine oxidation as a variable modification. Peptide IDs were validated by the PeptideProphet algorithm in Philosopher. Validated peptide spectral matches at a 1% false discovery rate were used to generate a spectral library in which proteins were assembled. Differential abundance analysis was performed using a two-sample t-test assuming equal variances and p-values corrected for multiple hypotheses using the Benjamini-Hochberg method. Data were log2 transformed and normalized by equalizing medians. Precursor areas for significantly upregulated proteins were then divided into two groups at the median and queried for an association with survival by cox regression analysis in all stage II samples (N=38). **Results:** 945 proteins assembled from 6226 peptides were identified. An overexpression of eosinophil peroxidase (EPX) was associated with LVI having a p-value of $2.45x10^{-5}$ (adjusted p-value of 0.023195) and a fold change of 1.28x. A higher expression of EPX was associated with a 26% increase in survival. **Conclusions:** Eosinophil peroxidase has been identified as a potential therapeutic target, having implicated association with survival in stage II colon cancer. A quantitative measurement of eosinophil peroxidase could possibly be used as a predictor of re-occurrence and metastatic potential in stage II colorectal cancer patients, who may benefit from more aggressive treatment options such as adjuvant chemotherapy. As demonstrated by this study, reanalysis of publicly available datasets can yield novel information of clinical value.

A-245

Cross-Sectional Analysis of Serum 1,5-Anhydroglucitol and Covid-19 in T2D Patients

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Background: Hyperglycemia worsens the prognosis and severity in Covid-19 patients.

Objective: To assess the impact of glycemic control on COVID-19 severity.

Methods and Findings: Data from 460 individuals collected in Spain between April and July 2020 was analyzed in a cross-sectional study: healthy, Sars-Cov-2 negative (group 0, N = 197), Sars-Cov-2 positive, with mild COVID-19 symptoms (group 1, N = 113), Sars-Cov-2 positive, with severe COVID-19 symptoms who required hospitalization (group 2, N = 150) at Hospital Universitario Cruces in Bilbao, Spain. Fasting blood glucose, HbA1c and serum 1,5-anhydroglucitol (1,5-AG, indicator of hyperglycemic excursions over the prior 1-2 weeks) were measured. Differences in 1,5-AG (normal range >10µg/mL) were observed between all three groups (mean 1,5-AG of 21.19, 18.99, 14.64 µg/mL, respectively) when compared by an unpaired t-test. 1,5-AG levels across groups decreased with increasing severity, with the hospitalized patients (group 2) showing the greatest difference when compared to healthy individuals, p value < 0.0001. Logistic regression analysis showed that 1,5-AG had a mild positive association with increased COVID-19 severity (group 0 vs group 2: AUC = 0.69, p value < 0.0001). A previous T2D diagnosis did not show association with COVID-19 severity (group 0 vs group 2: AUC = 0.58, p value < 0.0001). Analysis of patients with a previous diagnosis of T2D showed a robust positive association between 1,5-AG and COVID-19 (group 0 vs group 2, AUC = 0.79, p value 0.008). There was no association between HbA1c or fasting glucose with severity. Combination of 1,5-AG and HbA1c was similar than 1,5-AG alone (AUC = 0.80, p value 0.028). Consistent with these findings, there was a significant correlation of 1,5-AG to Covid-19 severity (r = -0.42, p value 0.008) with no significant correlations to HbA1c (r = 0.20, p value 0.220) and fasting glucose (r = 0.23, p value 0.159).

Conclusions: Glycemic fluctuations in T2D patients may contribute to severe COVID-19. Measurement of serum 1,5-AG in T2D patients might help clinicians quickly identify diabetes patients at risk of severe COVID-19. These patients may benefit from more intensive management, treatment and vaccine prioritization.

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A-246

A Multiparametric NMR-GFR Estimating Equation improves CKD classification in Liver Disease

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Background: Accurate assessment of glomerular filtration rate (GFR) is critical to decision making in individuals with liver disease. Renal impairment is common in association with liver disease and the degree of renal dysfunction impacts decisions on drug dosing, therapeutic interventions, and suitability for liver transplantation. Altered hemodynamics in liver disease often results in overestimation of GFR when using creatinine based GFR estimating equations. Recently, we have developed a novel GFR equation, which utilizes serum myo-inositol, valine and creatinine quantified by nuclear magnetic resonance spectroscopy (NMR) in combination with Cystatin-C, age and sex (GFR_{NMR}). This equation outperforms many GFR estimation methods in chronic kidney disease. We hypothesized that GFR_{NMR} would lead to improved CKD classification in the setting of end-stage liver disease. **Methods:**We compared various GFR estimation equations including GFR_{NMR} in end-stage liver disease patients scheduled for orthotropic liver transplantation (OLT) in a multicenter retrospective study with renal tracer clearance as GFR reference method. In n=78 liver recipients, renal tracer clearance (mGFR) was measured in preparation of OLT as part of normal clinical routine to assess kidney function. Stored serum was analyzed for Cystatin C and NMR quantified the biomarkers myo-inositol, valine and creatinine in a single simultaneous measurement. Analytes were used to estimate GFR based on $CKD\text{-}EPI_{crea}$, $CKD\text{-}EPI_{cys}$, $CKD\text{-}EPI_{crea\text{-}cys}$, or GFR_{NMR}. **Results:** $CKD\text{-}EPI_{crea}$ was the least accurate with a P_{30} value of 0.46 and a MAE of 20.4 ml/min/1.73m². $CKD\text{-}EPI_{cys}$, was better with a P_{30} of 0.56 and a MAE of 17.7 ml/min/1.73m². The $CKD\text{-}EPI_{crea\text{-}cys}$, which has been established to lessen the effects of age, sex and race to improve GFR estimation, had a P_{30} value of 0.81 and a MAE of 11.8 ml/min/1.73m². GFR_{NMR} across the entire range of GFR showed good accuracy to measured GFR with a P_{30} of 0.83 and a MAE of 11.7 ml/min/1.73m². McNemar testing corrected for multiple testing revealed highly significant differences ($p<0.001$) in P_{30} values for GFR_{NMR} compared $CKD\text{-}EPI_{crea}$, $CKD\text{-}EPI_{cys}$. GFR_{NMR} correctly staged 49/78 (62%) patients across the GFR range. Only 39/78 (48%) of individuals were correctly classified by creatine only estimating equation CKD-EPI2009, a net reclassification improvement of 14%. **Conclusion:** Kidney function is an important consideration for determining liver transplant allocation. Compared to gold standard renal tracer clearance, GFR_{NMR} has been shown to highly correlate with mGFR and proved better at accurately stratifying individuals into CKD classes in the setting of liver disease.

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Anti-Spike, Not Anti-Nucleocapsid Assay Method is Needed Following COVID-19 Vaccination

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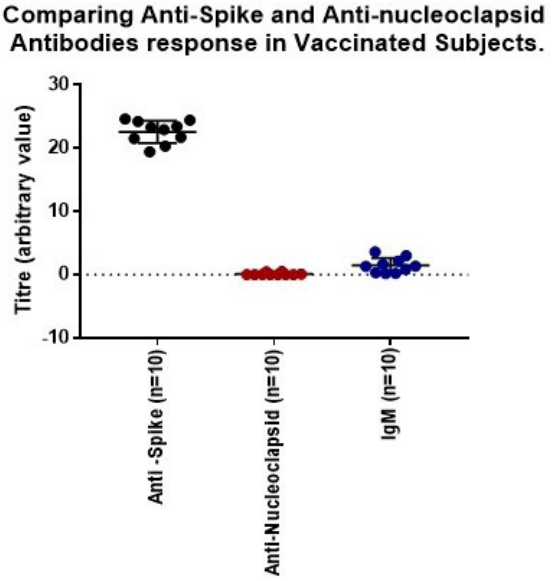
AbstractBackground: An increasing number of the world population has been vaccinated in recent months. Still, confusion remains among patients and providers on which test method would best determine if anti-SARS-CoV-2 antibodies have been

produced in response to vaccination. This study aimed to determine if positive anti-spike SARS-CoV-2 antibodies might explain negative antibody results in vaccinated individuals who had tested negative for antibodies using anti-nucleocapsid assays. **Methods**: We tested serum samples from ten vaccinated individuals, thirteen randomly chosen patients with nucleic acid amplification test (PCR or Rapid ID NOW)-confirmed SARS-CoV-2 infection and two randomly selected patients with no history of vaccination or infection on both our institution's Ortho-Clinical Diagnostics VITROS anti-spike IgG and Abbott Architect anti-nucleocapsid IgG assays. **Results:** All ten vaccinated subjects (100 %) were negative for anti-nucleocapsid antibodies but positive for anti-spike antibodies. In contrast, most (ten out of thirteen) of the subjects (77 %) with test-confirmed infection had detectable anti-nucleocapsid IgG antibodies, and all had detectable anti-spike IgG antibodies (100%). For the infected patients, antibodies were measured approximately one-and-a-half months after nucleic acid amplification test positivity. For the two patients who had not been infected or vaccinated, their serum samples were negative on both IgG assays and the DXI anti-spike IgM assay.**Conclusion:** COVID-19 vaccination is associated with specific stimulation of anti-spike antibodies, reflective of how the mRNA vaccines were developed to encode spike protein-specific genetic information. Thus, the anti-spike-specific assay method is a more reliable assay to determine post-vaccination serologic immune response. However, both anti-nucleocapsid and anti-spike assays are sensitive tests for detecting post-COVID-19 infection.



A-248

Thyroglobulin (Tg) measured by LC-MS/MS <and> immunoassay in Tg autoantibody positive samples: An 8 year observational data review

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Background: Thyroglobulin (Tg) measurements in serum/plasma are used to monitor patients after treatment for differentiated thyroid carcinoma (DTC). Endogenous anti-Tg autoantibodies (Tg-AAb), present in many patients, may interfere with sandwich Tg immunoassay (IA) measurements and cause false-negative results. This presents a challenge when monitoring patients for DTC recurrence. In an earlier study where Tg was undetectable by IA in Tg-AAb positive samples, Tg concentrations were at or above 0.5 ng/mL in 23% of samples when measured using LC-MS/MS method, which overcomes Tg-AAb interference (ClinChem 2013, 59:982-990). To evaluate this relationship in a larger, historical dataset, we retrospectively reviewed Tg concentrations in over 75,000 Tg-AAb positive samples over two 8 year periods and compared the distributions of those measured by LC-MS/MS to IA. To date, there are no long-term comparative studies assessing the distribution of Tg concentrations in Tg-AAb positive samples analyzed by these two methods. **Methods:** Historical Tg and Tg-AAb data reported by a national reference laboratory was reviewed. Distributions of Tg results determined by IA (Beckman DxI 800, 2004-2011, n=34,390, 82.2% women) were compared to LC-MS/MS (ClinChem 2013, 2013-2020, n=41,188, 81.5% women) in Tg-AAb positive samples. During the observational study period, both assays used Beckman IA calibration standards. The lower limit of quantitation (LOQ) was 0.5 ng/mL (LC-MS/MS) and 0.1 ng/mL (IA). Tg-AAb were measured using IA; positive status was defined as results exceeding the manufacturer's reference interval. **Results:** Previous Tg method comparisons using Tg-AAb negative serum samples demonstrated good agreement between LC-MS/MS and IA. Mean/median Tg-AAb concentrations in the LC-MS/MS dataset were 193/21.5 IU/mL and 244/54.5 IU/mL in the IA set. Mean/median Tg concentrations in the LC-MS/MS dataset were 13.0/0.5 ng/mL and 37.6/0.4 ng/mL in the IA set. Within the Tg concentration range 0.5-5 ng/mL, statistically significantly higher concentrations were measured using LC-MS/MS (mean/median 1.92/1.60 ng/mL, n=8,804) than IA (1.81/1.40 ng/mL, n=6,822), p<0.0001. The total prevalence of Tg concentrations between 0.5-5 ng/mL was 21.4% and 19.8% (difference 1.5%) for LC-MS/MS and IA, respectively; among samples with elevated Tg-AAb titers (>10 IU/mL), the difference in prevalence was higher: 30.4% and 26.4% (difference 4%) for LC-MS/MS and IA, respectively. The most significant difference in prevalence of Tg concentrations by LC-MS/MS and IA was within the ranges of 0.5-0.6 ng/mL (prevalence 1.9% vs. 1.6%); 1.0-1.9 ng/mL (7.1% vs. 5.9%); and 2.0-4.9 ng/mL (7.9% vs 7.6%). **Conclusions:** In a direct comparison using Tg-AAb negative samples, LC-MS/MS agreed with IA for Tg. Review of historic Tg data in Tg-AAb positive samples during 8 year periods of routine use of these methods demonstrated a greater prevalence of samples with Tg concentrations between 0.5-5 ng/mL in the LC-MS/MS data set. This indicates possible underestimation of Tg by IA in Tg-AAb positive samples, as expected. This effect is most clinically relevant when monitoring for disease recurrence in samples containing low Tg concentrations. Confirmation of these patterns in large longitudinal data sets reinforces the difference between, and need for, high sensitivity LC-MS/MS methods that can counter the effects of Tg-AAb on Tg measurement.

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Analytical Performances of HLADQA1*05 and TPMT/NUDT15 Gene Variants for Thiopurine Induced Myelosuppression in Combination with Anti-TNFs

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Background: Thiopurines (azathioprine, 6-mercaptopurine) are often prescribed with Anti-Tumor Necrosis Factors (Anti-TNF) to mitigate the risk of immunogenicity and formation of anti-drug antibodies. We have developed a pharmacogenetic panel combining HLADQA1*05 (rs2097432), a known predictor of immunogenicity to Anti-TNF with genetic variants in TPMT and NUDT15 that are known to increase the risk of Thiopurine Induced Myelosuppression (TIM). The objective of the study was to establish the analytical performances of this pharmacogenetic panel in Inflammatory Bowel Disease (IBD). **Method**: Genomic DNA was isolated from whole blood (QIAsymphony® DSP DNA Mini Kit). HLADQA1*05 (rs2097432) and inherited NUDT15 variants (rs116855232; rs147390019; rs186364861; rs746071566(ins)) were determined using real time PCR genotyping assays (TaqMan® based fluorescence-quencher technology). Inherited TPMT variants (rs1800462, rs1800460, rs1142345) were determined using PCR coupled with multiplex Allele Specific Primer Extension (mASPE) on the Luminex® 200™ system. All testing was conducted in a clinical laboratory accredited for genetic testing. The performance of the panel in identifying subjects with heightened risk for TIM during Thiopurine monotherapy or in combination with Anti-TNF was established in a population of 377 consented IBD patients. Performances are presented in aggregate or by self-reported ancestry. **Results**: All genetic assays perform with 100% repeatability, reproducibility and accuracy in genomic DNA derived from whole blood. HLADQA1*05 (rs2097432) carrier status was detectable in 44% of IBD subjects (n= 164; 44.7% Caucasians and 33.3% in Asians and Latino combined). As expected NUDT15 variants were most common in Asian and Latino ethnic groups as compared to Caucasians (20.5% vs 1.7% p<0.01) (**Table 1**). Among Caucasians, 9.8% presented increased risk of TIM and would be ineligible for combination therapy with Anti-TNF. **Conclusion**: We have established the analytical validity of a genetic panel that may facilitates the identification of IBD with heightened risk for TIM either alone or in combination with anti-TNFs.

Table 1	Inherited variation in TPMT/NUDT15 and HLA DQA1*05 Status for Combination Therapy in IBD				
	N	**% carrier HLADQA1*05**	**% carrier TPMT**	**% carrier NUDT15**	**% carrier TPMT/ NUDT15**
All Subjects	377	43.5%	8.2%	3.4%	11.1%
Caucasian	338	44.7%	8.3%	1.5%	9.8%
Asian / Latino	39	33.3%	7.7%	20.5%	23.1%

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Darwin Theory of Survival of the Fittest-Inspired Exponential Enrichment and Point-of-Care Detection of Rare Mutant Alleles

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Background: The identification of tumor-associated nucleic acid fragments in body fluids is challenged by their low abundance and sequence homology with the vast background of nucleic acids from healthy cells. Thus, mutant allele fraction enrichment is essential for timely, sensitive detection of clinically critical, rare mutant alleles. Recently, programmable endonucleases (i.e. CRISPR-Cas and prokaryotic Argonaute) have been utilized for rare mutant allele enrichment. Although these endonucleases preferentially cleaves wild type alleles with specific guide design, it also cleaves, albeit, to a lesser degree off-target mutant alleles. While off-target and target cleavage rates depend on the guide RNA design, programmable endonuclease variant, and assay conditions, samples with low mutant allele fraction (e.g., 0.01%) contain just a handful of molecules and any loss of critical biomarkers would compromise assay's sensitivity. Furthermore, not all programmable endonucleases are productive. By some estimates, fewer than 90% targets are cleaved. For example, if an assay cleaves only 90% of the wild type alleles, the mutant allele fraction can be enriched by less than 10-fold.

Methods: Inspired by Darwin theory of survival of the fittest, we propose a simple assay that combines cleavage with concurrent polymerase amplification to overcome the shortcomings of the cleavage only assays. Our assay amplifies concurrently mutant alleles of interest and wild type alleles in the presence of relatively high concentration of guided endonuclease. The endonuclease in our system plays the role of a predator while the nucleic acids are the prey with the wild type alleles much more vulnerable than the mutant alleles. While the copy numbers of both the wild type and mutant allele increase with time, the latter at much greater rate, alleviating any concerns of losing valuable biomarkers. We used CRISPR-Cas9 to demonstrate the concept. To match the optimal operating temperature of the spCas9, we use here Recombinase Polymerase Amplification (RPA) as our amplification assay and dub our combined cleavage – amplification assay Programmable Enzyme-Assisted Selective Exponential Amplification (PASEA).

Results: By taking advantage of the programmable CRISPR-Cas9 endonuclease system, and combining its specific cleavage of wild-type alleles with RPA, we have demonstrated that ultrarare somatic mutant alleles (mutant allele frequency [MAF] 0.01%) can effectively be enriched into a large fraction (MAF 70%, a 7000-fold enrichment) within 20 min. Moreover, the addition of our specially designed Exo-RPA probe enabled fluorescent real-time detection of rare somatic mutant alleles at a satisfactory sensitivity (MAF 0.1%); and specially designing RPA primers with short amplicons enabled concurrent detection of both cell-free (tumor) DNA and RNA. We incorporated this real-time PASEA into a multifunctional microfluidic chip device to enable point-of-care testing of rare mutant alleles. Furthermore, to demonstrate the application capabilities of real-time PASEA in resource-limited clinical settings, we analyzed 108 tissue samples and 10 blood samples from cancer patients. Our results demonstrated equal performance relative to clinical ARMS-PCR and next-generation sequencing.

Conclusion: As a simple, efficient, and rapid enrichment and analysis method for rare mutant alleles, we expect PASEA could be used in liquid biopsy for cancer diagnosis in resource-limited settings.

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Variability of *PIK3CA* mutations in breast cancer tumor samples analyzed by NGS: the importance to amplify the screening regions allowing patients to benefit from target therapy

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Background:*PIK3CA* mutations are present in more than 30% of all breast cancers, and have been suggested as a predictive biomarker for PI3K-selective inhibitor treatment in ER and HER2-positive patients. Four mutations (E542K, E545K, H1047R and H1047L) have been described as hotspots based on real-time PCR studies. However, other methodologies such as liquid biopsy and next generation sequencing (NGS) made important contributions for increased sensitivity and amplitude.

Methods: We analyzed *PIK3CA* mutations in 184 FFPE samples from breast cancer patients by using a targeted next-generation sequencing assay (Oncomine Focus™).

Results: Around 20% of the samples studied didn´t have quality enough to proceed the screening probably due to pre-analytic issues. Among the analyzed samples, 34,25% presented mutations in *PIK3CA*. From the total analyzed samples (146), we identified 26% of H1047R, 16% of E545K, 14% of E542K, 14% of N345K and 30% of other mutations that could not be detected using other methods like real time PCR. Besides that, around 30% of the samples had two or more mutations and none copy number variation was detected. The N345K mutation called our attention since it´s not always targeted among the hotspots and it was the fourth more frequent in this pilot study similar to the study in Martínez-Sáez *et al.* 2020. According to preclinical models, this mutation confers a gain of function and it has shown increased sensitivity to PI3K pathway inhibition. Moreover, COSMIC and OncoKB datasets consider it pathogenic. The presence of two or more mutations seems to increase the sensitivity to alpha-specific PI3K inhibitors and since the percentage found in the samples presenting two or more mutations is considered high, so it's important to use a method that allows their detection.

Conclusion: Molecular testing through NGS is a powerful tool, specially the use of panels that includes CNVs and allows the investigation of non-hotspots sites for point mutations in the same screening. Even with the CNVs frequency detection low, when they are present, they can contribute to PI3K/AKT vias in Breast Cancer. There are different methods used to detect *PIK3CA* alterations including NGS, Real time PCR and Digital PCR, each one have their own limitations and advantages. These results reveals that in order to be able to detect double or more mutations, even rare mutations, the NGS approach is indicated. Therefore identifying more patients that could benefit from target therapy. It´s important to encourage more studies and clinical trials involving rare point mutations and non-hotspots regions.

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Analytical Performance of Automated EGFR Mutation Idylla assay for Lung Cancer targeting targeted therapy

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Background: In lung cancer, there are two main histological subtypes: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Over the past decade, it has become evident that NSCLC subsets can be better defined at molecular level by recurrent rearrangements of "drivers" that occur in several oncogenes. Mutations can be found in all NSCLC histologies (including adenocarcinoma, squamous carcinoma and large cell carcinoma) and in smoking, ex-smoker and never-smoking patients. Patients with adenocarcinoma who have never smoked, have the highest incidence of rearrangements of EGFR, HER2, ALK, RET and ROS1. It is important to note that inhibitors of small molecules targeted are already available or in development for defined subsets, in molecular terms, of patients with lung cancer. The presence of deletion in exon 19 in EGFR (48%) and the L858R mutation in exon 21 (43%) is a predictor of the therapeutic benefit derived from EGFR tyrosine kinase inhibitor (TKI) therapy. Therefore, effective molecular diagnostic methods capable of correctly identifying mutations in EGFR gene are necessary for targeting target therapies. **Objectives:** To describe a analytical validation of EGFR Mutation Idylla assay in solid tumor samples for routine diagnostics implementation.

Methods: 30 Formalin-Fixed Paraffin-Embedded (FFPE) tissue section samples from lung tumor were used. Out of these, 30 cases, 10 have not mutations in the EGFR gene (WT samples; 33.33%), seven had point mutations (23.33%) and 13 had indel mutations (43.33%). These results were previously obtained by New Generation Sequencing (NGS) for EGFR gene. The sections were then sent for histopathological assessment. The Idylla method was carried out according to manufacturer recommendations. Briefly, 5 µm FFPE tissue section are loaded by a sterile forceps directly in

the cartridge then placed in the Idylla system allowing integrated DNA extraction and mutational analysis. The samples included in the study ranged of 15-90% to tumor area and allele frequency of mutations ranged from 5-98%. These data were used to assess the sensitivity of the test. Intrassay and interassay precision analyzes were also carried out.

Results: Invalid results were excluded from analysis. Overall, the agreement of results between two methodologies was 7/9 for WT samples (77.8%), 6/7 for point mutations samples (85.7%) and 9/10 for indel mutations samples (90%). The most frequent variants found in individuals with mutations in EGFR gene and that are targets for therapeutic targeting have been correctly identified, such as EGFR DEL19 and EGFR L858R. Samples with tumor area ≤15% and allelic frequency ≤5% are not detectable in the assay. The experiments performed to evaluate the precision demonstrated optimal repeatability and reproducibility.

Conclusion: EGFR Mutation Idylla assay proved to be an efficient test for identifying mutations in EGFR gene, thus enabling the targeting of approved target therapies, improving treatment opportunities. Due to its fully automated and easy to interpret process, Idylla system is capable of being integrated in molecular biology laboratories at different levels and allows the release of results in a short period. However, greater attention is necessary for samples with reduced tumor area and allele frequency.

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Identification of Cytokines, Chemokines, and Growth Factors in Breast Cancer Pleural Effusions

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Background: Pleural effusions are a valuable source for obtaining metastatic tumor cells to develop patient-derived organoid models for research and functional precision medicine. We have observed that tumor cells, obtained by thoracentesis of metastatic breast cancer patients, have high tumor viability (greater than 80 percent) when the cells are kept at 4C in the endogenous fluid from the patient. Here, we analyze the metastatic fluids from breast cancer patients to determine if there are differences in the cytokine/chemokine/growth factor composition across cancer patients and between patients with malignant vs non-malignant effusions. **Methods:** The Luminex xMAP ELISA platform was used to protein expression profile 63 unique effusions/ascites samples from patients with metastatic cancer using the Th1/Th2 Cytokine & Chemokine 20-Plex Human ProcartaPlex Panel 1 and Growth Factor 11-Plex Human ProcartaPlex Panel. All malignant fluid samples were run in duplicate. The assay also included synthetic multiplexed controls for each analyte; and biologic controls including plasma from 6 healthy individuals (3 men and 3 women) and 4 non-malignant effusions. **Results:** Out of the 31 analytes profiled, 28 were reproducibly measured in the analytic multiplexed controls. The top 3 principle components in the expression data account for the majority (approximately 60 percent) of the variability. **Fig. 1** shows a hierarchical clustering for a subset of the samples across the 15 most informative growth ligands (cytokines, chemokines, and growth factors). **Conclusion:** The characterization of cytokines, chemokines and growth factors found in the metastatic environment reveals the uniqueness of each person's cancer and identifies tumor signaling pathways that could foster cell viability, proliferation, and migration. In addition, these growth ligands have the potential for being used to develop customized media for *ex vivo* assays in functional precision medicine.

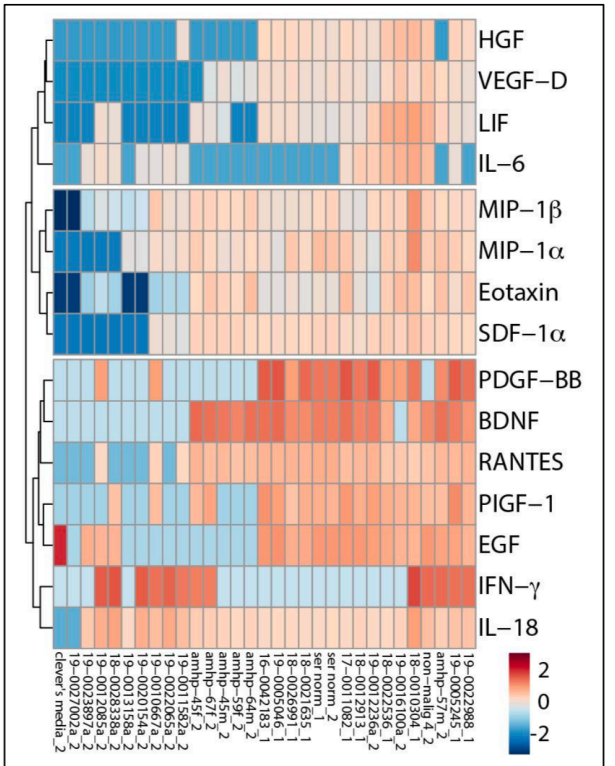

Fig. 1. Hierarchical clustering of the most variably expressed cytokines/growth factors in PE/ascites fluids. Although each sample had a unique expression profile, plasma and serum samples were more similar in composition than malignant effusions/ascites even when there were matching pleural effusion and plasma samples from the same cancer patient. Fluid samples from consecutive effusion taps from the same patients with metastatic disease showed tight cluster association.

Wednesday, September 29, 2021

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

Data Analytics and Informatics

B-001

Empirical Evaluation of the Precision of Slopes in Passing-Bablok Regression Analysis

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Background: Passing-Bablok regression analysis may be utilized for evaluation of potential bias between reagent lots. Evaluations are often comprised of measuring analyte concentrations of a set of patient samples that span the analytical measurement range for two reagent lots. Evaluation of lot acceptability includes parameters such as slope of the regression fit (a measure of bias), and r^2 value of the regression fit (a measure of fit imprecision). The bias may often be considered acceptable if the slope of regression line falls within 0.90-1.10. The objective of this study was to experimentally assess the reproducibility (imprecision) of the slope of Passing-Bablok regression analysis in relation to an acceptance criterion of 0.90-1.10.

Methods: Two reagent lots of the Beckman Coulter Access anti-thyroperoxidase (TPO) antibody and alpha-fetoprotein (AFP) assays were compared by Passing-Bablok regression analysis. For each assay, twenty patient samples with concentrations spanning the analytical measurement range (AMR) of the assays were tested eight times for each reagent lot. Independent Passing-Bablok regression analyses for each set of measurements were performed using Analyse-it for Microsoft Excel. The second reagent lot was calibrated prior to the start of testing, and after the fourth set of sample measurements. Testing was performed on a single UniCel DxI 800 instrument, and both reagent lot measurements were performed on the same day.

Results: Individual Passing-Bablok regression analysis of the TPO antibody assay yielded eight regression fit slopes ranging from 0.939 to 1.099, with mean slope being 1.018 (standard deviation (SD) of 0.051, coefficient of variation (%CV) of 5.0%). The mean slope of the four regressions from the first calibration was 1.049 with SD= 0.041, while the mean slope following the second calibration (n=4) was 0.983 (SD= 0.036). Passing-Bablok analysis of the AFP assay yielded eight regression fit slopes ranging from 0.863 to 0.971, with the mean regression fit slope being 0.911 (SD of 0.040, and %CV of 4.4%). The mean regression fit slope of the first calibration was 0.877 with SD= 0.009, and the mean slope was 0.954 (SD= 0.014) following the second calibration. All fit r^2 values for both assays ranged from 0.978 to 1.000, indicating acceptable precision and regression fit. In the lot to lot evaluation of the TPO assay minimal mean bias (+1.8%) was observed and all eight slopes felt within 0.90-1.10. In the AFP assay where substantial negative mean bias was observed (-8.9%), four out of the eight regression fit slopes fell outside the 0.90-1.10 fit slope acceptance criteria.

Conclusions: Imprecision between repeated Passing-Bablok regression slope determinations exhibited a CV of 5.0% for the TPO assay, and 4.4% for the AFP assay. This observed regression slope imprecision indicates that, in the theoretical absence of lot to lot systemic bias (slope of 1.00), ≥95% of regression fit slopes would fall within the acceptance criteria of 0.90-1.10, as the limits represent ≥2 SD of slope imprecision. Imprecision in regression slopes should be considered in the application of regression fit analysis, the ability of regression fits to detect bias, and in the establishment of acceptance criteria.

B-002

Introduction of a Web Based Laboratory Management Dashboard

A. J. Gammie. *Ortho Clinical Diagnostics, Pencoed, United Kingdom*

Background: Data analytics has the potential to improve patient care, save lives and lower costs. The key objective of these applications in healthcare is to extract insights for better informed decisions. **Methods:** Ortho Clinical Diagnostics has a long history in using e-Connectivity® to remotely monitor analyzer performance. Ortho provides customers with key performance insights through Ortho Plus, a secured online portal. Insights are available for the ORTHO VISION® and now available for the VITROS® systems via ORTHO PLUS℠. Users can log in via a secured website and see their data on a PC, tablet or phone and across single or multiple sites. **Results:** The VITROS Performance Dashboard includes overall productivity measures such as sample and test productivity with a 7-day moving average trendline. Workload balance information to assist in optimizing analyzer and assay usage; assay and reagent efficiency metrics. Quality information such as hemolysis, icterus and turbidity rates monitor sample quality and identify trends giving customers the insight to know how many results were reported without the need for a repeat blood draw. Quality is monitored by providing information that highlights when QC is performed to ensure optimal timing for productivity and compliance. Other quality indicators include, "first pass yield" demonstrating how often a result is reported the first time without unnecessary repeat testing. For infectious disease testing, weekly positivity rates and signal-to-cut-off (S/CO) results show the value of each test within the lot number, further demonstrating assay quality. The on-analyzer time information displays the time from first sampling to completion and allows the user to look at performance in multiple ways. **Conclusions:** The VITROS Performance dashboard is currently based on key analyzer performance data and will be expanded to cover more aspects of overall laboratory performance, including inventory management in future iterations.

B-003

Enhanced Sebia Phoresis protein electrophoresis reports incorporating immunoglobulin results automatically downloaded to Sebia instruments from Optilite (The Binding Site) instruments using Data Innovations (DI).

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

Background: Myeloma patients are evaluated using electrophoresis (SPEP) performed by the Capi3® Sebia system. SPEP reports are printed for pathologist interpretation. Optilite®, made by The Binding Site, immunoglobulin and free lights results are important for interpretation. Pathologists spend 30 seconds per case retrieving Optilite results from the LIS. 1,635 SPEP cases during 4 M-F work weeks, or 82 cases a day, show that pathologist must spend 42 minutes a day looking up results. Pathologists found that it would be highly beneficial to have the immunoglobulin and Free Lights results on the SPEP reports.**Methods:** First setup Phoresis, the Capi3 software: To make the Optilite tests available in the SPEP Template add them to the Worklist by labeling "Comment Field" columns with Optilite test names. In the Report Template, add the new worklist fields. DI sends the Optilite data in the format "test code: result"; delete the header text for each template item to avoiding redundant headers.Second, in Data Innovations (DI): Accomplish result routing using the DI Cluster function: In DI Cluster Setup, create Clusters which consists of an Optilite and all Capi3 instruments. In DI, Connection Assignment, for each Optilite, add all the Sebia Capi3 connections as destination lines. Create rules to suppress unwanted status messages, test comments, and specimen comments.Next, also in DI, use the Phoresis Worklist column headers as duplicate user friendly test codes. The numerical LIS (Beaker) test codes remain. Add the new test codes to the DI Sebia Phoresis configuration. Do not map new test codes in DI Optilite configuration. In the DI Sebia Phoresis configuration map the new test codes to themselves. (Example IgA mapped to IgA). In the DI Sebia configuration, Driver Properties, Driver Configuration tab, list the new test codes in the "Build Test Results into Comments Fields" boxes. In the DI rules engine, Optilite configuration, create a rule whose syntax means: If: A result for the corresponding LIS test code exists; Then: Add the new test code and make the result of the new test code equal to the result of the corresponding LIS test code. In the DI data stream place the new rule in "Incoming result", "Before Message Queued Internally".Lastly, now that test results are setup to send from Optilite to Sebia, prevent the new test codes from going to the LIS and causing fatal interface errors. Create a rule in the LIS Configuration and place it in the data stream location: "Outgoing result", "Before Message Sent to this Connection". Create a rule that means always suppress the tests listed in the rule's value list.**Conclusion:** The new SPEP report includes the Immunoglobulin and light chain results. Pathologists comment that the new report contributes to patient safety and enhanced workflow. Without DI rules, Sebia SPEP comments can associate with Optilite results going to the LIS, as well as cause intermittent order failure from the LIS to the Optilite. Validation/accuracy checks were performed by comparing LIS screenshots, Optilite printouts, and the Sebia report.

B-005

Reference Change Values of Hospitalized Patients differ from those of Healthy Subjects: It's Time to Get Real

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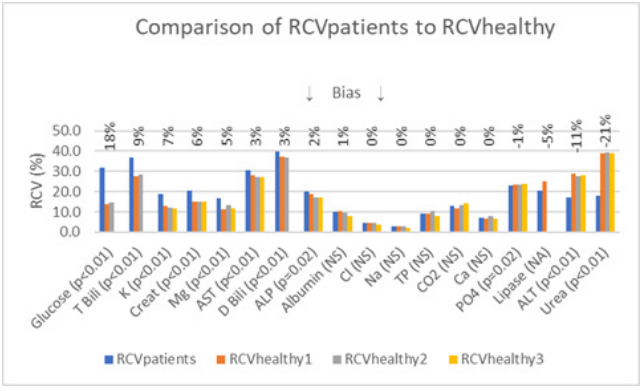
Background: Serial differences between intra-patient consecutive measurements can be transformed into Taylor series of variation vs time with the intersection at time=0 (yo) equal to the total variation (analytical + biological + preanalytical). With small preanalytical variation, yo, expressed as a percentage of the mean, is equal to the multiplicand of the RCV calculation: $(CV_a^2+CV_b^2)^{1/2}$ We determine the RCV of patient data for 18 analytes and compare them to healthy subjects' RCV.**Methods:** We analyzed 653 consecutive days of deidentified Dartmouth-Hitchcock Roche Modular general chemistry data (4.2 million results, approximately 60% inpatient and 40% outpatient). The serial patient values of 18 analytes (Figure) were transformed into 95% 2-sided RCV ($RCV_{Patient}$). 3 sets of $RCV_{Healthy}$ were calculated from 3 different Roche Modular analyzers'quality control summaries and a single set of curated CV_b derived from typical biological variation studies using healthy subjects. **Results:** The Figure compares $RCV_{Patient}$ to $RCV_{Healthy}$. For bicarbonate, calcium, chloride, sodium and total protein, the RCVs are statistically equivalent. Increased variation was found for glucose, direct and total bilirubin, creatinine, magnesium and AST. ALT and urea demonstrated lower variation **Discussion:** Water is not restricted In most healthy subject's biological variation experiments and we propose that water and food intake is better controlled in hospitals, resulting in reduced urea RCV. The healthy ALT CV_i of 9.3% originated from an IFCC study of 34 females and 35 males with unrepresentative average BMIs of 21.3 and 24.4, and average ALTs of 20.3 and 25.8 U/L, respectively. The patient ALT averaged 37 with a yo of 2.3 U/L with a resulting CV_i of 6%. It is time for the laboratory community to embrace working with and understanding variation in patients.



B-006

Evaluation in 2020 of the impact of flexibilization of fasting for the determination of the lipid profile in a sample of dyslipidemic patients in clinical laboratory practice.

L. N. Ribeiro, D. R. Ramadan, S. Tufik, M. C. Feres. *Associacao Fundo de Incentivo a Pesquisa, Sao Paulo, Brazil*

Background Currently, laboratory practice with more modern equipment and methodologies, the condition of not fasting, does not promote interference of lipemia in the results of laboratory tests. In the absence of many studies that show the superiority of these methodologies in laboratory practice in terms of clinical impact. The authors of this study aimed to evaluate the results of lipid profiles of a group of dyslipidemic patients who had dosages of these parameters in both conditions, fasting for 12 hours before 2014 and with easing of fasting in recent years. The main purpose of the authors is to evaluate, during the use of flexibilities of fasting and with new technologies, whether there was any impact on the diagnosis of dyslipidemias. **Material and Methods**: Retrospective observational study in which 58 patients were selected from a database in a large laboratory, who underwent lipid profile dosing before and after fasting easing and who had some type of dyslipidemia. For the tests of Cholesterol (Chol), HDL, LDL and Triglycerides (TG) performed before the flexibilization of the fast was used. o Beckman ® Analyzer - Coulter AU 5800 and after flexibilization - Atellica 1600- Siemens ® Analyzer. The method was automated colorimetric enzyme. To evaluate the sigmas metrics, different EQA references were used, such as RiliBÄK, Biological Variation, EFLM and CLIA. The reference values adopted were according to the Update of the Brazilian Guideline on Dyslipidemias and Prevention of Atherosclerosis - 2017. **Statistics:** Student's t-test was performed to compare the lipid profile in the two conditions and Pearson's (r) agreement test between the cases diagnosis of dyslipidemia. **Results:** Student's t-tests between fasting conditions and after fasting flexibility did not show any statistically significant difference in the studied parameters. Pearson's correlation tests (r) between the two conditions (JxF) were: Cholesterol, r = 0.988; LDL - C, r = 0.985; HDL - Col r = 0.953; VLDL, r = 0.648 and TG, r = 0.742. **Conclusion:** Pearson's correlation tests showed moderate indications of agreement for VLDL and TG between the two periods. This difference is probably due to the treatment that patients with hypertriglyceridemia followed during the years of treatment. This study did not control any variables because the objective was to validate the recommendations in clinical practice after years of flexibility. The study reinforced the recommendations of the National Pathology and Cardiology Societies in accordance with international recommendations, regarding the flexibility of fasting to determine the lipid profile. The results of this study did not show significant differences between the two conditions and the concordance tests did not impact the diagnosis of dyslipidemia in clinical practice.

B-007

Using big data to determine Reference Change Value (RCV) of ferritin, iron, total iron binding capacity and iron saturation in patients with iron deficiency.

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Background: Biologic variation (BV) and analytical variation (AV) are used to calculate RCV, an analyte's minimum change that is statistically significant. While BV is usually assessed in healthy subjects, it is not clear whether BV differs in subjects with iron deficiency. We use a unique methodology that generates from consecutive patient data the total variation [preanalytical+BV+AV]) [Clinical Biochemistry. 2017;50:936-41]. Here, we calculate between day and between week RCVs for iron-related tests. **Methods:** A data repository provided 88,000 total iron binding capacities (TIBC) and iron %saturations, 92,000 serum irons and 346,000 ferritins measured over 5 years in Ottawa Hospital inpatients and outpatients. For each analyte, we tabulated the pairs of intra-patient results that were separated by 3 hour intervals, 0-3, 3-6, 6-9, 9-12,⋯ up to 48 hours and weekly intervals up to 12 weeks. . The standard deviation of duplicates (SDD) of the paired analyte determinations was calculated for increasing time intervals. The graphs of SDD vs. time interval are linear and the y intercept represents $(AV^2 + BV^2)^{½}$. The between day and between week y intercepts were then expressed as percentage of the mean and then multiplied by the factor $2^{1/2}$ x 1.96 to provide RCV($p<0.05$). **Results:** The Table shows that the tested population has a high prevalence of iron deficiency. The RCV for weekly iron and ferritin in these iron deficient patients are 75% and 87%, respectively. Not surprisingly, the short term and long term variations of TIBC and iron saturation are roughly equivalent. **Discussion:** The increased variation in the week-to-week measurements is probably due to patients being drawn at variable times during the week with the resulting diurnal variation increasing the variation of the measured analyte. Use of these SDD-calculated RCVs should be incorporated into decision support software.

Between Days				
Test	Pt. Av.	yo	100 yo/Pt. Av.(%)	Ref. Change Value (%)
Iron, umol/L (RI 10 to 30)	14.2	2.7	19.0	53
TIBC, umol/L (RI 45 to 80)	57.2	4.7	8.2	23
Iron Saturation(%) (RI 20 to 50%)	27.4	7.3	26.6	74
Ferritin, ug/L (RI 20 to 320)	15.3	3.6	23.5	65
Between Weeks				
Test	Pt. Av.	yo	100 yo/Pt. Av. (%)	Ref Change Value (%)
Iron, umol/L (RI 10 to 30)	14	3.77	26.9	75
TIBC, umol/L (RI 45 to 80)	56.6	5.04	8.9	25
Iron Saturation(%) (RI 20 to 50%)	27.6	7.2	26.1	72
Ferritin, ug/L (RI 20 to 320)	15.3	4.82	31.5	87

B-008

Evaluating the Design and Performance of the Aptio Automation System Using Discrete Event Simulation

P. Zhao[1], D. Cooley[2], K. Cruse[1], S. McDonald[2]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]Siemens Healthcare Diagnostics, Oakville, ON, Canada*

Objective: This study aimed to assess the design and performance of the Aptio® Automation in clinical laboratories using a Discrete Event Simulation (DES) model. **Background**: A Discrete Event Simulation (DES) model was developed on Extend-Sim to give Siemens Healthcare Consulting Solutions (HCS) and lab managers an in-depth view of Aptio Automation system performance, specifically regarding throughput of the instruments connected to the track and method turn-around-times (TAT). The simulation model served as a tool for assessing the impact of instruments and automation modules on the laboratory Key Performance Indicators (KPIs). In this study, the following Aptio Automation configuration was analyzed: one Sysmex® CS-5100 Hemostasis System, one ADVIA Centaur® XPT Immunoassay System, two Dimension Vista® 500 integrated chemistry and immunoassay systems, one Input/Output Module for sample loading/unloading, two Centrifuge Modules, two Decapper Modules, one Sealer Module, and one Refrigerated Storage Module. The daily workload of the laboratory is 850 tubes and 8000 tests produced over 24 hours. The main KPI for the laboratory is Troponin-I-V TAT within 60 minutes from receipt of sample in the laboratory to result. **Methodology**: The simulation model used as input 24-hour laboratory information system (LIS) raw data of the scenario created using annual test volumes and the hourly distribution of the clinical laboratory accessions. Outputs of the simulation were compared with the actual performance of the Aptio Automation obtained from annual live system performance and workflow assessment reports. The following KPIs were compared to validate the results of the simulation: 1. TATs (mean, median, and 95th percentile) measured from loading of tubes on Aptio Automation to test results; 2. tube and test throughput; 3. an estimate of the analyzer utilization.**Results**: Daily workloads in the simulation model and Aptio Automation data from live system performance and workflow assessment reports were very similar (850 total tubes in the live system report vs. 838 in the simulation). The peak production hour occurred at 10:00 a.m. for both the simulation and live system reports. At the peak hour, 75 tubes were processed in simulation, and 74 tubes were processed in the live system report. In the simulation, 95th percentile TAT for Troponin-I-V was estimated at 29.4 minutes, and the mean TAT was 23.9 minutes. In the live system report, 95th percentile of TAT was 23.0 minutes, and mean TAT was 23.0 minutes. The peak hour instrument utilization for Troponin-I-V was 81% for Dimension Vista 500 -1 and 81% for Dimension Vista 500-2 in simulation. The live system performance report shows a peak hour instrument utilization of 89.62% for Dimension Vista 500 -1 and 95.64% for Dimension Vista 500 -2. **Conclusion**: In this study, the Discrete Event Simulation model predicts a very similar system performance to that achieved with the installed system. The model demonstrated its ability to evaluate Aptio Automation systems design and accurately predict its throughput and performance in clinical laboratories to the level of Key Performance Indicators.

B-009

Investigating epidemiological and geospatial trends in illicit drug use using laboratory data.

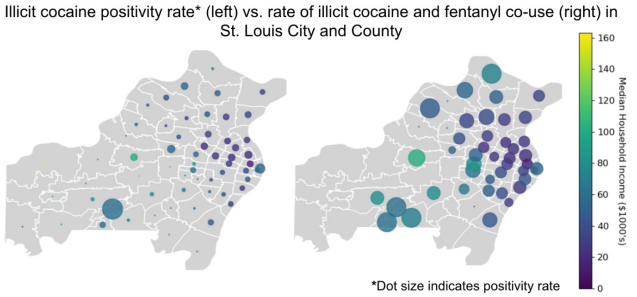
V. Azimi, C. W. Farnsworth, M. M. Budelier. *Washington University in St. Louis School of Medicine, St .Louis, MO*

Background: The rate of drug overdose deaths in the United States doubled from 6.1 deaths per 100,000 in 1999 to 21.6 deaths per 100,000 in 2019. Proactive drug use surveillance is crucial to informing timely and effective public health and safety responses. Our goal was to design an approach for combining laboratory, clinical, and demographic data to derive insights into drug use epidemiology in the St. Louis region.

Methods: We performed a retrospective review of urine drug screens (UDS) immunoassay results ordered from our emergency department from September 2019-August 2020. UDS results and ordering location were extracted from the laboratory information system. Clinical data, including patient demographics (zip-code, gender, age) and prescribed medications, were extracted from the electronic medical record and aligned to laboratory results. Publicly available population-level demographic data, including median household income was obtained from the United States Census Bureau and aligned by zip-code. Positivity rate (#positive screens/ #total screens) and co-positivity rate (e.g. #co-positive screens for fentanyl+cocaine/ #positive screens for cocaine) were calculated for each drug. Results were excluded if the patient had a concurrently prescribed medication of the same drug class.

Results: We identified 6,604 unique encounters with a UDS for which laboratory and clinical data exist. Analysis of UDS results identified socioeconomic disparities in the positivity rate for illicit cocaine use (more common in low income zip-codes), as well as illicit fentanyl and cocaine co-use (more common in high income zip-codes).

Conclusion: We used hospital UDS results to identify demographic and geospatial trends in illicit drug use. Given the real-time nature of laboratory data, this approach serves as an early proof-of-concept for using hospital laboratory data to elucidate real-time trends in population health metrics as well as epidemiological surveillance, with the potential to inform policy-making and optimal resource allocation of health and social services.



B-010

Test Utilization Study of Guideline Compliant Diagnostic Workup for Multiple Myeloma

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Background: Multiple myeloma is a hematologic malignancy that is often characterized by diagnostic delays. A systematic report highlighted that about 50% of patients with multiple myeloma experience a wait time greater than three months before diagnosis. Additionally, it is estimated that patients with multiple myeloma may seek primary care more than three times before referral to a specialist. Diagnostic delays are multifactorial, and the complexity of ordering appropriate clinical laboratory tests is a likely contributor. Clinical laboratories are well-positioned to identify gaps in testing as recommended by clinical practice guidelines and can identify mechanisms to improve test utilization to reduce time to diagnosis. This study's objective was to evaluate current myeloma test ordering patterns across different medical specialties to identify those that could benefit from targeted education regarding guideline-compliant testing for patients suspected to have multiple myeloma.

Methods: Laboratory orders for serum protein electrophoresis (SPE), serum immunotyping (SIT), serum-free light chains (SFLC), random urine protein electrophoresis (URPE), 24-hour urine protein electrophoresis (U24PE), and urine immunofixation electrophoresis (UIFE) received by TriCore Reference Laboratories from December 31, 2018, to December 31, 2019, were retrieved from the laboratory information system. Testing ordered within 24 hours was considered a single ordering event for the purpose of this study. Patients with an existing diagnosis of multiple myeloma or completed work up determined by ICD10 code C90.0 or bone marrow biopsy prior to 12/31/2018 were excluded. Lab test order combinations were grouped by medical specialty and compared to the tests (SPE+ SFLC) recommended by the International Myeloma Working Group guidelines for initial multiple myeloma screening.

Results: There were 4,206 individual test orders and more than 30 different combinations of screening tests received from 2,268 unique patients. The top three combina-

tions were SPE plus SFLC (41.5%), SPE alone (16.5%), and SFLC alone (8.7%). Other various test combinations accounted for the remaining 33.2% of orders. Overall compliance with screening guidelines was 41.5% across all specialties. By medical specialty, compliance adherence was as follows: 83.7% for hematology/oncology, 36.8% for internal medicine, 9.7% for family/general practice, 8.1% for rheumatology, 2.7%, and for nephrology.**Conclusion:** These results substantiate that reference laboratories can help identify clinical areas that might benefit from education on appropriate screening for improved guideline adherence and overall patient care. However, because we only looked at laboratory results, it is possible that some patients who had already been diagnosed with multiple myeloma may have been included in the study, consequently decreasing the accuracy of our analysis. Nonetheless, the findings of this study revealed overall low compliance with screening guidelines, especially in the groups that do not specialize in treating multiple myeloma (internal medicine, general practice, rheumatology, and nephrology). In order to decrease patient's time interval between screening and seeing the specialist, it is imperative to empower the providers who are generally seeing these patients first to order the most effective testing combination for screening.

B-011

Use of Artificial Intelligence for Effective Test Utilization and to Increase Reimbursement

R. Shrestha[1], Z. Kara[2], T. Hamill[3], S. Sajo Beqaj[4]. *[1]Patients Choice Laboratories, Indianapolis, IN, [2]Medical Database, Irvine, CA, [3]UCSF Department of Laboratory Medicine, San Francisco, CA, [4]UltimateDx Laboratories, Los Angeles, CA*

Background: The quality of laboratory investigations not only depends on testing methodology but also on the provider choosing the right tests to assist clinical decision-making. Unfortunately, it is estimated that at least 20% of the 5 billion lab orders submitted annually are inappropriate. Studies have shown that overutilization and/or underutilization of laboratory tests occurs 20.6% and 44.8% of the time, respectively. This inappropriate testing not only leads to incorrect or delayed diagnoses but also significantly adds financial burden. Use of an appropriate laboratory test utilization management system can significantly overcome the misutilization of laboratory investigations. Currently, Laboratory Decision System - LDS® developed by Medical Database is the only automated test utilization management system that uses evidence-based guidelines and industry best practices to assist healthcare providers understand, select, order, and optimally utilize tests for disease diagnosis and management. This algorithm-based testing selection and ordering database rates and scores potential tests for any given disease and assigns an easily interpretable numeric (1-10) and color-coded score based on clinical relevance, medical necessity, and testing indication. Importantly, every order using LDS will also have right CPT and ICD-10 codes assigned to meet the medical necessity and improve reimbursement. Therefore, in this study, we evaluated the performance of LDS as a testing utilization management system and its ability to improve reimbursement.

Methods: A total of 96,170 laboratory requests comprising 374,423 test orders were analyzed from a reference laboratory. Of these, 814 tests were accompanied by an invalid ICD10 code and 44,671 tests or were accompanied by ICD10 that are described by Medicare as "never covered" because of inability to meet medical necessity. 160,449 tests were subject to an Medicare policy review from which 112,400 tests met coverage criteria and 48,049 tests did not. These orders were then reevaluated using LDS®, which can be accessed from www.medicaldatabase.com, to determine if the system would have improved test selection and reimbursement.

Results: Of the original test order sample, 342,699 tests (91.5%) had an associated LDS score. Of these scored tests, 178,962 (47.80%) met coverage and 163,737 (43.73%) failed to meet coverage, according to the LDS Ranking System. Importantly, LDS provided recommendations for alternative diagnostic ICD10 codes or tests which could have aided physicians in choosing a more appropriate test or submitting a different ICD10 diagnostic code to meet medical necessity. Based on a subset composed of the first 10,000 claims reviewed, the LDS recommended 9,637 tests (96.4%) with an alternative ICD10 code or test with a score above 5, meeting medical necessity. Of these, 7755 tests (80.5%) were recommended by the LDS system which would meet Medicare policies, demonstrating that LDS system would correct inappropriate orders if employed as a testing utilization management system.

Conclusion: There is an urgent need for a laboratory decision support system which can aid providers in selecting the right test for each disease or condition, while assigning the correct ICD10 code to meet the medical necessity. LDS can assist providers in making appropriate utilization decisions while also supporting laboratories in reimbursement.

B-012

Automated Interpretation of Serum Protein Electrophoresis

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Background: Serum protein electrophoresis (SPE) plays a critical role in diagnosing and monitoring patients with clonal plasma cell disorders. SPE data consist of one-dimensional traces with characteristic peaks that correspond to defined protein populations. Interpreting these profiles requires manual inspection, which is labor-intensive and subjective. To address this, we developed a machine learning model to predict the SPE diagnostic labels assigned to clinical samples.**Methods:** 6,737 traces from SPE performed on a Sebia Capillarys 3 were used for modeling. The seven target labels were: no apparent monoclonal peak, abnormal alpha-2, abnormal beta-1, abnormal/possible abnormal beta-2, and abnormal/possible abnormal gamma. For each trace, candidate peaks were identified, and 107 morphological features were extracted. Samples were split 80/20 into training/testing sets, and the following models were trained for binary (normal vs. abnormal) and multiclass (specific label) classification tasks: k-nearest neighbors, penalized logistic regression, random forest, and gradient boosting machine. Hyperparameters were tuned using repeated cross-validation or Bayesian optimization. Area under the receiver operating characteristic curve (AUC-ROC) and precision recall curve (AUC-PR) were calculated on the test set. Data processing and modeling were implemented in R (v4.0.3) using tidymodels (v0.1.2).**Results:** The best binary classification was obtained with penalized regression, with an AUC-ROC of 0.985 and AUC-PR of 0.993 (Table). At fixed sensitivities of 0.90, 0.95, or 0.99, the specificities of penalized regression were 0.97, 0.92, or 0.73, respectively. The best multiclass classification was obtained with the gradient boosting machine, with an AUC-ROC of 0.978 and AUC-PR of 0.895. Errors were predominantly traces with possible abnormal peaks predicted as normal (or vice versa).**Conclusion:** Features used by laboratorians to interpret SPE can be automatically extracted and used to accurately predict SPE diagnostic labels. In practice, these tools could reduce manual review time and standardize interpretations. This approach is generally applicable to one-dimensional data produced for multiple applications.

Table: Model Performance

Classification Task	Model	AUC-ROC (95 % CI)	AUC-PR (95% CI)
Binary	K-nearest Neighbors	0.948 (0.937, 0.958)	0.976 (0.970, 0.981)
	Penalized Logistic Regression	**0.985 (0.980, 0.990)**	**0.993 (0.990, 0.995)**
	Random Forest	0.981 (0.976, 0.986)	0.991 (0.987, 0.993)
	Gradient Boosting Machine	0.985 (0.980, 0.989)	0.992 (0.990, 0.995)
Multiclass	K-Nearest Neighbors	0.938 (0.913, 0.952)	0.799 (0.758, 0.835)
	Penalized Logistic Regression	0.972 (0.957, 0.978)	0.847 (0.809, 0.880)
	Random Forest	0.974 (0.961, 0.982)	0.867 (0.829, 0.905)
	Gradient Boosting Machine	**0.978 (0.966, 0.984)**	**0.895 (0.868, 0.923)**

B-013

Optimization of "Moving Average" Parameters On the Architect C Systems in a Multi-Site Inpatient and Outpatient Oncologic Population

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Background: Traditional quality control (QC) methods assess the accuracy of an instrument at a given time. "Moving Averages", (MA) or patient-based real-time quality control, can be used to monitor instrument performance in real-time, offering greater instrument performance monitoring compared to traditional QC methods. Determining ideal MA parameters is a tedious task that requires analyzing result variability across a large data set. Using MA Generator, a commercially available software program, large data sets can be easily analyzed to determine ideal parameters to afford precise instrument performance. We have used MA Generator to determine the ideal moving average parameters across 4 analytes in a large multisite healthcare system comprising of oncology patients in both the inpatient and outpatient settings.

Methods: Sodium, chloride, potassium, and calcium were analyzed using either the Abbott Architect c4000, c8000, or c16000 analyzers. Results were monitored across 20 distinct analyzers in 10 separate hospital labs and collated into the Instrument Manager (IM) middleware software. Initially, results were recorded continuously for 14 days and a base mean and standard deviation (SD) was established. Rules were created in IM to afford either warning or error flag when results shifted 1SD (warning) and 2SD (error) from the mean. To optimize these warning and error flags, the analyte mean and SD was optimized through analysis using MA Generator software. Over 3 months' worth of patient results for the aforementioned analytes were imported into the software. MA Generator parameters were set to match the total allowable error (TaE) and total allowable bias of each analyte. Mean and SD determined by the MA Generator was compared to the previously established base mean and SD. With the new parameters determined, IM MA protocol settings were configured so that notifications were sent to staff when shifts outside these parameters occurred.

Results: Average number of entries per site into the MA Generator data set was as follows: sodium:13,900 entries, chloride:13,300 entries, potassium: 15,000 entries, calcium: 13,700 entries. This led to the following optimization from our initially established mean and SD to the MA Generator established mean and SD. Sodium: mean: 138.78 to 138.40 mEq/L, SD: 2.95 to 2.72 mEq/L (TaE <3%), chloride: mean: 104.6 to 104.2 mEq/L, SD: 3.54 to 3.09 mEq/L (TaE<5%), potassium: mean 4.41 to 4.10 mEq/L, SD: 0.46 to 0.32 mEq/L (TaE <10%), calcium: mean: 9.15 to 9.08 mEq/L, SD: from 0.58 to 0.56 mEq/L (TAE <10%).

Conclusion: Through the use of MA Generator software, our institution was afforded enhanced detection of shifts in analyzer performance in real-time, allowing for immediate instrument intervention (troubleshooting, recalibration, etc.). These analytical issues would typically go undetected for several hours - until the next time QC was analyzed. This software worked well in both physiologically variable inpatient, and more physiologically stable outpatient populations. Moving averages are a novel solution that immediately detects analytical issues before patient results are compromised. MA Generator software simplifies the historically arduous task of determining moving average parameters.

B-014

Need for benchmarks- mitigating false-positives rate by monitoring prenatal screens

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Background: Prenatal screening is helpful in managing the pregnancy, determining the outcome of pregnancy, planning for possible complications with the birth process, and finding conditions that may affect future pregnancies. Population-based screening of women for open neural tube defects (NTD), Down syndrome, and Trisomy 18 present special challenges to lab testing. Often, these women who test positive are offered counseling and followed with genetic testing. At our institution for second-trimester screening (performed between 15-22 weeks), we use the following cut-offs: NTD (2.01MoM - multiples of median), Down syndrome (1:270 risk ratio), and Trisomy 18 (1:100 risk ratio). All MoM values are adjusted to take into account- maternal race, maternal weight, diabetic status, and smoking. The College of American Pathologists (CAP) advocates labs that perform prenatal screening use a stratified approach to monitoring screen performance at least bi-annually or optimally quarterly. Usually, 1-3% of the screen-positive women are true positives in their index screen test, which is dependent on a combination of markers and trimester of screen performed. The brunt of the screening process falls on the women with false-positive outcomes that may include unnecessary stress, additional testing, costs, and unnecessary procedures that may risk fetal life. Therefore, it is very critical to obtain the right balance between true positives by detecting the target-oriented disorder and minimize false-positive rates. Herein, we aim to monitor the performance of the prenatal screen positives by established benchmark. Furthermore, our purpose is not only to serve as a continuing measure of assay quality, but also serves to check the appropriateness of medians, the accuracy of gestational dating, and the distribution of maternal age. Method: We performed a 5-year retrospective analysis of second trimester prenatal screens performed at our institution. In addition, we established benchmarks for each disorder and analyzed the demographics of the maternal population along with the factors that have confounding effect on the prenatal screen. Results: The data from 2016 to 2020 showed 8,847 women were screened for serious disorders. Our data shows the following race demographic breakdown - African Americans (41%), White (41%), Others (4%), Unidentified (15%). Out of 8,847 women screened: 3% of the women were overweight (>280lbs), 3% of the women identified themselves as smokers and 2% were identified as diabetic. The overall unique disorder percentage is 3% of the 8,847 women screened for serious disorders. The mean age of the women who tested positive for the disorder is 28 years. The benchmark for the screen-positive rate for each disorder is as follows: NTD (3.5%), Down syndrome (1.4%), and Trisomy 18 (1%). In the last quarter of 2020, the Trisomy 18 screen positive rate increased by 0.8%. This might account for a discrepant rate and consequently, a corrective action was appropriately taken. After making appropriate median adjustments the first quarter of 2021 showed a decrease in Trisomy 18 by 0.6%. Conclusion: It is extremely critical to establish benchmarks that will be used for ongoing monitoring of prenatal screening performances, maintaining low false-positive rates and making appropriate adjustments when warranted.

B-015

Implications of a novel emergency department sepsis diagnostic test for costs and outcomes

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Background: Sepsis is common, deadly, and costly, causing 270,000 deaths and costing $1.5 billion among US patients annually. Most cases of sepsis present in the emergency department (ED), where rapid diagnosis remains critical and challenging. The IntelliSep Index (ISI), a novel diagnostic analyzing characteristics of white blood cell structure, provides a reliable early signal for sepsis, potentially improving on current sepsis diagnostic tools, such as procalcitonin (PCT). This study performed a cost-consequence analysis of ISI relative to PCT for early sepsis diagnosis in the ED. **Methods:** A decision tree analysis was performed comparing ISI to PCT—two sepsis diagnostic testing strategies that can be used in the ED in patients with signs or suspicion of infection. Model parameters included prevalence of sepsis, sensitivity and specificity of diagnostic tests (both ISI and PCT), costs of hospitalization, and mortality rate stratified by diagnostic test result. Mortality and prevalence of sepsis were estimated from best available literature. Costs were estimated from the health system perspective, based on an analysis of a large, national discharge data set, and adjusted to 2018 US dollars. Outcomes included expected costs and survival. The decision tree model applied test characteristics for both ISI and PCT to a hypothetical cohort with suspicion for sepsis, in order to estimate differences in cost and mortality when either ISI or PCT is utilized for sepsis identification. **Results:** Assuming a confirmed sepsis prevalence of 16.9% (adjudicated to Sepsis-3), the ISI strategy had an expected cost per patient of $3,742 and expected survival rate of 95.05%, while the PCT strategy had an expected cost of $4,657 per patient and an expected survival of 94.96%. ISI was both less costly and more effective than PCT, primarily because of fewer false negative results. This result was robust in in one-way sensitivity analyses of prevalence, cost of the diagnostic tests, and costs of hospitalization. **Conclusion:** Compared to PCT, the ISI may provide health systems with a higher value diagnostic test in ED sepsis evaluation. Specifically, the ISI strategy improved stratification of very high and very low risk patients, with fewer false negative results. Additional work is needed to validate these results in clinical practice.

B-016

A Tale of Two Tests: Pandemic Impact on Point of Care Testing Use and Patient Management

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Background: Our health system deploys point of care (POC) testing in a wide range of clinical settings. We perform 2.5 million annual POC tests in waived and non-waived settings, using more than 1200 POC devices. Our program includes outpatient waived testing programs in Coumadin monitoring using waived PT/INR testing as well as diabetes management through waived POC Hemoglobin A1c (HbA1c) testing. Many patient visits to primary care settings were cancelled, deferred and/or rescheduled to virtual when pandemic precautions were implemented in March 2020. The impact on routine patient care was significant in many aspects of healthcare; we sought to evaluate the effect of the pandemic precautions from the perspective of the POC testing program.Methods: Our PT/INR monitoring devices (Roche Coaguchek XS) and HbA1c platforms (Abbott Afinion) are networked to the patient medical record (EPIC) via middleware systems (TELCOR and RALS). Test data was exported from middleware repositories and patient information was removed. Using a combination of MS Excel and open source analysis programs (R and Orange), the data was filtered to remove non-patient results (QC, competency, etc.) and then analyzed for test volumes and changes in the distribution of test results. Results that indicated a decline in successful patient management (INR>3.5, HbA1c > 9%) were classified as poorly controlled and the fraction of these results were compared to historical trends.Results: Our pre-pandemic outpatient testing programs included INR monitoring of patients on Coumadin (approximately 11,000 tests/month) and management of diabetes patients who did not get a HbA1c test result from the lab prior to a doctor's visit (approximately 4500 tests/month). In April and May of 2020 ("early" pandemic), INR test volumes dropped to about 60% of pre-pandemic levels then gradually increased. However, during the period from January to July, the percentage of poorly controlled patients increased from 10.3% to 13.1%. For HbA1c, test volumes dropped significantly in March (50%) and dramatically lower in April (3%). The test volumes slowly recovered from May to July, but they were still only 50% of the pre-pandemic levels. The percentage of poorly controlled patients increased from 7.8% to 9.5% in March but quickly returned to the historical level in April.Conclusions: The decrease in POC test volumes after implementation of pandemic precautions was quantified in this analysis. The drop in POC HbA1c testing could be due to several factors, including patient confidence in their diabetes self-management, or the reluctance of the high risk COVID19 patient population to seek in-person care at this stage of the pandemic. The fraction of poorly controlled patients briefly increased, but returned to historical levels within a few months. The drop in INR testing volume was not as dramatic, perhaps due to the acute risks of poor management, the brevity and relative safety of Coumadin clinic visits, or the temporary nature of treatment for many anti-coagulated patients (e.g. pre-procedural). Deferred care and less frequent testing likely account for the increased fraction of poorly controlled patients.

B-017

Algorithms Developed using Long Short-term Memory Machine Learning Models Can Appreciably Improve Cancer Screening with Tumor Biomarkers

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Background: Annual screening for cancers in asymptomatic populations by evaluating tumor marker (TM) levels is common throughout the world. Applying machine learning (ML) derived algorithms to improve the predictive value of a set panel of tumor markers for cancer screening has previously been demonstrated to be useful. In real world settings, however, the number of tumor markers tested often varies as a function of availability, cost and clinical preference. It is therefore necessary to develop machine learning algorithms that can analyze incomplete tumor marker panels to predict cancer risk. Furthermore, as more individuals take advantage of annual cancer screening protocols, there is great potential of further enhancing predictive power by incorporating serial TM values in such algorithms. Long short-term memory (LSTM) is an artificial recurrent neural network architecture that employs feedback connections allowing it to process both single point as well as serial data and to allow for missing values and as such is well suited for the development of cancer screening algorithms.

Methods: TM, demographic and other clinical data from real-world cancer screenings were collected from asymptomatic cohorts at two independent medical centers between May 2001 and Dec 2019. The cohorts contained 163,184 individual subjects, including 785 subsequently diagnosed cancer cases. This data was used to train and validate cancer screening models using the LSTM framework. The resulting algorithms assign prediction scores that are used to classify individual cases into risk groups. This algorithm is further tested using inputs with missing TM values. Additionally, the relationship between time-to-cancer diagnosis and the ML prediction was evaluated by Cox-regression analysis.

Results: A risk prediction algorithm has been developed using an LSTM model which can accept one, two three or four TM values from a panel consisting of CEA, AFP, PSA, and CA199. The AUROC of this algorithm for screening cancers is 0.831 (95% confidence interval: 0.827-0.835) when all markers are present, and decreases when fewer markers are used down to an AUCROC of ~0.746 depending on the choice of the marker. By comparison, a single threshold method, which simply predicts cancer on the basis of any of the four markers being above a standard cutoff value yields an AUCROC of 0.628 (0.591-0.665). Using the results of this LSTM algorithm to correlate prediction scores with time-to-cancer diagnosis by Cox-regression analysis, identified strategy of subsequent follow-up, age, AFP, CEA, and CA19-9 levels as informative features for risk stratification.

Conclusion: A predictive algorithm has been developed to stratify cancer risk in asymptomatic populations. The algorithm accepts as input 1, 2, 3 or 4 single time point TM values and can thus be applied widely to many subjects around the world. The risk scores assigned by this algorithm generally correlate well to time to diagnosis in those individuals who ultimately develop cancer supporting the use of proactive clinical follow-up for all individuals with higher scores.

B-018

Automating HIV Viral Load Results Reporting from an Electronic Laboratory Information System to Antiretroviral Therapy Data System using eLabMessenger and DHIS2

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Background and Purpose: Automation of HIV Viral Load Results Reporting is regarded as critical for handling Viral Load data. However, owing to the difficulties of implementing interoperability through health information systems (HIS), electronic viral load results are often printed and re-entered into the Antiretroviral Therapy (ART) Data System. This study demonstrates how DHIS2 Tracker, eLabMessenger, and the Roche Cobas Amplilink Software can be combined to capture HIV patients' Viral Load (VL) requests from ART clinics and transmit them to eLabMessenger, with the eLabMessenger also automatically submitting the patients' VL results back to the DHIS2 Tracker through eLabMessenger interface client with the Roche Cobas Amplilink Software.

Methods: We deployed the eLabMessenger and DHIS2 tracker in a test environment at the Korle-bu Teaching Hospital, Accra, Ghana Viral Load Testing Laboratory. DHIS2 Tracker was configured to push demo HIV patients' Viral Load (VL) test requests from ART clinics using the DHIS2 Tracker VL test request form, and eLabMessenger was set up to receive them. The eLabMessenger interface client then pulls the HIV patients' Viral Load results from the Roche Cobas Amplilink Software and sends them back to the DHIS2 Tracker.

Result: The DHIS2 Tracker VL test request form was used to automatically relay HIV patients' Viral Load (VL) test requests from ART clinics to the eLabMessenger. The eLabMessenger interface client then successfully pulled the HIV patients' Viral Load results from the Roche Cobas Amplilink Software and submitted them back to the DHIS2 Tracker. The Viral Load results for patients were correctly received by DHIS2 Tracker.

Discussions: According to the findings, HIV patients' Viral Load (VL) test results can be submitted automatically from eLabMessenger to DHIS2, removing the need for manual data entry. The effectiveness of this test would aid in determining the effects of incorporating an automatic delivery of HIV patients' Viral Load (VL) test results in order to minimise the number of human resources required to meet reporting needs while also improving data accuracy, completeness, and timeliness. These results show that expanding electronic HIS will further improve health care quality, M&E, and public health management.

B-019

Clinicians' Probability Calculator to convert pre-test to post-test probability of COVID-19 based on method validation from each laboratory

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Background: Despite best efforts, false positive and false negative Covid-19 test results are unavoidable. Likelihood ratios can convert a clinical opinion of pre-test probability to post-test probability, independently of the prevalence of the disease in the test population. The authors created an online calculator to overcome the drawbacks to Fagan's Nomogram, which is typically used with the likelihood ratios and is cumbersome for front-line use and does not include confidence intervals.

Methods: The authors examined results of PPA (Positive Percent Agreement, sensitivity) and NPA (Negative Percent Agreement, specificity) from 73 laboratory experiments for molecular tests for SARS- CoV-2 as reported to the FIND database 6 , and for two manufacturers' claims in FDA EUA submissions.PPA and NPA were converted to likelihood ratios to calculate the post-test probability of disease based on the clinical opinion of pre-test probability. Confidence intervals were based on the number of samples tested. An online calculator was created to help clinicians identify false-positive or false-negative COVID-19 test results.**Results:** Laboratory results from the same test methods did not mirror each other or the manufacturer. Laboratory studies 6 showed PPA from 17% to 100% and NPA from 70.4% to 100%. The number of known samples varied 8 to 675 known patient samples, which greatly impacted confidence intervals.

Conclusions: Post-test probability of the presence of disease (true-positive or false-negative tests) varies with clinical pre-test probability, likelihood ratios, and confidence intervals. The "Clinician's Probability Calculator" creates reports to help clinicians with an estimation of the post-test probability of COVID-19 based on the testing, the laboratory-verified PPA and NPA.

Link for the calculator: https://awesome-numbers.com/post-test-probability-calculator/

B-020

Indirect Estimation of Reference Intervals for Serum Thyroid Stimulating Hormone (TSH) in Pakistani Neonates from Mixed Distributions using Truncation Points and the Kolmogorov-Smirnov Distance (KOSMIC)

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Background: Serum TSH reference intervals (RIs) are methodology, population and age specific. However, the ethical and practical challenges restrict the establishment of pediatric RIs using conventional approaches advocates the use of indirect data mining based algorithms. This study was carried out to estimate the reference interval of neonatal serum TSH in Pakistani population using an indirect approach. **Methods:** After exemption by the institutional ethical review committee, all the serum TSH results with date of collection from neonates aged up to 1 months for both genders, over a 6-year period from 2013 to 2018 were extracted from the laboratory information management systems database. To study the age dynamics, the dataset was further stratified into 0-5 days and 6-30 days. The diversified dataset comprised of both diseased and normal cases. Samples obtained during clinical care from hospitalized subjects as well as those subjected to routine screening and outside referrals performed regardless of the clinical indication were included. In case of multiple test request form the same individual, only the first sample's results was included in the final analysis. Serum TSH was analyzed by Chemiluminescence immunoassay (CLIA) on ADVIA® Centaur™, Siemens platform. The laboratory performs to the best of standards for both internal and external quality assurance and is accredited by the College of American Pathologists.A validated tool by the German Society of Clinical Chemistry and Laboratory Medicine's Working Group on Guide Limits (DGKL) was utilized for the data analysis. The built in algorithm functions upon diminishing the variations due to the estimated parametrical distribution and truncation, using the Kolmogorov-Smirnov-distance followed by a Box-Cox-transformation. A statistical program which is implemented within a software package from the DGKL (https://www.dgkl.de/verbandsarbeit/arbeitsgruppen/entscheidungsgrenzen-richtwerte/) is available to calculate the Box-Cox transformation parameter lambda (λ), the truncation interval, and the parameters of the Gaussian distribution Mu (μ) and sigma (σ). Fine grained details of the statistical analysis utilized are available from Zierk J et al. To estimate confidence intervals, bootstrapping of the input dataset was undertaken. Subsequently, the lower and upper reference intervals with their 90% confidence interval (CI) were derived for the two age groups. Moreover, a comparison of our derived serum neonatal TSH from Mayo clinical laboratory and Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) recommended values was also done. **Results:** A total of non-duplicate 82299 neonatal serum TSH tests were retrieved over a period of 6 years, including 88 % (n=70788) aged 0-5 days and 12 % (n=11511) ranging from 6 days to 1 month. The estimated RIs for the first age partition was 0.7 (90% CI 0.6-0.8) to 15.5 (90% CI 12.9-16.2) and for the second group 0.7 (90% CI 0.5-0.9) to 7.8 (90% CI 6.1-9.9) uIu/mL. **Conclusion:** This study revealed age related trends in serum TSH. The study advocates the need for population specific RIs owing to the significant variations noted on comparison with previously published literature. Precise RIs becomes vital particularly when serum TSH is undertaken as a confirmatory test for presumptive positive results on newborn screening for congenital hypothyroidism.

B-022

Sex specific reference value for fasting insulin and HOMA-IR index in healthy adults of Rio de Janeiro using Roche-COBAS platform

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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, non-alcoholic liver disease and polycystic ovary syndrome. Insulin and especially Homeostatic Model Assessment Insulin Resistance index (HOMA-IR) are relatively simple and reliable noninvasive markers of IR. Given the importance of these markers, and the considerably variation of insulin reference interval (RI) between manufactures of commercially available kits, we believe it is essential to establish a reliable RI for these markers in our population. **Objectives:** The aim of this study was to determine RI of fating insulin and HOMA-IR index in adults living in Rio de Janeiro -Brazil and to verify if there was any difference of RIs between genders. **Design and methods:** Fasting serum insulin levels of 204388 subjects submitted to blood sampling in 2019 in the city of Rio de Janeiro were obtained retrospectively through access to a big data base 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. Exclusion criteria were body mass index (BMI) ≥ 25 kg/m^2, glucose ≥ 100 mg/dL, A1C ≥ 5,7 % , triglycerides ≥ 150 mg/dL and HDL < 50 for women and < 40 mg/dL for men. After applying exclusion criteria, 31780 "healthy" subjects, 26628 women (84 % and 5152 men (16 %), above 20 years were included (range 20 to 98 and average 42 years). **Statistics:** Kolmogorov-Smirnov test was used to verify if insulin values were normally distributed. As distribution was not normal, logarithmic transformation was applied. Student's t test was used to verify difference between genders. For evaluation of statistical significance, p <0.01 was considered. After exclusion of outliers, the central 95% insulin concentrations were considered for determination of RI. **Results**: Overall 95% RIs for fasting insulin were, respectively, 2.5-13.0, 2.3-12.6 and 2.4-12.9 μU/mL in women, men and total population with no statistical difference between genders (p=0.4). HOMA-IR index RIs were, respectively, 0.42-3.17, 0.4-3.0 and 0.42-3.15 in woman, men and total population again with no statistical difference between genders (p=0.2). **Conclusion:** As we did not observe a statistically significant difference in insulin reference values and HOMA-IR index between genders, we conclude that the values found for the total population can be used for both genders. Thus, we suggest the following RIs for our population: fasting Insulin: 2.4-12.9 μU/mL and HOMA-IR index: 0.42-3.15.

B-023

Using Natural Language Processing to Improve Discreet Data Capture from Interpretive Cervical Biopsy Diagnoses at a Large Healthcare Organization

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Background: Different terms have been used to describe inflammatory, borderline, and precancerous changes of the cervical epithelium. Current descriptive terms include atypia, dysplasia, cervical intraepithelial neoplasia (CIN), squamous intraepithelial lesion (SIL), among others. Changes to the squamous epithelium are defined by either two or three tiers of severity: low and high grade SIL, as defined by the Lower Anogenital Squamous Terminology (LAST); mild, moderate and severe atypia and dysplasia; and low, moderate, and high grade CIN (CIN terminology). In addition,

descriptive text is often added to modify or clarify CIN or SIL diagnostic terminology. Descriptive phrases, such as "cannot exclude," or "small focus of," may lack clarity, risking misclassification and inappropriate management as defined by the three tier CIN terminology (ASCCP). Non-standardized terminology and narrative text may also hinder data capture from the final diagnostic report. While the use of synoptic reporting has begun to address this issue, most anatomic pathology diagnoses remain unstructured. Accordingly, patient management often relies on a clinician's interpretation of a narrative, non-structured pathology report. At Kaiser Permanente Northern California (KPNC), we derive an actionable outcome by manually processing 35,000 colposcopy reports annually. To automate this process, we developed natural language processing (NLP) algorithms to extract interpretive biopsy diagnoses and generate a discreet outcome using CIN terminology. Here, we report the accuracy of the algorithms compared to the manual abstraction adjudicated by expert pathology coding of discreet categories. The NLP approach is intended to automate and expedite translation of interpretive text to a single-most severe, and thus actionable CIN diagnosis, augmenting and expediting the manual review and coding process to ensure appropriate treatment. **Methods:** There were 35,847 colposcopy pathology reports identified from August 2019 to July 2020 through our Laboratory Information System (LIS). We developed and applied an NLP algorithm to each colposcopy pathology report, inclusive of all biopsies and/or ECC within each report. From all biopsies within each report, the algorithm selected only the highest severity CIN; specifically, the diagnosis that would dictate post-colposcopy patient management. We then compared the NLP algorithm generated CIN diagnosis with the highest severity CIN reported in the original diagnostic text for each colposcopy pathology report. We defined significant non-concordance between the two as any difference that would lead to different patient management, e.g. CIN2 vs CIN3 represents a significant difference in patient care among women of childbearing age, whereas CIN2-3 vs CIN3 does not. Evaluation of the NLP algorithms' performance was measured by calculated precision, recall and F-score. **Results:** The NLP algorithms yielded a precision of 95.7%, a recall of 92.5% and an F-score of 94. Additionally, we estimated the time to evaluate each monthly biopsy file was significantly reduced from 30 hours to 0.5 hours. **Conclusion:** A set of validated NLP algorithms applied to colposcopy pathology reports can rapidly and effectively assign a discreet, actionable diagnosis using CIN classification. Moreover, discreet diagnostic data encoded as CIN terminology can be used to automate analytics for quality, surveillance, and research purposes.

B-025

Performance Characteristics of a Novel Theoretical Control Method for the Prostate Health Index (*phi*) Multi-Analyte Assay

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Background: Multi-analyte assays with algorithmic analyses (MAAAs) are assays that combine multiple analytes and demographic results into a calculated diagnostic index. The Prostate Health Index (*phi)* is one MAAA used to estimate prostate cancer probability prior to biopsy. Currently, like other MAAA assays, imprecision and bias in *phi* itself are typically indirectly monitored by established quality control (QC) of individual assay components. It is unknown if this approach yields reliable quality control of *phi* itself, as *phi* is simultaneously affected by three PSA components [*phi*=(p2PSA/freePSA)x(totalPSA)$^{0.5}$]. In order to more directly assess and monitor *phi* imprecision and bias, a novel calculated theoretical *phi* QC (t*Phi*C) metric was utilized. **Methods:** Intra-assay (60 measurements/level) and inter-assay (20 measurements/level, 20 days) precision of three QC levels per analyte were determined by measurement of total (tPSA), free (fPSA) (Immunoassay Liquichek, Bio-Rad, Irvine, CA), and p2PSA (Beckman Coulter, Brea, CA) on a Unicel DxI 800 immunoassay instrument (Beckman Coulter, Brea, CA). The mid control levels for tPSA and p2PSA and high control level for fPSA were used to generate a t*Phi*C that exhibited *phi* values (~25) associated with moderate prostate cancer probability. Routine QC measurements of all control levels for each component and for t*Phi*C were monitored for several weeks of daily operations (84 QC runs/level). The 1_{3s} Westgard rule was used to assess for QC failure in both t*Phi*C and components. Finally, to investigate potential effects on *phi* stemming from index component bias, hypothetical component bias was introduced in theory, and the resulting impact on t*Phi*C bias was determined.**Results:** In general, observed intra- and inter-assay *phi* imprecision was higher than that of the components. For example, intra-day *phi* %CV imprecision for the mid control level determinations were 6.7% for calculated *phi* versus 4.3%, 4.5%, and 3.9% for tPSA, fPSA, and p2PSA, respectively. For monitoring of t*Phi*C over time, the inter-assay %CV (mean) obtained was 7.3% (24.5) as compared to 4.8% (3.8 ng/mL) for the tPSA mid control, 3.9% (14.1 ng/mL) for fPSA high control, and 3.9% (177.8 pg/mL) for p2PSA mid control. However, no t*Phi*C failures were observed using a %CV of 7.3%, so the average component %CV of 4.2% was instead utilized for t*Phi*C QC assessment. In the course of monitoring t*Phi*C and component QC, twelve total QC run failure events (1_{3s}) were observed. Six QC failures involved only t*Phi*C failure, three involved only component QC failure, and three involved both component and t*Phi*C QC failures. Changes for t*Phi*C were also calculated for hypothetical changes in individual component bias. When tPSA, fPSA, and p2PSA were individually changed by +1 SD, the corresponding t*Phi*C SD changes were +0.56, -0.89 and +0.92. When both tPSA and p2PSA was increased by 1 SD, and fPSA was concurrently decreased by 1 SD, a more pronounced t*Phi*C change of +2.53 SD was observed.

Conclusion: *phi* is generally less precise than its component assays. The use of a t*Phi*C potentially complements QC of individual index components. In theory, theoretical calculated controls could be further applied to other MAAAs.

B-026

Association of Vitamin B6 Status with Health Conditions in the US Population

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Background. Vitamin B_6 (VB6) is a cofactor in numerous biologically important enzymatic reactions that regulate metabolism of proteins, carbohydrates, and lipids. Although VB6 is present in many foods, deficiency is common and associated with a number of diseases and conditions. Pyridoxal 5′-phosphate (PLP) is the principal biologically active form of VB6; PLP measurements are used as a marker of VB6 status. We evaluated the distribution of PLP concentration to assess VB6 status in various health conditions found in the US population. **Methods.** We reviewed historic data on PLP concentrations measured in consecutive routine patient samples (tested 2017-2019, n=12,815), for which International Classification of Diseases (ICD) codes were provided with the test request. Out of the entire set, in 6296 (mean/median age 55/56 years; 67% female) specimens the ICD-10 codes corresponded to conditions known to be associated with VB6 deficiency. PLP was measured using a validated LC-MS/MS method; the assay imprecision was <10%. The established reference interval for PLP is 20-125 nmol/L. **Results.** Measured PLP concentrations by ICD-10 code group, concentration distribution, and comparison of mean PLP concentration in disease groups with mean PLP from a group of healthy adults are summarized in Table 1.**Conclusions.** We observed statistically significant differences in distribution of PLP concentrations between healthy adults and individuals with a number of conditions. PLP measured in specimens from patients with neurological symptoms, potential nutrient deficiency, undergoing treatment for deficiency, or monitored post-bariatric surgery was significantly higher than mean PLP concentration in the healthy adult population, indicating appropriate supplementation and monitoring of nutritional status. Specimens from patients with obesity or alcohol abuse/dependence showed low PLP concentrations. In these conditions, micronutrient deficiency is unlikely to be evaluated as part of routine medical care. Our data suggest that nutritional assessment may be an important component of health care for these patients.

Table 1.

ICD10 Code	Group	N	PLP (nmol/L) Mean	PLP (nmol/L) Median	Central 95%	Difference (nmol/L)	Significance (p<0.05)
Z00.00	Healthy	670	72	39	9 - 338	na	na
K13.0, K13.70, K13.79, K14.0, K14.1, K14.4, K14.6, L21.9, L25.9, L29.9, L30.9, L71.0, R23, R26, R74.8	Suspected vitamin deficiency	356	114	60	10 - 452	42	0.0001
E67.2, E67.8	Megavitamin B6 syndrome/Hyper-alimentation	68	102	66	11 - 494	30	0.015
E43, E44.0, E44.1, E46, K90.0, K90.89, K90.9, K91.2	Malnutrition/Malabsorption	298	84	55	13 - 348	12	0.073
Z90.3, Z90.49, Z98.84	Post-bariatric surgery	1046	84	54	10 - 330	12	0.013
G56.92, G57.91, G57.93, G58.9, G59, G60.0, G60.3, G60.8, G60.9, G61.1, G61.89, G61.9, G62.0, G62.1, G62.2, G62.89, G62.9, G63, G64	Neuropathy	1838	83	50	10 - 356	11	0.012
R20.0, R20.2, R20.8, R20.9, R25	Abnormal sensation	1066	82	50	12 - 359	9	0.045
E53.1	Vitamin B6 deficiency	518	78	40	7 - 289	6	0.29
D53.8, D53.9, D64.9	Anemia	166	73	47	9 - 294	1	0.95
E51.9, E53.8, E53.9	Vitamin B group deficiency	156	70	39	3 - 370	-2	0.82
G40.019, G40.209, G40.219, G40.319, G40.909	Epilepsy	46	70	29	12 - 354	-2	0.92
E50.9, E61.7, E61.8, E63.8, E63.9	Multiple nutrient deficiencies	162	68	48	12 - 236	-4	0.59
E66.01, E66.3, E66.9, Z68.35, Z68.38, Z68.41, Z68.42	Obesity/ BMI>35	546	51	28	8 - 307	-21	0.0001
F10.10, F10.20, F10.21, F10.230, F10.24, F10.29	Alcohol abuse/dependence	30	36	36	6 - 102	-36	0.043

B-027

Derivation of a predictive equation for stress level assessment that objectively measures short-term stress

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Background: In recent years, many stress-related substances (e.g., IgA and cortisol) have been identified from non-invasive saliva, but it is difficult to set standard values due to large individual differences. In order to investigate the causes of short-term stress accumulation, it is necessary to objectively quantify stress-related substances that change over a short period of time in order to clarify the causes of long-term stress. For this purpose, it is important to select effective stress biomarkers together with subjective stress assessment by questionnaires and blood tests.**Aim:** To derive a prediction equation for the stress level caused by short-term stress by using only a few objective test items. Methods: Eighteen healthy adult volunteers (9 males > for 9 females) underwent short-term stress testing using the Uchida-Kleppelin test (15-minute computational load). TMD scores by POMS and salivary stress markers (amylase, cortisol, IgA, IgG, DHEA) and blood glucose levels using a simple blood glucose meter were measured as objective stress.**Results:** As objective changes before and after stress testing, salivary IgA and amylase increased. Multiple logistic regression analysis combining the results of subjective assessment with objective measures showed that cortisol and amylase tended to be associated with the results of TMD score. In addition, the relative 3-hour average blood glucose level (Sum180) increased, but suppressed rise in blood glucose levels (Differential calculus) (Figure 1).**Discussion:** Previous findings have reported that stress increases blood glucose levels, but this study showed the rise in blood glucose was gradual. This may be due to glucose consumption in the brain by calculation.**Conclusion:** We were able to derive a prediction equation for subjective stress level from cortisol, gender, and amylase in short-term stress. When monitoring stress-induced blood glucose, it is necessary to consider not only the effects of diet, but also the effects of glucose consumption.

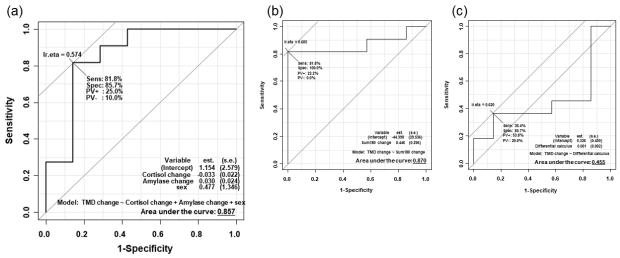

Figure 1. ROC analysis with short-term stress factors as objective variables
(a) Multiple logistic regression (Cortisol and Amylase and Sex)
(b) The relative 3-hour average blood glucose level (Sum180)
(c) Differential calculus of blood glucose levels (Differential calculus)

B-028

A Systematic Review and Meta-Analysis of Lipid Signatures in Post-Traumatic Stress Disorder

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Background: Studies investigating lipid profile in post-traumatic stress disorder (PTSD) have yielded mixed results. The aim of this meta-analysis was to provide a comprehensive synthesis of the evidence examining lipid levels in PTSD patients as compared to healthy individuals. **Methods:** We performed meta-analyses of the studies comparing lipid levels between PTSD patients and healthy individuals by searching Embase, Ovid Medline, Scopus, PsycINFO, and Cochrane databases. Search period encompassed all studies included in these databases up to March 2020. We extracted outcomes of interest, including mean and SD of lipid profile as well as relevant study and participant characteristics. Meta-analyses were performed using random-effects models with the restricted maximum-likelihood estimator to synthesize the effect size assessed by standardized mean difference (SMD) across studies. **Findings:** 8657 abstracts were identified, and 15 studies were included for inclusion in meta-analysis. Levels of lipid parameter total cholesterol (TC) was higher (**SMD= 0.57, 95% CI 0.27; 0.87, p=0.003)**, low density-lipoprotein (LDL) (**SMD= 0.48, 95% CI= 0.19; 0.76, p=0.004**), and **triglyceride (SMD= 0.57, 95% CI= 0.27; 0.87, p=0.003**) in PTSD patients compared to healthy controls. No changes in the high density-lipoprotein (HDL) (**SMD=-0.25, 95% CI=-0.89; 0.39, p=0.39**) and very low density lipoprotein (VLDL) **(SMD= 0.83, 95% CI=-0.60; 2.25, p=0.085)** was found in PTSD patients compared to healthy controls. Heterogeneity (I2) among studies was high (≥75%).

Interpretation: This meta-analysis found some robust effects indicating that PTSD is associated with increased levels of lipids TC, LDL, and TG. No changes in HDL and VLDL were found in PTSD patients when compared to healthy people. Cholesterol is an important part of the "lipid raft" that floats in synaptosomal membranes and is vital for neuron-to-neuron signaling. Cholesterol is also important for myelination of neurons; therefore dysregulation of cholesterol levels contributes to white matter abnormalities in PTSD patients. Low cholesterol in the brain synaptosomal membrane decreases the number of serotonin receptors. These mechanisms could both reduce the number of serotonin receptors and reduce binding, contributing to mood symptoms. This study will be useful for the physicians when considering the assessment of lipid profile in PTSD patients to reduce the cardiovascular mortality and morbidity.

B-029

There is no relationship between vitamin D levels and COVID19

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There is no relationship between vitamin D levels and COVID-19Background: Wonders if immunomodulatory actions of 25 OH vitamin D (VD) would have a role in susceptibility to SARS-CoV-2 infection and if there is a higher COVID-19 prevalence and severity in patients with lower VD levels. **Methods:**This study was done through an observational, retrospective big data laboratory approach with a network that extends over a large part of Brazil to compare VD between subjects with and without a diagnosis of COVID-19. The survey of all *reverse-transcriptase polymerase chain reaction* (RT PCR) tests for SARS-CoV-2 was computed from

January 2019 to February 2021. Measurements for VD were computed from one month before the RT PCR test. RT-PCR COVID-19 test was done by different platforms with similarys pefrformances and VD was mesured on the Advia Centaur Siemens on the Architect Abbott. The subjects were characterized as hypovitaminosis D following the criteria of the Endocrine Society Clinical Practice Guideline(2011): < 30 ng/mL (<75nmol/L). VD were described in mean and standard deviation. **Results:**Data were collected from 52293 subjects, all ages, who underwent RT-PCR SARS-CoV-2 and VD tests. Of these, 12281 were RT-PCR positive. There was a predominance of females (66,4%) and in the age group ≥ 20 and <60 (73%). Hypovitaminosis D was observed in 31,9% of subjects in SARS-CoV-2 positive group and in 32,9% of negative group. There was no statistical difference in these results evidenced: 18.2±5.0 versus 17.8±4.9ng/mL, respectively (p>0.05). There was also no statistical difference between groups with normal values of VD: 34.0± 17.2 versus 33.8±17.9ng/mL, respectively (p>0.05). **Conclusion:** There was no significant difference between VD in subjects with both hypovitaminosis D and normal dosages in the groups with and without COVID-19 analyzed.

B-030

Implementation of Global Diagnostics Network Project: an interaction between DASA and QUEST

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Background: Interface between the equipment and the Laboratory Information Management System (LIMS) allows us to effectively manage samples and associated data to provide quality care to our clients (patients). Besides all our equipment's had an interface with the LIMS, we still have a problem when we don´t execute determinate test. In this case, the process is to send the sample to the reference laboratory. We used a whole manual procedure that involves a work map generation in our LIMS, manual registration on the website and when the result is ready, we need to transcribe to our system. This limitation it was a factor for the growth in the number of tests. Thus DASA, through the partnership with the QUEST, in the GDN (*Global Diagnostics Network)* project, carried out a review of exams, prices and deadlines as well as the creation of an interface between these two laboratories to facilitate the process of sending tests.**Methods:**Data were extracted from the automated system of production, considering six tests - Acetylcholine Receptor Blocking Antibody (BACC), Acetylcholine Receptor Binding Antibody (LACC), Acetylcholine Receptor Modulating Antibody (MACC), Streptococcus pneumoniae Antibody (IgG) 14 Serotypes (ANPN_COMP), Hantavirus Antibody IgG/IgM (HANT) and HPV mRNA E6/E7 (E6E7) send to QUEST between the period from August/19 to December 2020. We send a total of 12620 tests in this period.**Results:**In August, we had a turnaround time (TAT) medium of 19 days while in December it was 7 days. Regarding the interface, the results of these tests automatically return and the analysis of the result is done in our system of production. Now, any difference of methodology or value reference, LIMS blocks, until an analyst check with the QUEST report, that is available by image (pdf) in our LIMS. Other advantage was when the test is not performed (TNP), this communication returns automatically and the analyst can check the necessity of recollect, eliminating the necessity to receive an email from QUEST, making contact with the patient more effective. We also had a reduction in the number of people in the sector because of automation process besides we have a saving of R$ 84.525 dollars through this project. **Conclusion:**The interface besides reduces the TAT and with all the results above, ensure more quality to the process, reduce human error; and also will allow us to increase the number of tests performed in 2021 whose expected increase of which is 15%. The next step is to achieve complete automation of all tests sent to the QUEST, further improving our results.

B-031

TSH reference interval for the Brazilian CentralWest pediatric population

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Background:Reference intervals (RI) are essential to adequately interpretate laboratory results, and in some instances, they must be adjusted for sex and age for guiding the good clinical practice. In this scenario, for accurate clinical decision on the diagnosis and management of thyroid diseases in pediatrics, it is important to evaluate the need of establishing specific RI for some age groups. Clinical and Laboratory Standards Institute (CLSI) recommends that RI could be established through indirect data, retroactively identifying acceptable reference populations. This procedure is especially recommended for groups in which restrictions or difficulties for sample collection apply, such as infants, children and adolescents.Objective: To determine the thyroid-stimulating hormone (TSH) RI for the Brazilian Central-West pediatric population using a laboratory database, according to CLSI Guidelines. Methods:This is a retrospective study carried out in a private laboratory. It was accessed the database containing the TSH results from 87,791children and adolescents, aged 3 to 19 years, whose samples were collected between Jan/2017 and Nov/2020. Exclusion criteria included subjects with positive anti-thyroid peroxidase (TPOAb), anti-thyroglobin (TGAb) and/or TSH receptor (TRAb) antibodies; TSH below 0.1 mIU/L or above 10 mIU/L; issues with records data; and individuals who had more than 3 tests in one year, suggesting high probability of thyroid dysfunction. Serum TSH, TPOAb TGAb, and TRAb were all determined by electrochemiluminescence immunoassays on the Roche Modular Analytics platform. The R statistical software was used to run the analyzes and Kolmogorov-Smirnov normality test was used to access the type of distribution. When TSH did not present a normal distribution, LOG10 transformation was used. After removing outliers, RI was defined as the central interval between the 2.5^{th} and 97.5^{th} percentiles, with a 95% confidence interval. Student's t test was used to decide whether RI adjustment by sex should be applied.**Results:**Out of the total, 591 TSH tests were eligible: 227 from 3 to 10-year-old individuals (group 1), and 364 from 11 to 19-year-old individuals (group 2); being 315 (53.2%) females. Group 1 presented a non-normal distribution, median age of 7.1 years for females and 7.3 years for males; with a TSH RI of 1.0 to 6.1 µIU/mL). (min/max 0.6-6.7 µIU/mL). Group 2 presented a normal distribution, mean age 15.6 years for females, and 15 years for males; with a TSH RI of 0.8 to 5.4 µIU/mL (min/max 0.6-8.3 µIU/mL). No statistical difference was identified between males and females for both groups. No ethnicity stratification was performed, due to the intrinsicracial miscegenation of the Brazilian population. **Conclusion:** For the pediatric Brazilian Central-West population in this study, the TSH RI differs from the one currently in use for the adult population (0.270-4.20 µIU/mL on Roche® Elecsys platform). TSH RI also differs between children and adolescents from the studied group. Such data highlights the importance of recognizing TSH RI and interpreting each patient's results according its population. This step fully contributes to a more qualified standard of care each individual deserves in clinical assistance.

B-033

Analysis of the coexistence of SARS-CoV-2 and Dengue viruses in the Brazilian population

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Background: The outbreak of coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide. Since the first case detected in Brazil on February 25, 2020, the number of COVID-19 cases has been on a rapid rise. In the same period, the country has been also facing a dengue fever epidemic, an *Aedes*-borne endemic tropical disease caused by the dengue virus (DENV). Is often observed that the number of cases of dengue fever increases at the beginning of the years due to the rainy season and high temperatures, climate factors that affect DENV vector abundance and contribute to an increase in the number of infections. According to the Brazilian Ministry of Health, the incidence of dengue cases revealed an upward trend, since almost 1 million infections were observed in 2020. The impact of both COVID-19 and dengue epidemics could have devastating consequences for the population and health service providers, which would be responding to concomitant emergencies. In this context, the Pan American Health Organization encourages countries to continue the epidemiological surveillance and provide reports of suspected and confirmed dengue cases in order to contribute to the implementation of public health policies 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:** This study is based on data from patients who underwent tests for the detection of SARS-CoV-2 and DENV in the assistance routine of Instituto Hermes Pardini (IHP), from all country, since March 2020 (data collected up to 2021-04-12). The data were treated using the R programming language and were plotted by the municipality on an interactive map using the Leaflet and Shiny libraries. The data presented were the total of tests performed and the total of positive tests/total of tests performed (positivity rate) for COVID-19 and Dengue fever. Also, an alert icon is used to inform which municipalities have positive results for the two tested conditions. The determination of the icon size and the graph were calculated according to the formula $Dengue\ positivity\ rate \times COVID\text{-}19\ positivity\ rate \times 100$. This

value was used to express the difference between the regions evaluated. **Results:** Map data is updated weekly and presented on Shiny App. Up to the present date, 2,289,758 tests have been carried out for both morbidities in the IHP care routine, being that 96.9% from COVID-19 and 3.1% from Dengue. A peak of positivity was observed between March and April 2020, due to the seasonality of the Dengue virus. **Conclusion:** Based on the data presented, in the current scenario of COVID-19 and the endemic history of Dengue, an alert should be kept on new peaks for the same period in 2021, since the public health scenario in Brazil is more critical than in the beginning of the year analyzed. Therefore, epidemiological surveillance is very important in monitoring diseases and controlling public health.

Laboratory Management and Leadership

B-034

Determining the Best Course of Action to Manage Interference in Drug Testing by Immunoassay

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Background: Immunoassays (IAs) are often used to detect drugs and drug metabolites in urine but are subject to interference. For two pain management drug panels performed in our laboratory, we run IAs on the Beckman Coulter AU5810. Immunalysis IAs are used to screen for carisoprodol, ethyl glucuronide, and tramadaol (with cutoffs of 100 ng/mL, 500 ng/mL, and 200 ng/mL, respectively). The Siemens Emit II Plus IAs are used to screen for barbiturates, cocaine, methadone, phencyclidine, propoxyphene, and cannabinoids (with cutoffs of 200 ng/mL, 150 ng/mL, 150 ng/mL, 25 ng/mL, 300 ng/mL, and 20 ng/mL, respectively). Between September and December 2018, there were 601 results reported as interference, most commonly for ethyl glucuronide (n=443). Currently, results are reported as interference leaving it to the provider to decide if additional testing needs to be requested. Our objective was to evaluate and compare different approaches to resolving interference results with respect to effectiveness, cost, and workflow integration.**Methods**: Urine samples (n=100) with interference results for one or more analytes were collected from September to December 2018. They were diluted times two or times five with distilled water and tested again using the nine IAs. Results were evaluated for resolution of interference, if analytes previously reported as "present" in the neat sample (no interferences) remained "present" after dilution, and if analytes with interferences became "present" upon dilution. All analytes newly identified as "present" after dilution were confirmed by liquid-chromatography tandem mass-spectrometry (LC-MS/MS). Additionally, a cost analysis comparison for diluting samples versus sending samples directly to LC-MS/MS was performed.**Results**: Interference was reported for 254 results of the 100 samples collected (mean±SD=2.5±2.0). After dilution, interferences for 191 results were resolved with a times two dilution and all were resolved with times five dilution. Of 30 analytes that were "present" in the neat sample (no interferences), 28 were still "present" and two were negative after times five dilution. After times five dilution, 23 samples with interferences had "present" results including ethyl glucuronide (n=11), cannabinoids (n=8), cocaine (n=2), barbiturates (n=1), and tramadol (n=1). LC-MS/MS results confirmed the presence of the drugs in all samples revealed after dilution. Considering labor, reagent, and supply costs for the top three IAs with interference, the cost of diluting a sample and rerunning one assay was determined to be $3.07±0.58 (mean±SD). Due to analyzer limitations, on-board dilutions were not an option. In comparison, the average cost of one LC-MS/MS assay was $6.44±1.53.**Conclusion**: Dilution of the urine samples resolved all interferences observed in the IAs represented in our study and was more cost effective than directed LC-MS/MS testing.

B-035

Revisiting a Core Laboratory Technique: New Manual Dilution Process Yields Results

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Background: In the modern day core chemistry laboratory the majority of specimen dilutions are performed via automated chemistry/immunochemistry analyzers. However, there is still a need to perform manual dilutions in some circumstances. Examples of these scenarios include when an automated dilution is not available, investigation of potential analytical issues, and/or off-label dilution to extend the clinical reportable range. Upon attempted establishment of the latter, significant imprecision was noted. This lead to a laboratory-wide process improvement for manual dilutions. The manual dilution procedure was updated to include a more standardized process, vortex mixing, and a calculation/manual entry re-checking process. Further, hands-on training was provided to all staff. **Methods:** To determine whether the new process was effective, all staff in the laboratory performed an example manual dilution before and after implementation. Creatine kinase (CK, Roche Diagnostics, cobas c702) was selected as the example manual dilution (1:21 with normal saline solution). A plasma pool was separated into aliquots with a numerical identifier. Staff were told to perform the stated dilution and provide the calculated result with the identifier. However, all aliquots were the same material. Statistical analysis was then performed utilizing Microsoft Excel and EP Evaluator. **Results:** Fifty-one staff performed the before (control) experiment. The mean of the material was 5,669.6 U/L [95% confidence interval (CI): 5502.3 – 5836.9 U/L], the standard deviation (SD) was 594.7 U/L (95% CI: 497.6 – 739.3 U/L), and the coefficient of variation (CV) was 10.5% (95% CI: 8.4 – 12.6%). After the new process and training was initiated, approximately 10 months after the first experiment, the manual dilution was repeated by staff with a fresh plasma pool. The mean of the material was 4,834.6 U/L [95% CI: 4726.1 – 4943.0 U/L], the SD was 365.3 U/L (95% CI: 303.0 – 460.1 U/L), and the CV was 7.6% (95% CI: 6.0 – 9.1%). An f-test statistical analysis was performed to compare the 2 variances. At α=0.05, the variances were significantly different. One limitation of the study was the different sample pools and therefore different means for CK. However, the CV was reduced by 27.6%. **Conclusion:** A robust manual dilution process is important to ensure the quality and consistency of results from manually diluted specimens. To this end, several new processes were implemented and inconsistent practices standardized in a core chemistry laboratory. These changes lead to a significantly reduced variation in manually diluted CK results.

B-036

Is there a continued need for routine assessment of folate status?

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Background: Folate is involved in single-carbon-transfer-dependent biosynthesis of some amino acids and nucleic acids required for efficient cellular activities including erythropoiesis. Low blood folate level has been reported in folate deficiency anemia. In 1996, the U.S. Food and Drugs Authority mandated the fortification of all foods with folate, hence the rarity of folate deficiency. The choose wisely organization recommended the withdrawal of the laboratory measurement of blood folate. This study reviewed the utility for the continued folate test requests at a large safety net teaching hospital. **Method**: Laboratory findings for RBC folate, vitamin B12, iron studies, ferritin, and hemoglobin were obtained for a six month's period as part of a test utilization review effort at Parkland Memorial Hospital. All measurements were performed in-house except for RBC folate which was sent to a reference laboratory. Hemoglobin (a component of complete blood count profile) was measured using Sysmex® automated analyzer (Sysmex, IL). Iron studies and ferritin were performed using Cobas® automated chemistry analyzers (Roche Diagnostics, IN, USA). **Results:** Of the 251 RBC folate measurements performed, 31(12.3%) had low values, suggestive of folate deficiency. All patients with low folate also had low hemoglobin. Results showed all vitamin B-12 samples analyzed were within normal limits, 44 normal and 175 low Hemoglobin, 107 normal and 30 low ferritin, 65 normal and 67 low iron levels. 25 patients had normal folate, in the presence of low ferritin, low hemoglobin and low iron, characteristic of iron deficiency anemia. Further, 34 subjects had normal folate in the presence of normal ferritin, low hemoglobin, and low iron, indicative of iron deficiency anemia in conjunction with preexisting conditions such as chronic infection or inflammation, chronic liver disease, or malignancy. Analysis showed 2 and 5 patients had low folate levels in the presence of low ferritin, low hemoglobin, low iron and low folate in the presence of normal ferritin, low Hb, and low iron levels respectively, suggesting coexistence of folate and iron deficiency anemias. **Discussion:** Low RBC folate and hemoglobin levels consistent with folate deficiency anemia, still persist even in this era of folate fortification. Our findings reveal a higher frequency of low folate than low vitamin B12 in a patient population presenting with anemia. Normal RBC folate is useful to assertively rule out folate deficiency anemia when hemoglobin, and either ferritin or iron levels are decreased. Low RBC folate is an independent predictor of folate deficiency, an implausible diagnosis without the measurement of RBC folate, demonstrating the continual need for the retention of the test. Though rare based on our data, the coexistence of folate and iron deficiency anemias persists. In **conclusion**, the present study showed that RBC folate remains clinically valuable and improves the diagnosis, while reducing the incidence of misdiagnosis of folate deficiency anemia. The future of this research is to expand the present data,

explore the clinical relationship of methyl malonic acid, homocysteine and folate to distinctively discriminate other causes of anemia and further substantiate the clinical utility of RBC folate.

B-037

Utility of capillary electrophoresis for hemoglobinopathy screening in anemic adult patients

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Background: Hemoglobinopathy screening is an essential tool in the workup of anemic adult patients. Several methods are available to separate hemoglobin variants including high-performance liquid chromatography (HPLC), acid or alkaline gel electrophoresis, and capillary electrophoresis (CE). The preferred method for hemoglobinopathy screening in anemic adult patients and subsequent result interpretation are not well established due to insufficient guidance from professional societies. In this retrospective study, we evaluated the utility of CE as a screening method to rule out clinically significant hemoglobin variants. **Methods:** During a twelve-month period, 373 adult patients without previously identified hemoglobin variants had hemoglobinopathy tests prompted by anemic red blood cell (RBC) indices through a comprehensive algorithm, which uses both CE (Capillarys, Sebia, Paris, France) and HPLC (laboratory-developed test) with reflex to more advanced variant identification such as mass spectrometry and genetic analyses. **Results:** The study population mainly consists of patients with hematologic malignancies (26%) and anemic pregnant women (25%). Categories of abnormal results are shown in **Table 1**. All of the abnormal results are recognized by CE alone, although HPLC and other methods help identify the abnormal variants and confirm diagnosis. The most frequent condition was isolated elevation of hemoglobin F spanning 1.0-6.8% attributable to bone marrow stress from chemotherapy in leukemia patients. Decreased hemoglobin A2 fractions were seen in alpha thalassemia or iron deficiency. **Discussion:** In summary, our study shows that CE as the first line of screening method would rule out major hemoglobinopathies in adults. Isolated elevation of hemoglobin F up to 6.8% in patients with hematological malignancies is associated with chemotherapy rather than underlying hemoglobinopathy and thus does not need further hemoglobinopathy workup. Abnormal hemoglobin A2 fractions, on the other hand, are likely indicative of thalassemia. CE therefore offers an automated, quantitative and reliable option for rapid hemoglobinopathy screening in anemic adult patients.

Table 1. Summary of hemoglobinopathy screening results in adult patients during a 12-month period.

Total screened	373
Abnormal results	121
Elevated hemoglobin F	40
Hemoglobin S trait	32
Decreased hemoglobin A2	18
Increased hemoglobin A2 (Beta thalassemia minor)	17
Hemoglobin C trait	5
Heterozygote A2 prime	4
Hemoglobin E trait	2
G-Philadelphia	1
Both F and A2 elevation	1
Hemoglobin Q-India	1

B-038

Evaluation of a Batched-Extraction Method for Measurement of Sirolimus, Tacrolimus, and Cyclosporine on the ARCHITECT i2000SR

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Background: Measurement of sirolimus, tacrolimus, and cyclosporine using the ARCHITECT immunoassay analyzer requires a manual offline extraction before analysis. Individual extraction of samples puts clinical laboratory staff at risk for ergonomic injury. We evaluated the analytical performance of a batched-extraction method for measuring sirolimus, tacrolimus, and cyclosporine in whole blood using the ARCHITECT i2000SR.**Methods:** We developed a batched-extraction method and evaluated the analytical performance of the ARCHITECT sirolimus, tacrolimus, and cyclosporine immunoassay after individual and batched extraction. Analytical evaluation included linearity, imprecision, analytical sensitivity, and method comparison. Linearity was assessed by diluting high and low value patient samples with manufacturer provided diluent. The between-day imprecision was assessed by analyzing three levels of QC material and three patient samples in duplicate across 5 days. Analytical sensitivity was assessed by verifying the manufacturer's claimed LOQ by serial dilution of low value samples. Deidentified residual whole blood samples were collected from patients who had undergone transplantation and received either sirolimus (N = 76), tacrolimus (N = 169), or cyclosporine (N = 170) and analyzed following individual or batched extraction. Data were evaluated using Deming regression analysis and Bland-Altman plots. **Results:** The batched extraction method showed a linear response across the AMR with R^2 values of 0.9955, 0.9932, and 0.9953 for sirolimus, tacrolimus, and cyclosporine, respectively. The total between-day imprecision for sirolimus was between 2.58 – 3.13% for QC material and between 2.35 – 4.48% for patient samples. For tacrolimus, the total between-day imprecision was between 2.70 – 3.77% for QC material and between 2.96 – 7.26% for patient samples. For cyclosporine the total between-day imprecision was between 7.89 – 12.41% and 7.96 – 9.44% for QC material and patient samples, respectively. Analytical sensitivity at 20% CV were determined to be 0.82 µg/L, 0.48 µg/L, and 7.68 µg/L for sirolimus, tacrolimus, and cyclosporine, respectively. Deming regression analysis showed agreement between individual and batched extraction methods for sirolimus, tacrolimus, and cyclosporine with a Pearson's r value of 0.985 (slope: 1.03, intercept: -0.13), 0.993 (slope: 1.00, intercept: 0.17), and 0.99 (slope: 1.01, intercept: -4.62), respectively. The Bland-Altman plot revealed a bias of 1.29% (95% CI: -0.58% to 3.16%) for sirolimus with an upper level of agreement (ULOA) of 17.2% (95% CI: 4.00% to 20.42%) and lower LOA (LLOA) of -14.63% (95% CI: -17.83% to -11.42%). In addition, the Bland-Altman plot revealed a bias of 2.07% (95% CI: 1.06% to 3.07%) for tacrolimus with an ULOA of 15.00% (95% CI: 13.28% to 16.72%) and LLOA of -10.87% (95% CI: -12.59% to -9.15%). Bland-Altman analysis revealed a bias of -1.56% (95% CI: -2.99% to -0.12%) for cyclosporine with a ULOA of 16.94% (95% CI: 14.49% to 19.4%) and LLOA of -20.05% (95% CI: -22.51% to -17.6%). The ULOA and LLOA for sirolimus, tacrolimus, and cyclosporine overlapped with our predefined total allowable error of ±15%. **Conclusion:** The batched extraction method for sirolimus, tacrolimus, and cyclosporine were not equivalent to individual processing using 15% as allowable limit. Additional studies are required to confirm this observation.

B-039

Virtual QC Crossovers

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Background:

When a laboratory receives a new lot number of quality control material, the target value for each analyte must be established. This is true even for assayed controls as the assay ranges are usually too wide for direct use in the laboratory's quality control program. The normal approach is to evaluate at least 10 QC samples over 10 days to determine a target mean in a crossover study. This is costly in terms of time and materials as the QC target is not established until the end of the study.

Here we present a method of establishing a QC target that requires an initial 2 samples evaluated on the first day of a 'virtual' crossover study, using the historical bias of the laboratory with respect to its peers when a suitable peer group exists on the new lot of QC material.

Methods:

The initial estimate of the new QC target (L_{pred_new}) is given as:

$bias = L_{mean\_old} / G_{mean\_old}$

$L_{pred\_new} = bias * G_{mean\_new}$

Where L_{mean_old} is the laboratory mean of the old lot number, G_{mean_old} is the peer mean of the old lot number and G_{mean_new} is the peer mean of the new lot.

As samples of the new lot are evaluated the estimate of the new QC target is updated by:

$W_1 = (N_T - N_Q)/N_T$

$W_2 = N_Q/N_T$

Updated $L_{pred\_new} = W_1 * L_{pred\_new} + W_2 * L_{mean\_new}$

Where N_Q is the number of samples run so far and N_T is the total number of samples to be run – usually 10.

The SD of the new lot (L_{SD_new}) can be estimated as $L_{SD\_new} = (L_{mean\_new} * CV_{old}) / 100$ Where CV_{old} is the CV of the old lot.

Results:

Results from a virtual crossover computation example:

$L_{mean\_old} = 34.3$

$G_{mean\_old} = 36.1$

bias = 0.95

$G_{mean\_new} = 34.5$

Initial estimate (L_{pred_new}) = 32.780For an alkaline phosphatase dataset of 33, 32, 33, 32, 34, 32, 33, 33, 31, 34, here are the updated estimates for the mean of the new lot after each datapoint is added.

$L_{pred1} = 32.802$ (0.9*32.780 + 0.1*33).

$L_{pred2} = 32.741$ (predicted target after 2 datapoints)

$L_{pred3} = 32.719$

$L_{pred4} = 32.631$

$L_{pred5} = 32.716$

$L_{pred6} = 32.686$

$L_{pred7} = 32.706$

$L_{pred8} = 32.741$

$L_{pred9} = 32.704$

$L_{new\_mean} = 32.727$ (actual target – mean of 10 datapoints).

Conclusion:

For labs that have participated in an inter-laboratory peer program that are starting a new lot of QC material, virtual crossover studies provide labs a good initial estimate of their QC target before evaluating the new material. In many circumstances, only a few samples are required to refine the initial QC target estimate to the point that it can be used, while further refinements are made during the normal course of operation.

B-041

Workflow Impact with Bio-Rad's InteliQ QC on Beckman Coulter DxC 700 AU Instrument

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Background: The emergence of automated instrumentation has been a growing trend in the past decade. As a result, there has been more demand for automated method to run quality control(QC). In 2021, Bio-Rad introduced a suite of InteliQ controls addressing the need for QC automation in major platforms, including Beckman Coulter's DxC700AU.

The objectives of this study is to test if the Bio-Rad InteliQ Controls function properly on the Beckman Coulter DxC700AU chemistry analyzer from a hardware/software perspective and identify QC workflow efficiencies gained (reduction in processing steps and time savings) when compared to the current method of using QC conventional glass vials.

Method: Over a 5-day period, the InteliQ controls were stored in DxC700AU designated QC racks in an offboard refrigerator and loaded onto the analyzer when QC was needed to be performed. This process was compared to the current QC process where the control material from vials is aliquoted into insert cups for each different control used. The study was conducted at AMITA Medical Center at Hinsdale, IL, U.S.A and Bolingbrook, IL, U.S.A.

Result

	QC Pre-Run Process	QC Post Run Process		Total Time
Current Process	6 min 21 sec	26 sec		6 min 47 sec
InteliQ Process	1 min 27 sec	1min 18 sec		2 min 45 sec
		Time Savings		4 minutes 2 sec

Both sites saved an average of 4 minutes (59% average time savings) in the daily QC Pre-Run and Post-run process utilizing InteliQ. According to the time collection table, each operator spent 6 minutes 47 seconds for the vial process and about 2 minute 45 seconds with the InteliQ process. Both operators observed that the InteliQ process eliminate the need to transfer material to insert cups and the need to dispose the insert cup from the rack in each QC run.

Conclusion InteliQ streamlined their workflow and saved each lab an average of approximately 4 minutes (59% average time savings) per QC run. It is estimated a laboratory can save up to 24 hours per year based on a one QC run per day.

B-042

POC Blood Gas Testing in Critically Ill Patients with and without SARS-COV2 Infections

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Background: UK HealthCare (UKHC) launched point of care (POC) blood gas and whole blood chemistry testing in the Medical Intensive Care Unit (MICU) in January 2020 as a pilot demonstrating reduced laboratory turn-around times (TAT) for rapid clinical decision making. Driven by a multidisciplinary Blood Gas Task Force, the goals were to reduce 1)TAT, 2)specimen recollections, and 3)specimens sent through the pneumatic tube system to the core laboratory, a known cause for preanalytical errors that compromise specimen integrity. Two months into the pilot, UKHC admitted Kentucky's first Sars-CoV2 positive patient. The Blood Gas Task Force pivoted to expand POC blood gas testing in the dedicated COVID ICU. Goals for testing were to provide rapid TAT, reduce specimens in the pneumatic tube, and implement a process to minimize staff exposure to COVID and conserve personal protective equipment (PPE).**Objectives:** To assess the effects of task force POC Blood gas placement and novel testing protocols on TAT, specimen recollections and COVID exposure in Medical and COVID ICUs.**Methods:** Three interventions were implemented to optimize patient care and minimize Sars-CoV2 exposure: 1)Blood Gas Task Force created to plan, implement, and review POC blood gas testing; 2)POC analyzer placement; 3) updated blood gas collection procedure in COVID ICU.The multidisciplinary Blood Gas Task Force, a team of Information Technologists, financial analysts, RTs, and clinical laboratorians, ensured appropriate changes to EMR, data monitoring, staff training, instrument validations, and procedural updates. POC analyzers were first placed in the MICU. TAT and specimen recollections were monitored before and after the pilot. The task force also addressed the COVID-19 pandemic. The pilot's goals aligned with COVID ICU requirements with additional goals. The collection process in the COVID ICU changed to reduce staff exposure and conserve PPE. Rather than having an RT handle specimens from collection to result, an RN or RT would collect the specimen and pass it to an RT outside the room. The collector remained in room until the results posted and could recollect a new specimen if necessary without using additional PPE.**Results:** The MICU pilot goals were achieved within two months. 65% of MICU blood gases were performed at POC by RT. Before the pilot all MICU blood gases were collected by nursing and performed in the central laboratory. The average blood gas TAT was 16 minutes and 6% of specimens required recollection for compromised specimen integrity. Post implementation of POC Blood gas testing the TAT was 6 minutes and <2% of specimens required recollection. POC testing in the COVID ICU reduced risk of COVID contamination of the pneumatic tube system, sending 67% fewer specimens to central laboratory. The COVID ICU specimen collection process change reduced exposure and conserved PPE. The COVID ICU performed ~22 POC blood gases daily, and the process change spared one-plus set of PPE per blood gas and one or more staff exposures.**Conclusion:** Implementation of a multidisciplinary task force to direct POC blood gas testing resulted in improved patient care and allowed for rapid transitions during a global pandemic.

B-043

Multidisciplinary Approach to Optimizing Laboratory Test Ordering while Capturing Lost Revenue Through Process Improvement

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Background: Laboratory testing is a growing and evolving field. New/improved technology, innovation in testing techniques, automation, and genetic testing research has provided clinicians with a variety of testing options for their patients. Many of these tests are expensive, and insurance providers require documentation of medical necessity before reimbursement is approved. As new tests are validated, insurance providers implement guidelines for reimbursement and require insurance preauthorization before testing is performed. Claims are denied without preauthorization, leading to lengthy appeal processes, which may still ultimately end with a denial. Patients who have testing performed without preauthorization risk receiving large bills when their insurance denies coverage. At our institution in FY19, laboratory tests performed without preauthorizations accounted for over $1,100,000 in lost revenue.

Objectives: To evaluate the effectiveness of a multidisciplinary committee to optimize laboratory test ordering while ensuring insurance reimbursement.

Methods: A multidisciplinary committee with members from laboratory, finance, Information Technology, revenue management, and genetics convened in 2018 with the goal of reducing lost reimbursement due to no preauthorization. The committee implemented three interventions: 1) identify laboratory CPT codes responsible for largest denials, 2) implement IT solution to automatically flag and hold laboratory test orders requiring preauthorization until authorization is obtained, and 3) centralize and standardize preauthorization staff and training. The committee began by reviewing laboratory testing denial data to identify the most frequently denied tests and locations with the largest denials. Revenue management expanded the existing centralized preauthorization team through executive support, standardized training for all staff responsible for obtaining laboratory preauthorizations, and defined a central location to document approvals. IT automated order holds for tests requiring preauthorization. A new "hold for preauth" status within the EMR was developed to provide a visual indication to phlebotomy teams not to draw labs until the pre-auth hold is cleared. Additional executive support added a laboratory genetic counselor position and genetic counselor assistant in Pediatric Genetics Clinic who were essential to ensuring that tests that did not automatically flag were also obtaining preauthorization.

Results: The process developed by this committee ensured frequently denied tests are flagged within the EMR. A hold is placed on orders requiring preauthorization ensuring all staff can see a pre-auth is pending if patients present to phlebotomy. Preauthorization staff document preauthorization numbers which are then added the insurance claim after the patient returns for testing. Through the efforts of this committee, $660,000 in reimbursement was recaptured in FY2020. Monthly denials dropped from $100,000 to $40,000. The number of CPT codes denied dropped from 112 in 2019 to 64 in 2020.

Conclusion: The committee continues to meet to review denials and determine which new tests are added to the workflow. Through this multidisciplinary effort, we are able to identify new tests that are being denied and implement a solution to prevent patients from receiving large bills for these tests and revenue loss for our institution in a timely manner. We have had great success with this process and have sustained these gains through continued engagement of committee members.

B-044

Addressing Rigor and Reproducibility by Applying QC/QA Principles to Preclinical Laboratory Research

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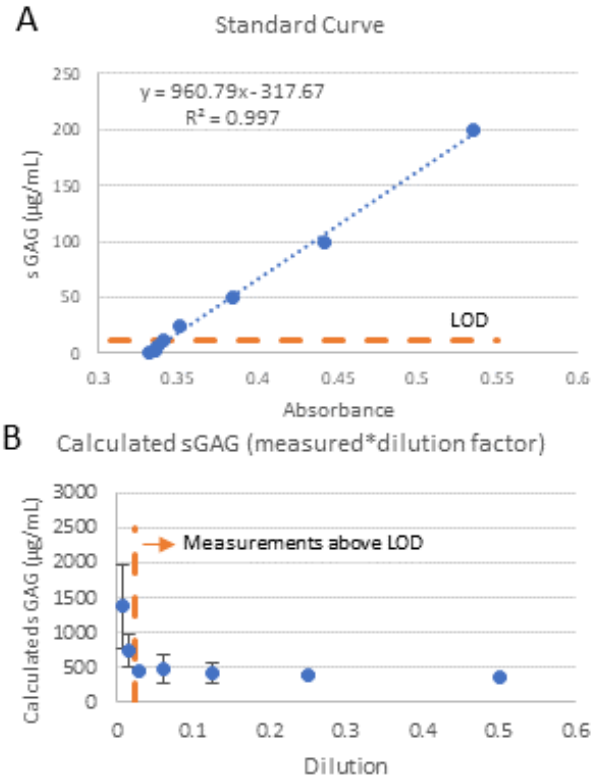
Background: While quality control (QC) and quality assurance (QA) practices are standard procedure in clinical laboratories, QC/QA practices are far less common and not standardized in research laboratories. Validation of assay performance within a research lab for every sample type could be a powerful tool to improve rigor and reproducibility, which has recently become a priority for NIH funding. To this end, we performed a validation experiment for the dimethylmethylene blue (DMMB) assay, which is commonly used in orthopedic preclinical research to measure extracellular matrix proteoglycans in musculoskeletal tissues. **Methods:** A standard curve was prepared by serial dilution of chondroitin sulfate in papain digestion solution with standards ranging from 200µg/mL to 3.125µg/mL, according to a commonly used protocol. Limit of detection (LOD) was calculated as mean(0 standard) + 4*SD(0 standard). A serial dilution of an unknown sample prepared by digestion of meniscus tissue in papain was assayed, and concentration was determined by multiplying sample measurements by the dilution factor. **Results:** LOD was determined to be 11.4µg/mL. Measurement of multiple dilutions of an unknown sample were concordant above the measured LOD (RSD 11.5%), but not when including measurements between the LOD and lowest standard (RSD 60%). **Conclusion:** Measured LOD for the DMMB assay was higher than the lowest points on the standard curve of a commonly used protocol. Measurement of an unknown sample diluted below the measured LOD produced spurious results, which would not be recognized in a research lab without performing in-lab method validation for the sample types used. This is a completely plausible scenario which could contribute to significant error in published results for preclinical research. Establishing standards, training, and incentives to increase adoption of assay validation and QC/QA practices in preclinical or basic science research should be a focus of efforts to improve rigor and reproducibility.



(A) Standard curve for DMMB assay. As prepared according to the protocol, two standards are below the measured LOD.
(B) calculated concentration of sGAG (µg/mL) of a serial dilution of an unknown sample. Measurements below the LOD but within assay range according to the protocol return spurious results.

B-045

Investigation of unacceptable proficiency testing results: Identification of interferant in PT sample

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Background: Proficiency testing (PT) is an integral part of verifying the reliability and accuracy of results produced by clinical laboratories, including those that perform toxicological testing. Lighthouse Lab Services manages quality assurance for 44 urine toxicology labs; 22 of these labs test for sample adulteration (creatinine and/or pH) using various methodologies. A round of proficiency testing was performed and labs with unacceptable results for creatinine requiring corrective and/or preventive action were identified. After exploring possible clerical, basic technical, and methodological causes, three laboratories repeated a low creatinine concentration (0.3 mg/dL, 0.3 mg/dL, 2.1 mg/ dL) outside the acceptable range (17.3-42.4 mg/dL) for the same PT sample. **Methods:** Issues with quality control, calibration, internal standards, and re-

agents were investigated, and studies to rule out sample precipitation were completed. Dilutions studies were performed with varying concentrations of the PT sample diluted with either a patient urine sample or acceptable dilution reagent to determine if an interferant was present. **Results:** It was identified that all three labs used an enzymatic assay for detection of creatinine via endpoint measurement of a quinone-based dye, indicating a possible issue with the combination of the PT sample and the given method. From the dilution studies, an uncharacteristic dilution pattern was identified, validating the possibility an interfering substance. The PT provider was contacted and all work-up of the unacceptable PT result was provided. Through the reporting process, it was found that the problematic creatinine sample contained 5% glutaraldehyde. Review of assay package inserts did not identify 5% glutaraldehyde as a known interferant. **Conclusion:** Glutaraldehyde is the active ingredient in commercial adulterant products like UrinAid and Clear Choice. Glutaraldehyde is known to cause false negative results via interaction with enzymes used in immunoassays for drug identification. The assays used by the three labs with unacceptable results employed an enzymatic method, and it was inferred that a similar mechanism could be at work, resulting in a very low creatinine concentration. Our data was reviewed by the PT provider's scientific committee, and the unacceptable score for creatinine was resolved. Despite the PT issue being resolved, this prompted us to determine if 1) glutaraldehyde adulterated samples would be detected by the system we have in place and 2) if additional testing/ criteria would be needed if glutaraldehyde-adulterated samples were received. In this instance, glutaraldehyde adulteration caused creatinine to be close to zero for the three labs and outside our normal range (20-200 mg/dL). This would still indicate that the sample was adulterated, even though we are not specifically testing for glutaraldehyde. PT is an integral part of ensuring quality for the laboratory; however, it is important to realize that an aberrant or unacceptable result does not always mean that the system is not working properly. Thorough investigation of all unacceptable PT results is a necessary component to identify quality assurance issues outside the scope of laboratory result accuracy.

B-046

CDC's OneLab Network as a Unified Response to COVID-19 Laboratory Training Needs

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Background: The COVID-19 pandemic has demonstrated in an unprecedented way the importance of the connections and dependencies between public health, clinical, and commercial laboratories. CDC created the OneLab Initiative to bridge, train, and sustain a capacity-building community among the US clinical and public health laboratory workforce.

Methods: A OneLab Network was established in February 2021 to support the initiative's short-term goals of: 1) identifying and responding to urgent COVID-19 continuing education/training needs; and 2) widely disseminating free education and training resources to help learners meet challenges related to the COVID-19 pandemic response. Over time, the network will serve as an ongoing learning community, connecting professionals with a role in the training and continuing education of the clinical and public health laboratory community. A training needs assessment consisting of an electronic survey and seven focus groups was conducted February 5-26, 2021, to gather feedback on COVID-19 training needs from the OneLab Network.

Results: As of March 23, 2021, network membership consisted of 1,767 people, with the majority (75%) of members working primarily in a laboratory setting. Professional organizations, government, academia, and others across the US are also represented. Members have a variety of responsibilities in developing, delivering, recommending, or providing content for continuing education of clinical laboratory professionals who are internal or external to their organizations. Approximately 65% of members spent at least half of their current work focusing on COVID-19 testing and response. A total of 372 members (33% of network members at the time of launch of the training needs assessment) participated in either the online survey or a focus group. After qualitative thematic analysis, 12 critical education and resource gaps were identified, including gaps related to test validation, testing guidelines, regulations and compliance, and emergency response and risk management. Three primary audiences to target training and education resources were categorized: laboratory directors, new clinical laboratory hires providing surge support, and developers of laboratory training/continuing education resources.

Conclusion: In response to these findings, CDC is in the process of creating additional publicly available COVID-19 laboratory resources in a variety of modalities and widely disseminating them through a tailored learning management system. In the long term, the OneLab Initiative will establish a sustained laboratory learning community to collectively support rapid and coordinated large-scale emergency responses.

B-047

Efficiency gain with Automatic Quality Control function on Atellica Solution

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Background: Before the Atellica Solution system was equipped at Hanoi Medical University Hospital, the quality control procedure for chemistry and immunoassay testing had included many painstaking manual steps for QC preparation, performance and validation. This procedure wasted time, costs and had error-prone daily QC preparation which could lead to patient results delay. The aim of this study was to demonstrate time saving and efficiency gain on managing quality control (QC) procedure using Auto-QC function of Atellica Solution system along with the QC/Cal onboard storage feature when compare with manual QC management

Methods: The manual and automatic QC procedures were concurrently performed in five days on Atellica Solution system. Time and QC results of ten assays chosen (Glucose, Urea, Cholesterol, ALT, AST, TSH, fT3, fT4, AFP, CEA) were recorded to evaluate time and cost saving by using Auto-QC function comparing to manual quality control procedure. QC result correlation between Auto-QC and manual QC was evaluated to demonstrate the performance of QC is within stability claim when using QC/Cal onboard storage feature.

Results: Time for QC procedure per day was reduced by 60.92% (29.45 minutes) for Chemistry testing and 60.16% (36.95 minutes) for Immunoassay testing. Total cost for QC procedure per year of Chemistry and Immunoassay testing was reduced by 85.54% (754.30 USD) (Chart 1). QC result correlation between Auto-QC and manual QC with r = 0.09 to 1.00.

CH: Chemistry analyzer.
IM: Immunoassay analyzer.
The costs are follow market price and Salary table of the latest staffing ratio for medical personel (2020).
317 working days per year

Chart 1. Cost saving by using Auto-QC function of Atellica® Solution comparing to manual QC procedure per year

Conclusion: The Automatic QC function along with the QC/Cal onboard storage feature on Atellica Solution eliminates laborious and error-prone daily QC preparation; saves time for more valuable tasks; saves money by eliminating material waste from manual QC procedure including eppendorfs, sample tips, tubes and cup for daily containing QC material. It makes reliable QC results available for staff to review when they arrive, reduces biocontamination risk, improves QC management and laboratory workflow.

B-048

Demand management intervention to avoid redundant measurement of gamma glutamil transpeptidase and alkaline phosphatase in primary care

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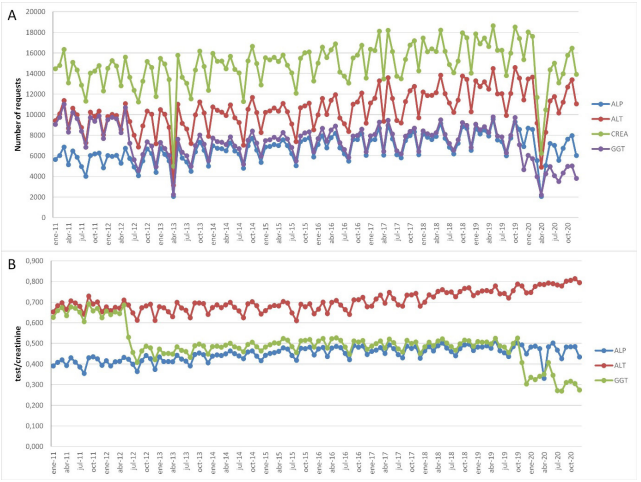
Background: Liver tests can be restricted to alanine amino trasferase (ALT) and alkaline phosphatase (ALP) when the purpose of testing is to exclude liver disease in primary care. However the demand for gamma glutamil traspeptidase (GGT) is still elevated in primary care. The purpose of the study was to show GGT and ALP demand from primary care along ten years, and report an intervention to diminish the GGT measurement when concomitantly requested with ALP.

Methods: The intervention was conducted from November 1st 2019 to January 31st 2021 at a 370-bed suburban community Public University Hospital that serves a population of 234,551 inhabitants. The laboratory receives samples from primary care patients. Requests are ordered electronically and reports sent out from the Laboratory Management Information System (LIMS) to the patient's electronic medical record. The intervention was designed in a meeting between laboratory professionals, general practitioners (GPs) and members of the hospital board. The primary care request for GGT and ALP in a period of 10 years was shared; also the monthly request of both markers related to that of serum creatinine (GGT/CREA; ALP/CREA). The agreed intervention consisted in the LIMS automatically cancelation of GGT when requested concomitantly with ALP, and non-previous GGT elevated value. The GGT request in pre and post interventions period was compared, and the number of GGT cancelled and subsequently no measured counted, and savings calculated.

Results: 874384 GGT were measured; 1261018 and 806412 ALT and ALP were requested respectively in 10 years. The monthly ratio of the three markers related to creatinine (figure), showed a decrease in GGT/CREA when the intervention implementation. Out of the requested 57614 GGT, 38167 (66.2%) were not measured. 7633.4€ were saved in reagent. **Conclusion:** In agreement with GPs and making use of LIMS and patients data base, GGT demand can be corrected.



B-049

Laboratory Medicine: A Decision Maker Hub

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Background: To share a new laboratory model based on laboratory knowledge, meaningful use of information technology, and partnership with clinicians, to lead the appropriate use of laboratory testing and clinical decision making in the diagnosis of as-yet-undiagnosed disease. More specifically, we evaluated the role of four different opportunistic interventions to diagnose certain asymptomatic disorders, by means of the automatic registration of appropriate laboratory testing according to different scenarios. **Methods:** This is a retrospective study to evaluate the impact of laboratory interventions based on adding serum calcium, glycated hemoglobin (HbA1c), serum B12 vitamin and serum magnesium (s-Mg) on the diagnosis of occult hyperparathiroidim (HPPT), diabetes, vitamin B12 deficit and magnesium deficit when a laboratory request and sample availability, including data from January 2012 to September 2020. The number of identified cases was counted. Also the number of patients with parathyroidectomy, in a pre-intervention (years 2011-2012) and post-intervention period (2018-2019); percentage of emergency department (ED) patients with hypocalcaemia (calcium corrected per albumin<7.5 mg/dL) readmitted in ED the first 30 days after hypocalcaemia detection, in years 2015-2016, before s-Mg measurement in ED, and also in 2019-2020; the number of intramuscular vitamin B12 prescriptions per 100000 inhabitants in pre- (2014-2015) and post- intervention periods (2019-2020); and regarding HbA1c, the proportion of patients in 2016 and 2019 with HbA1c values below 8%. Results in pre and post intervention periods were compared.

Results: Overall, the above strategies have so far identified 2063 patients with clinically relevant as-yet-undiagnosed disorders who would have otherwise remained occult, such as hyperparathyroidism, diabetes, and vitamin B12 or magnesium deficit. Table shows outcome results. **Conclusion:** We are facing a new laboratory model, a leading laboratory rather than a passive traditional laboratory, not just to intervene in clinical decision-making, but to make the clinical decision, through the identification of patients with occult disease.

Outcome indicators

Intervention	Outcome indicator	Pre-intervention period (period; result)	Post-intervention period (period; result)
Calcium	Number of parathyroidectomies	2011-2012; 10	2018-2019; 34
HbA1c*	Percentage of patients with HbA1c values <8%	2016; 32.5%	2019; 36.4%
Vitamin B12*	Number of intramuscular vitamin B12 prescriptions	2014-2015; 3009/100000 inhabitants	2019-2020; 4580/100000 inhabitants
Magnesium*	Percentage of ED patients with hypocalcemia readmitted the first 30 days after hypocalcaemia detection	2015-2016; 25%	2019-2020; 13%

*P<0.001

B-050

Scaling Decentralized Laboratories Using a Digital Platform to Meet Testing Demand During a Pandemic

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Background: High-performance molecular testing for SARS-CoV-2 became the cornerstone of infection containment when the World Health Organization declared COVID-19 a global pandemic on March 11, 2020. During this time, two major needs for curbing the spread of infection became apparent: the need for at-home specimen collection and testing infrastructure and the need for diagnostic laboratories with scalable capacity and rapid turnaround times. Our experience describes a decentralized approach to addressing these demands.

Methods: Thorough evaluations of prospective laboratory partners were performed and included a full assessment of instrumentation, quality management system, certifications, physical laboratory space (sq. ft.), reporting capabilities, staff, shift schedules, and throughput. Once completed, our organization facilitated digital technical integrations and monitored performance metrics including sample issue rate and turnaround time.

Results: Upon launch of a COVID-19 Test Collection Kit for healthcare providers (HCP) on March 23, 2020, two partner laboratories had been onboarded to support a laboratory reported capacity of up to 10,500 kits per week. With the anticipated increase in demand and Emergency Use Authorization (EUA) launch of a COVID-19 Test Home Collection Kit (Rx Only) product on May 15, 2020, a total of eight decentralized laboratories across the United States were onboarded. From the initial launch of the HCP offering to the present day, weekly capacity of partner laboratories was ramped up to support up to 910,000 kits/week—an increase of nearly 87 times the initial capacity across partner laboratories. Technical support in the form of a digital dashboard was created to support HCP services and collection events. Notably, total sample errors observed for specimens collected with the HCP collection kit were high in the initial three weeks following launch, a reported average of 17.8%. Upon implementation of an additional digital platform training session for HCP users starting April 7th, 2020, total sample errors declined to an average of 8.9%.

Conclusions: Onboarding new laboratory partners and expanding laboratory testing capacity across decentralized laboratories allowed for increased capacity and maintained agreed upon turnaround times. While vaccines become more widely available, the lessons learned from the rapid scaling efforts seen are not lost. Clinical testing laboratories may continue to experience these challenges in the future as newly identified SARS-CoV-2 mutants arise and the possibility of facing an epidemic or pandemic fueled by a new pathogen remains.

B-051

Plasma versus Serum: Which specimen type is most cost-effective in the community setting?

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Background: Our laboratory is a major academic reference laboratory that performs over 40 million chemistry tests/year. This laboratory provides community testing for a catchment population of 1.4 million people. Currently at our main laboratory, plasma is the specimen of choice for most routine general chemistry tests. This is in contrast to other major North American reference labs (e.g. Mayo Clinic Laboratories, Quest Diagnostics™) that use serum as the specimen of choice for routine general chemistry tests. Serum samples are "cleaner" specimen due to removal of fibrinogen. In addition, serum samples are less expensive than plasma samples because they do not contain heparin. However, the clotting process takes about 30 min as opposed to plasma, which can be used within 5 min. BD Vacutainer® Rapid Serum Tube™ (RST™), which only requires 5 minutes for the clotting process, is good but expensive alternative serum tube. The focus was to investigate if switching to serums was more cost effective for our high volume laboratory.

Methods: In order to explore cost-savings opportunities, two options for changing from plasma to serum specimen collection containers were considered for routine chemistry testing performed at the laboratory as well as specimen collection centers: 1) BD Vacutainer® Plasma Separator Tubes™ (PST™) to BD Vacutainer® Serum Separator Tubes™ (SST™) or 2) PST™ to SST™ and/or RST™. Study parameters were restricted to specimens received to the laboratory for routine general chemistry tests during a typical weekday from the 19 collection centers. Information regarding major costs associated with changing the primary collection container was obtained by consulting laboratory stakeholders (e.g. laboratory and community service senior technologists and managers) and the vendor (BD Life Sciences). The major costs included the type of tube and the labor associated with processing different tube types.

Results: The tube costs for SST™, PST™, and RST™ were $0.1756, $0.1824, and $0.600, respectively. On a typical weekday, the laboratory received 7,950 PST™ specimens from the collection centers. Based on unit specimen collection container costs, switching from the PST™ to the SST™ resulted in a cost savings of $56/day. However, the increased specimen handling time required for the clotting process requires staff to remain at the collection center after hours to complete the process. The minimum overtime costs associated with having to accommodate patients entering the facility near closing time was calculated to be $1,268/day. To overcome the need for overtime, the RST™ can be used in the final hour of operation. However, the increased cost of the RST™ over the SST™ during this one hour period is $293/day. Therefore, switching from PST™ to either SST™ or SST™ and/or RST™ will cost our laboratory an additional $237 to $1,212/day.

Conclusion: When compared to the SST™, despite a slightly higher unit cost, due to workflow requirements specific to the community setting, the PST™ is the more economical specimen collection container for routine general chemistry tests in our laboratory. Less expensive tube is not necessarily more economical.

B-052

Performance Evaluation for Automated Quality Control on Siemens ADVIA-2400

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Background:

By utilizing Siemens Aptio Automation and "QC on track" programme, automated quality control (Auto-QC) for two ADVIA-2400 analyzers were achieved. This study aimed to evaluate the performance of Auto-QC.

Method:

31 out of 38 assays, which were controlled by Bio-Rad Lyphochek Assayed Chemistry Control, were selected. In July 2020, 20 parallel QCs (2 concentrations) were run with manual and automated processes to evaluate the bias and precision for Auto-QC.

A 5ml Control was reconstituted and aliquoted into two common blood collection tubes for Auto-QC. One tube was frozen while the other was barcoded as Control and inserted via Input/Output Module and refrigerated in Storage Module of Aptio system. It was automatically retrieved and tested at 7am and 2pm daily and disposed after 10 tests. The frozen tube was then thawed and acted as new Control.

Auto-QC was fully deployed since August 2020. QC stats were compared between selected periods in 2019 and 2020.

Results:

The Bias% of the Auto-QC mean when compared with Bio-Rad Unity Interlab reports and manual QC means were below 1/2 TEa, and CV% differences were below 1/3 TEa. Thus, performance of Auto-QC passed the internal QC.

5-month QC comparisons for 6 representative assays are shown below.

Table.1

No.	Assay	TEa (%)	Concentration	Aug – Dec 2019			Aug – Dec 2020		
				CV (%)	Bias (%)	Sigma	CV (%)	Bias (%)	Sigma
1	LDH	11.0	M	0.86	1.21	11.38	0.93	0.59	11.17
			H	0.72	1.64	12.93	0.75	0.28	14.33
2	ALP	18.0	M	2.35	2.05	6.79	3.79	4.82	3.48
			H	1.63	1.11	10.37	2.35	2.26	6.70
3	AST	15.0	M	4.04	0.00	3.72	3.99	4.76	2.57
			H	1.27	1.91	10.30	1.36	1.65	9.80
4	K	6.0	M	1.17	1.31	4.02	1.13	1.36	4.11
			H	1.27	0.31	4.47	1.26	0.43	4.42
5	TG	14.0	M	1.54	2.74	7.31	1.92	0.97	6.79
			H	1.29	3.08	8.48	1.62	0.92	8.09
6	BUN	8.0	M	2.43	1.75	2.58	1.79	0.00	4.47
			H	2.34	0.29	3.30	1.70	0.18	4.59

TEa% was established by Ministry of Health of China and Bias% were collected from EQA reports.

Conclusions:

With the aid of Auto-QC, electrolyte, lipids, and metabolism assays' sigma levels were maintained or improved due to controlled and standardized QC process. However, Enzyme assays' sigma levels, except LDH (given cold denaturation phenomenon), decreased due to instability of enzyme activity. Enzyme assays' performance should be closely monitored with tighter QC rules applied. It was also possible to select appropriate QC frequencies and re-test/calibrate rules based on sigma levels.

Auto-QC also enabled shorter turnaround-time with 95% of first batch in-patient reports released 30 minutes earlier, and doubled the number of tests/5ml-control as dead volume was reduced (180±10µl/10tests Vs 200µl/test).

B-055

Abnormal low-density lipoprotein subfraction profile in young patients with congenital generalized lipodystrophy

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The dosage of LDL particles (LDL-p) presents greater predictive value in the evaluation of CVR, than the measurement of LDL-c cholesterol, independently of established risk factors. There are 8 types of LDL-c subfractions, 6 of which correspond to small LDL-c subclasses and are closely related to the accelerated atherosclerosis process. The most atherogenic phenotypic pattern appears to be pattern B, which small dense low-density lipoprotein (sdLDL) particles predominate. Congenital generalized lipodystrophy (CGL) is a rare autosomal recessive disorder characterized by the near-total loss of subcutaneous adipose tissue since birth, resulting in ectopic fat deposition and severe metabolic disturbances. Affected individuals develop severe insulin resistance (IR), diabetes mellitus (DM), hypertriglyceridemia, hepatic steatosis and earlier cardiovascular disease.The aim of this study was to evaluate the of sdLDL profile performed by ion mobility mass spectrometry methodology (CARDIO ID) in four patients with Congenital generalized lipodystrophy (CGL). We describe 5 cases (4 female/ 1 male), with ages ranging from 21 to 32 years old, with CGL due a mutation in AGPAT2 gene (4 female) and in BSCL2 gene (1 male). 4/5 patients had diabetes (patients 1,2,4,5), 1/4 (patient 4) had previous CV event. AGPAT2 patients presented LDL-c type B pattern and moderate to high cardiovascular relative risk. BSCL2 patient presented LDL-c type A and low relative risk. Cardio ID profile is described in table 1. These findings suggest that LDL dosage alone would not be sufficient to accurately identify patients at high cardiovascular risk and reinforce that LDL subfractions may be useful to detect residual CVR and guide more aggressive therapies and monitoring response to the treatment. Age and type of mutation may be factors implicated in the highest risk.

Table 1- CARDIO ID of four CGL patients.

Patient (Age yo)	LDL (nmol/L)	Small LDL (nmol/L)	Medium LDL (nmol/L)	Big HDL (nmol/L)	Pattern of LDL	Size of peak of LDL (angstrom)	Apolipoprotein B (mg/dL)	Lipoprotein a (mg/dL)
1 (32 yo)	1294	318	329	5694	B	213	127	12
2 (26 yo)	1789	364	197	6289	B	205	189	< 3,0
3 (24 yo)	1130	102	78	5122	B	196	112	13
4 (32 yo)	1298	284	193	4216	B	207	not available	not available
5 (21 yo)	778	90	153	5885	A	224	not available	not available

B-056

Management of Procalcitonin overuse in the Emergency Department through a computer algorithm based on C-reactive protein

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Background: The superiority of procalcitonin (PCT) compared to C-reactive protein (CRP) in the diagnosis of sepsis is clear, but our hypothesis was that CRP could be used to decide upon PCT measurement.

Our aim was to show the PCT demand from the Emergency Department (ED) over years and how through a computerized algorithm based on the CRP value, the PCT measurement can be decreased.

Methods: A cross sectional study was performed from January 1st 2017 to May 31st 2018 to design an intervention to not perform PCT measurement in the ED, based on CRP values. A PCT ≥ 1.5 ng/mL was agreed with ED providers as the value for clinical decision making, considering values <1.5 ng/ml as negative. We retrospectively reviewed all the PCT and CRP values in ED patients and calculated the diagnostic indicators for PCT at four different CRP cut-offs using the PCT as a "gold standard" (Table). From July 1st 2018 to April 30th 2020, the agreed strategy was established, the number of non-measured PCT was counted, and we calculated the economic savings.

Results: The selected CRP cut-off, to not measure PCT, was 0.8 mg/dL, as false negative results were 1%, and the p99 of PCT was 1.5 ng/mL (Table). In the post-intervention period 1091 PCT were not measured and $8457.2 were saved.

Conclusion: Selective PCT use in the ED, based on CRP values; designed by the clinical laboratory in consensus with requesting clinicians; decrease the number of PCT tests performed and generated significant economic savings.

	CRP<0.5 mg/dL	CRP<0.8 mg/dL	CRP<1.0 mg/dL	CRP<2.0 mg/dL
Column	I	II	III	IV
Negatives	779	1045	1179	1642
False negatives	7 (0.9%)	11 (1.0%)	17 (1.4%)	30 (1.8%)
Potential savings ($)	9226.7$	12377.2$	13964.4$	19448.2$
p99 of PCT (ng/mL)	1.00	1.50	1.96	3.37
Sensitivity (%)	98.76	98.05	96.98	94.67
Specificity (%)	20.20	27.06	30.41	42.19
PPV (%)	15.42	16.53	17.04	19.44
NPV (%)	99.1	98.95	98.56	98.17
LR+ (95% CI)	1.24 (1.21-1.26)	1.34 (1.31-1.37)	1.39 (1.36-1.43)	1.64 (1.58-1.69)
LR- (95% CI)	0.06 (0.03-0.13)	0.07 (0.04-0.13)	0.10 (0.06-0.16)	0.13 (0.09-0.18)

B-057

Continuing Education Program via Virtual Learning and Education (VLE) for Technologists of Chemical Pathology

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Introduction: The learning and education of laboratory technologists is an ongoing process. Before COVID-19 pandemics, educational sessions were through face-to-face lectures and small group discussions. During the surge of COVID-19 pandemic in Pakistan, all educational sessions were discontinued. **Objectives:** To develop a Continuing Education (CE) program in a Virtual learning environment (VLE), for technologists. The final objective was to assess the perception of technologists regarding the new CE program. **Methods:** This project was initiated at the section of Chemical Pathology, Department of Pathology and Laboratory Medicine in May 2020 following the guidelines of Plan-do-check-act cycle. The project team included faculty, technologists, QC Coordinator, information technology (IT), and laboratory manager. **Plan:** A team was developed, a shell was created in Moodle (VLE platform). Technologists trained on the VLE to design a course, develop educational content and assessment for CE program modules, orient technologists to attend CE programs via VLE. Perception of technologists about the CE program was carried out in the form of an e-questionnaire allowing analysis with excel. **Do:** Responsibilities of team members were defined, content and assessment developed, uploaded to Moodle, piloted by two technologists and one faculty. An enrollment method for students was created, and they were enrolled to participate in a certificate-based Basic QC module. This module was designed to focus on scientific knowledge (SK) and critical thinking (CT). The module was implemented from July 15th to September 30th, 2020. An e-questionnaire was used for feedback from the users before the final assessment and certificate generation. The questionnaire (five questions) was constructed in four subquestions to which respondents selected a point on a Likert scale. One question asked suggestions to improve this course. Survey completion was taken as informed consent for data to be used in the study. **Check**: Data (Formative assessment scores and feedback of users) was analyzed and shared with the team. **Act**: After the implementation of the module, the detailed report was generated. **Results:** During the three months 36 useable questionnaires were completed. 32 (88.8%) agreed that teaching methods were effective, in contrast, 4 (11.1%) were neutral. Total 31 (86.1%) agreed that the cursed level was appropriate for the level of training of technologists, while 5 (13.8%) were neutral. Seventeen (47.2%) technologist strongly agreed that the aims and objectives of the course were clear, while 15(41.7%) and 4 (11.1%) agreed and were neutral respectively. Thirty-one (86.1%) technologists agreed that the course improved their scientific knowledge and understanding of QC; in comparison, 5 (13.9%) were neutral. Total 33 (91.6%) rated their overall experience as very good, while 3 (8.3%) responded that they had a good experience. Total 24 (66.6%) suggested adding more modules and activities while 12 (33.3%) were given no comments. **Conclusion:** The findings conclude that the use of the VLE encourages independent learning and positive impacts from various contexts. They can; promote reflection, increase enthusiasm and confidence, improved readiness to learn, and more modules should be developed for *continuing* education.

B-058

Evaluation of the Roche Elecsys® SARS-CoV-2 S on Cobas® e801 Assay for Quantitative Total Antibody Detection

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Background: SARS-CoV-2, the causative viral agent of COVID-19, can be identified in respiratory samples with the detection of viral genomic RNA or viral proteins (antigens). However, obtaining respiratory samples is challenging because of variable viral loads. Contributing factors to variability includes the time of sampling with respect to the course of infection, the location of sampling, such as swabbing nasopharyngeal compared to nasal sites, and quality of collection. Other factors include sample degradation during transportation and storage and loss of sample during extraction procedures. Detection of antibodies against SARS-CoV-2 in serological assays is advantageous because the collection of serum samples is less time-sensitive and samples are more stable. Furthermore, processing and analyzing of samples in laboratories is amenable to automation. Most available serological assays enable qualitative detection of IgM and/or IgG antibodies. However, quantitative measurement of total antibodies is advantageous in that they are not impacted by differing rates of seroconversion and can add clinical value to aid in decision-making. Applications include assessing symptomatic patients with negative molecular test results, determining extent of exposure in the population, evaluating responses to vaccines, and estimating antibody titres in convalescent plasma. This study assessed the analytical performance of the Elecsys® Anti-SARS-CoV-2 S from Roche Diagnostics for quantitative detection of total antibodies against the Spike protein (S) of SARS-CoV-2 in serum and plasma.

Methods: Two Roche cobas® e 801 units were used to assess the analytical performance of the kit based on manufacturer's specifications. Quality control material (QC) from Roche Diagnostics was used to evaluate precision and external proficiency testing samples (n=3) from College of Accredited Pathologists (CAP) were used to evaluate accuracy. Positive (n=47) and negative (n=57) serum samples were used to assess precision at decision levels, linearity, assay concordance, and cobas e 801 unit correlations. Serum samples positive for other respiratory viruses but negative for SARS-CoV-2 (n=15) were used to assess cross-reactivity. A total allowable error of 25% was used.

Results: Overall precision of the assay was acceptable with coefficient of variation (CV) values of 0.7-1.8% and 1.0-2.7% at the decision cut off (0.80 U/mL). The analytical measuring range as specified by the manufacturer of 0.40-250.00 U/mL was linear and could be extended via autodilution of 1:20. The two cobas e 801 units correlated well (y-intercept of -0.977 and a slope of 1.018). The assay demonstrated 100% agreement with two previously validated qualitative assays, the Roche Elecsys anti-SARS-CoV-2 (anti-Nucleocapsid or N) assay on Roche cobas e 801 and the SARS-CoV-2 Total (COV2T) assay on Siemens Atellica® IM (anti-S) platforms. There was no cross-reactivity to serum from patients who tested positive to other respiratory viruses, including coronaviruses NL62 and HKU1 and influenza H1N1. Interferences of hemolysis, icterus, or lipemia was not significant. Serum samples were stable for up to 8 days (maximum number of days assessed) at room temperature.

Conclusion: The Elecsys Anti-SARS-CoV-2 S assay showed excellent analytical performance, permitting its application in clinical laboratories for aiding in outbreak surveillance, assessing past exposures, evaluating response to vaccines and supporting decisions on containment.

B-059

Evaluating Effectiveness of Delta Check Performance at a High-Volumes Clinical Laboratory

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Background: Data from recent studies suggest that the highest incidence of laboratory-related errors occur in the pre-analytical phase of laboratory testing. The Clinical Laboratory Standards Institute recommends that delta check programs should be monitored after implementation, to assess their effectiveness. Aim was to implement and evaluate the feasibility and effectiveness of Delta check at a high-volume clinical laboratory. **Material and Method:** This audit was conducted at the Aga Khan Clinical Laboratory, from December 2016 to January 2020, based on Plan, do check act cycle. Audit team included, Chemical Pathologist, Technologists, QC Officers, Information technology (IT) analyst and laboratory manager. **Plan**: A policy on 'Error detection for unusual results' was developed; delta check protocols and procedures were defined and a training & education program for all technologist was developed. **Do**: Initially the delta check was implemented on eight analytes (Creatinine, BUN, Sodium, Potassium, Total Bilirubin, Calcium, Phosphorous and Albumin) on middleware in Jan 2017. The delta limits were taken from Tietz textbook of Chemical Pathology, and delta percent change method was utilized. **Check**: Audit was done from May-August 2018. For feasibility technologist time consumed in evaluating a delta failure (DF) results was analyzed while for effectiveness, frequency of true DF was examined. To identify deficiencies gap analysis was done. **Act**: Developed a program for fulfilling the gaps and re-audit to assess feasibility and effectiveness was done in Jan 2020. **Results:** During Audit period, delta check was applied on CentraLink for 8 routine chemistry analytes, in four months period 0.07% (n=248 out to 350363) DF were observed, most common analytes were creatinine (30%, n=75) followed by Potassium (K) (23.4%, n=58) and BUN (24%, n=59) respectively. Few common reasons included patient dialysis, unsatisfactory specimen, fluid infusion and dehydration related changes. Daily 3-5 DF were observed and total technologist time taken for corrective actions was 30-50 minutes daily. On Gap analysis it was identified that reasons of DF were not documented, data was manually collected, CentraLink was unable to differentiate between serum and urine samples, no patient history available, turnaround time of reporting was increased, not implemented in labs at locations other than main hospital, different type of delta methods could not be applied in CentraLink. To overcome these gaps, corrective actions were taken process flow algorithms defined for each analyte, technologist trained and given access to view patient history, a program of delta check developed for implementation on in-house ILMS and re-audit was done. Delta limits were derived using 'Reference Change Values' formula and algorithms for corrective action were designed for each analyte. Hands-on training were conducted for all technologists and after that delta check was implemented on ILMS for only creatinine, in one-month period 2.7% (n=653 out of 23651) DF observed, with daily 20-30 and total technologist time taken for corrective actions was 60-120 minutes daily. **Conclusion:** The findings strongly suggest that the process of manual investigation of every delta check is inherently time-consuming, automated system of delta check identification, and algorithms, reduce error rate and time consumed in corrective actions.

B-060

Development of a validation and implementation plan for 50 blood-gas analyzers across a large geographic area

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Background: Laboratory directors sometimes face the task of implementing numerous point of care analyzers across multiple sites. In these cases, validation and implementation plans must be developed to efficiently utilize resources, meet project timelines and satisfy medical and accreditation requirements. We developed a validation and implementation plan for 50 GEM 5000 (Instrumentation Laboratory; IL) blood gas analyzers across 8 urban centers and 17 laboratories in the province of Alberta, Canada. **Methods:** A team of clinical biochemists developed a staged plan that satisfied requirements of the Clinical Laboratory Standards Institute (CLSI) for method validation. Goals of the evaluation were outlined, as well as logistical requirements. Type and quantity of evaluation samples, including quality control and patient samples for instrument comparisons (vs GEM 4000 analyzers), were selected based on clinical decision thresholds and analytical measuring range. Locations of different workup stages were identified, and the plan was finalized with technical and operational leads at each laboratory. **Results:** Two analyzers were obtained for a full, representative validation at a single site. This was done to determine whether the performance of the instruments justified purchasing the remaining analyzers. The evaluation included the following studies: between and within run imprecision (3 levels of control materials, 20 replicates each), linearity (5 levels in quadruplicate), and patient sample comparisons [150-300 samples in singleton for most analytes, 40 for glucose and chloride] to existing GEM 4000s on site. GEM System Evaluator Controls (levels 1-3) were used to evaluate imprecision whereas Instrumentation Laboratory PVP controls were used to evaluate linearity. Patient samples were obtained from routine blood gas workload at each site. Provided the GEM 5000 tests had bias, imprecision and total error favorably below total allowable error (TAE) set by the College of American Pathologists, the remaining 48 instruments were purchased and transported to a central site. Here, a 'Stage 1' evaluation was performed, which included abbreviated studies of within-run imprecision (3 levels, 10 replicates), linearity testing linearity (5 levels in duplicate) and patient sample comparisons (20-30 samples in singleton) to on-site GEM 4000s, as well as linearity (5 levels in duplicate). At this stage, linearity was evaluated using RNA medical CVC 123 and 223 calibration verification controls. If bias, imprecision and total error were favorably below TAE, analyzers were relocated to their final destinations for 'Stage 2' of the evaluation - which was to complete testing of the remaining controls and specimens required by CLSI and determine if movement of the analyzers affected performance. Studies included between run imprecision (3 levels in duplicate over 5 days) and patient sample comparisons (10-20 samples) to on-site GEM 4000s. If imprecision, bias or total error exceeded TAE for any test, clinical biochemists met to discuss potential explanations, to determine if additional investigations were required or if analyzers had to be replaced. Over several months, all GEM 5000s passed both stages of the evaluation and replaced existing GEM 4000s at each site. **Conclusions:** Multi-instrument validations and implementations require very careful planning and extensive clinical/technical/operational collaboration in order to succeed.

B-062

The importance of a Medical Consulting Center as a strategy for customer relationship management regarding altered tests results

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Background: The use of clinical laboratory test results in diagnostic decision making is an integral part of clinical medicine. Laboratory services are relatively safe and errors due to analytical problems have been significantly reduced over time. Even so, as a precaution, some altered results may not be released without repetition in a new sample. To avoid unnecessary recollections, a structured Medical Consulting Center (MCC) in the clinical laboratory, composed of doctors and biomedicals, has a key role in assessing the patient´s previous data and/or making a contact for recollection, to better understand if the altered result makes sense or not. Therefore, the aim of this study was to assess the percentage of patients that no longer needed a new collection after the MCC evaluation.

Methods: Retrospective evaluation of all recalls for analytical reasons, to confirm altered results, in a private laboratory located in Rio de Janeiro, Brazil, from January to December of 2020. Within this time interval, all cases that led to the request for a new

sample to confirm the result, were recorded, as well as all canceled sample requests. As a standard laboratory procedure, to define if the recall must be canceled or not, the MCC evaluates all recall cases either by checking patients´ previous data in the Laboratory Information System (LIS) and/or contact to either the patient or the prescribing physician to better understand the context in which the exam was requested.

Results: A total of 4,764 altered results led to recall for the result confirmation, due to analytical reasons. Of this total, 202 (4.24%) patients did not need a new sample collection after MCC evaluation. Of the total number of canceled sample requests, 11 (5.44%) were from urinalysis, 12 (5.94%) from biochemistry, 44 (21,78%) from toxicology and 135 (66.83%) from hematology.

Conclusion: Although a small percentage of all cases did not require a new sample collection after MCC evaluation, it is important to note that avoiding unnecessary recollections provides a faster release of the altered result, which allows a prompt medical decision regarding patient´s health. Moreover, it leads to a lower cost to the laboratory. Therefore, an MCC composed of experienced medical specialists, plays a role on the perception of value and reliability of laboratory results.

Laboratory Stewardship and Patient Safety

B-063

Pandemic-associated trends in measurement of HbA1c for children with diabetes mellitus

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Background: The COVID-19 pandemic has disrupted access to care, which has serious consequences for patients with chronic illness, such as diabetes mellitus (DM). Routine monitoring of hemoglobin A1c (HbA1c) is the standard of care in DM, yet, adhering to regular appointments may be infeasible during a pandemic.

Methods: We evaluated trends in patient encounters and laboratory testing for DM in a pediatric healthcare system for a six-month period pre-pandemic (March-September) in 2019 and during the pandemic in 2020. Descriptive statistics were performed for 17,367 patient encounters and 7,193 HbA1c results by point of care testing during these time periods. We also evaluated use of dried blood spots (DBS) for measurement of HbA1c by Vitros 5600 chemistry analyzer, establishing method linearity, precision, and correlation to whole blood (WB) HbA1c.

Results: COVID-related stay-at-home orders were associated with significant decreases in the number of in-person office visits and point-of-care HbA1c tests for patients with DM. A significant increase in > 14 % HbA1c was observed in May 2020 relative to 2019, but other measured HbA1c values did not differ.. HbA1c by DBS exhibited good correlation to WB measurements (r = 0.9889) and intra- and inter-assay precision from 0.5 - 3.5 %.

Conclusion: The COVID-19 pandemic was associated with poor adherence to regular monitoring of HbA1c in our healthcare system. As HbA1c remains the standard of care for DM in both children and adults, HbA1c measurement by DBS as a complement to telemedicine services may offer a means to address this gap in care.

B-064

Gamma Glutamyl Transferase Activity Has Limited Utility in Predicting Alkaline Phosphatase Isoenzyme Elevations

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Introduction: One way laboratories can add value is through the evaluation of send-out testing. Alkaline phosphatase (ALP) isoenzymes (isoenzymes) testing, in which ALP activity is assigned to hepatic, bone, intestinal, and/or placental ALP by various fractionation methods, is a common sendout. Gamma glutamyl transferase (GGT) activity has been used to triage such orders due to the shared mechanism of release of GGT and ALP. The underpinning assumption is that elevations in GGT coincide with hepatic isoenzyme elevations. However, this has not been systematically evaluated in recent literature. We performed retrospective analysis to determine the effectiveness of this approach. Methods: We obtained reports for GGT, ALP, and isoenzymes measurements spanning an eight year period and identified patients for whom all measurements were available within a seven-day span. We examined the ability of GGT measurement to predict isoenzymes elevations through 1) calculation of sensitivity and specificity in the context of institutional reference intervals and 2) receiver-operator characteristic curve analysis. Results: We identified 153 suitable patient specimens. Eighty-five percent of concurrent measurements originated from specimens collected within one day. GGT was insensitive (46.6%) but reasonably specific (85.0%) for hepatic isoform elevation. Measured GGT activity within reference limits was insensitive (59.8%) and nonspecific (46.4%) for identification of nonhepatic isoform elevation (area under the curve = 0.52). This owed partially to frequent concurrence of hepatic and nonhepatic isoform elevations. GGT elevation coincided with 42 observed elevations of nonhepatic isoforms. For performance against specific isoforms, see the Table. Discussion: In this setting, GGT measurement adds expense to patient testing while having little utility in predicting hepatic and nonhepatic isoform elevations. Given the finding that GGT identified hepatic elevations in less than half of cases, while hepatic elevations frequently occurred alongside nonhepatic elevations, we have discontinued the practice of requesting GGT measurements before confirming with isoenzymes testing.

Gamma Glutamyl Transferase Performance in Prediction of Alkaline Phosphatase Isoform Elevation

GGT[1] activity as predictive of:	AUC[2]	Sensitivity (%)	Specificity (%)	PPV[3] (%)	NPV[4] (%)	Accuracy (%)
Hepatic Isoform elevation	0.68	46.6	85.0	95.4	19.3	51.6
Any non-hepatic isoform elevation	0.52	59.8	46.4	65.9	40.0	54.9
Intestinal isoform elevation	0.64	81.5	47.6	25.0	92.3	53.6
Bone isoform elevation (electrophoresis)	0.47	55.4	40.0	52.3	43.1	48.4
Bone isoform elevation (ELISA[5])	0.59	54.2	28.6	56.5	26.7	44.7

[1]GGT, gamma glutamyl transferase; [2]AUC, area under the Receiver-Operator Characteristic curve; [3]PPV, positive predictive value; [4]NPV, negative predictive value; [5]ELISA, enzyme-linked immunosorbent assay

B-065

Improving Point-of-Care Testing Device Cleanliness: Nursing and Point-of-Care Partnership

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Background: Point-of-Care testing (POCT) devices must meet specific accreditation requirements surrounding device cleanliness. During a 2017 College of American Pathologists (CAP) accreditation inspection, POCT devices from two separate units were found to be visibly soiled by the inspection team. This inspection highlighted some opportunities for improvement surrounding POCT device cleanliness. **Methods:** After the citation was issued, monthly POCT device audits were implemented on over 200 POCT devices hospital wide to assess compliance. When a soiled device was identified, re-education was sent to the individual nursing unit. Continued audits showed no improvement, so a new approach was taken. The decision was made to work collaboratively with a group of nursing units to discuss and understand the various processes regarding POCT and to identify the root causes of continued non-compliance. Over the course of several weeks, process maps were developed for the nursing units involved in the project. Risk points were identified and mitigation steps were developed. Differences were identified in each unit's processes which indicated the need for a variety of resources that were better tailored to each scenario. Standard unit resources were developed in addition to ala carte options that units could utilize. Standard resources included developing new education to be used upon hire, cleaning requirement highlights and quiz questions added to all initial, 6 month, and annual POCT competencies, adding wipe acceptability to annual education, and continuing the audit process on all nursing units. Additional options involved self-audit calendars tailored to the unit's needs and visual indicators created for a variety of circumstances around where the devices may be stored including on shelves, tables, or in trauma bags. The POCT team also reached out to the disinfectant vendor to obtain wipe specific stickers that can be applied to devices. These stickers were color coded to the wipes and indicate proper contact time. Color coded wipe holders were also provided to ensure that the appropriate wipes were always available as missing wipes were frequently identified as an issue. Nursing units developed a mantra to be used when a device was found away from its home base - "if it's found, wipe it down". Once resources were rolled out, the POCT team increased audits to weekly for the purposes of prompt feedback. Due to the success of the project, these were rolled back to monthly after 3 months.**Results:** In 2018 and 2019, prior to starting the collaboration with the nursing units, 15 and 20 soiled POCT devices were found throughout the year, respectively. In 2020, after the collaboration with nursing units, only 6 devices were

found visibly soiled throughout the entire hospital for the year. **Conclusion:** Nursing and POCT teams collaborated to identify the root causes surrounding POCT device cleanliness. This improvement project not only identified underlying issues, but also helped improve communication between different clinical teams. This process improvement ultimately enhances the quality of patient safety and improving the overall patient care.

B-066

Implementation of POCT COVID-19 Testing using a Mesa Biomedical SARS-CoV-2 Testing Platform in a Labor and Delivery Department

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Background: In the spring of 2020, a global pandemic for Coronavirus disease 2019 (COVID-19) was declared by the World Health Organization (WHO). As patient care adjustments were made in the Johns Hopkins Hospital (JHH), the need for a rapid COVID-19 test result was identified in the Labor and Delivery (L&D) unit. Several methodologies and platforms for COVID-19 Point of Care Testing (POCT) were evaluated early in the pandemic, including antibody, antigen, and molecular platforms. The Accula™ SARS-CoV-2 Test, a molecular POCT, fit the needs of the L&D unit based on the time to results and ease of use. The goal of this project was to decrease turn-around times - measured from Order Time to Result Time, and improve the quality and safety of patient care. **Methods:** The Accula™ SARS-CoV-2 Test was utilized to assess nasal swab specimens collected from pregnant women being admitted to the L&D unit at JHH for the presence of SARS-CoV-2 viral RNA. All L&D RNs received extensive training from a POC Coordinator and/or approved unit trainer prior to independently performing specimen collection and patient testing. An RN would collect a single nasal swab from both patient nostrils then place into the labeled SARS-CoV-2 Buffer extraction solution for transport to the POCT Laboratory for testing. Additional steps are completed once in the POCT laboratory, including visual interpretation of the results. All results are documented on a paper patient result log, which was in turn used to document results in the Electronic Medical Record. In this workflow design, a single RN was responsible for the entire process, which, in turn, led to quicker result availability and rooming of the women in the proper location with applicable ventilation and PPE requirements to assist in the provision of high quality, safe care. Quality oversight activities performed by the POCT Office included monitoring of positivity rates, regular audits of all logs (QC, patient result, and maintenance), and swipe testing to check for the presence of SARS-CoV-2 viral RNA on the testing surface and Accula™ Docks. **Results:** Prior to the implementation of POCT COVID-19 testing, delays of greater than two hours between specimen collection and result availability were common as hospital operations adjusted in the early months of the pandemic. The availability of two Accula™ Docks at the POC allowed for an expedited process of specimen collection, testing, and resulting for women being admitted to the L&D Unit. The POCT method go-live occurred on 5/11/2020; in the months following, the average turnaround time was 48 minutes. Comparatively, in the month of February 2021, a decrease in the average TAT down to 0:35 minutes was noted. **Conclusions:** The Accula™ SARS-CoV-2 Test provided a viable solution for the Labor and Delivery Unit at JHH, with improvements noted in turnaround times and in the quality and safety of patient care. Even with an easy-to-use platform, however, robust quality oversight is key in ensuring success in the implementation and maintenance of COVID-19 testing at the Point of Care.

B-067

Implementation of POCT SARS-CoV-2/Flu A&B/RSV Testing using the Cepheid GeneXpert Xpress Testing Platform in a Tertiary Care Adult and Pediatric Emergency Department

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Background: In the spring of 2020, a global pandemic for Coronavirus disease 2019 (COVID-19) was declared by the World Health Organization (WHO). As adjustments were made to patient care in the Johns Hopkins Hospital (JHH), the need for a rapid COVID-19 test result was identified as immediate in the Adult and Pediatric Emergency Departments (EDs). The Cepheid GeneXpert Xpress was identified as fitting the needs of the Adult and Pediatric Emergency Departments due to throughput capacity, availability of the SARS-CoV-2/Flu A+B/RSV test cartridge, time to results, interfacing capabilities, and ease of use. The goal of this project was to increase the percentage of SARS-CoV-2 results available within 3 hours of patient arrival, improve turnaround times for STAT SARS-CoV-2 results, and improve the quality and safety of patient care for symptomatic patients presenting to either Emergency Department with influenza-like illness.

Methods: The implementation of the Cepheid Gene Xpert Xpress SARS-CoV-2 instruments in the adult ED was expedited with a goal of less than six week timeline, from conception to implementation. Testing was set up in a newly built ED point-of-care laboratory staffed by Certified Nursing Assistants (C.NAs) working 4-hour shifts. The ED laboratory was created specifically for this project and is staffed 12 hours a day, 7 days a week and processes 75% of the daily SARS-CoV-2 samples for both EDs. All testing personnel received extensive training from a Point of Care Coordinator prior to independently operating the Xpert Xpress analyzers. The operator responsibilities include documenting results on a patient result log, which is then used to be transcribed into the Electronic

Medical Record. Quality oversight activities performed by the Point of Care Testing Office include supply management due to extreme allocation limitations initially, monitoring of positivity rates, daily audits of all logs (QC, patient result, and maintenance), and swipe testing to check for the presence of viral material on the testing surface and GeneXpert Xpress systems, among others.

Results: The benchmark turnaround time for STAT tests performed from symptomatic patients via the Microbiology laboratory was at least 60% of test results available ≤3 hours of patient arrival in the ED. In the month of November 2020, prior to POCT implementation, an average of 66 tests were resulted per day, with 63.7% meeting the benchmark. By comparison, in the 3-month timespan following the December 2020 Go-Live with the POCT method, the following improvements in statistics were noted. From the Adult Emergency Department, an average of 39 tests were resulted per day, with a mean 87.3% of results being made available within 3 hours of patient arrival. Using median arrival to result time calculations for the time period of 12/8/2020 through 3/8/2021, the average turnaround time utilizing the POCT methodology was 98 minutes, improved from 160 minutes. The Pediatric ED saw a 93.4% rate benchmark in the same time period.

Conclusions: The Cepheid SARS-CoV-2 Test at the point-of-care provided a viable and effective solution for the Adult and Pediatric EDs, with improvements noted in turnaround times of results.

B-068

Continued Impact of Antimicrobial Stewardship Program In A Rural Community Hospital Three Years After Its Implementation: Fourth Year Results.

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Background: Effectiveness of antimicrobial stewardship program in a hospital environment has been proven valuable and is a federal requirement from Centers for Medicare and Medicaid. US hospitals are required to follow recommendations set forth by Centers for Disease Control and Prevention, Infectious Diseases Society of America, the Society for Healthcare Epidemiology for development and implementation of antimicrobial stewardship program. However, based on hospital size and resources available, there are inconsistencies in program implementation, viability and success. Described here is an antimicrobial stewardship program that was implemented in a rural community hospital with limited resources, absence of infectious diseases physician on staff and only *part time* availability of a PharmD clinical pharmacy specialist trained in infectious diseases. The program was ongoing for three years with successful results (described elsewhere) and this is a fourth year report on program impact.

Methods: Eastern Niagara Health Care Lockport Memorial Hospital in Lockport, NY is a 90 medical beds and 8 ICU beds hospital with hospitalist service, private physicians, and a medical residency program. A designated stewardship physician was a hospitalist attending due to the absence of infectious diseases physician on staff. Stewardship clinical pharmacy specialist was available only on part time basis due to limited resources. We also received support from the intensivist/pulmonologist and a physician chief of staff. Infection control nursing, laboratory supervisor, nursing supervisor, pharmacy director, IT support, medical residents, hospitalists and private physicians, as well as nursing and laboratory staff were educated regarding the program. New and ongoing antimicrobial stewardship policies and procedures were put in place. All antimicrobial stewardship policies and procedures were approved by P&T and Medical Executive Commitees. Antimicrobial policies and procedures put in place (in addition to the existing limited pharmacokinetic monitoring) included pharmacy-driven automatic renal dosing for all antimicrobials and antivirals, automatic pharmacy drived pharmacokinetic and laboratory monitoring, dosing adjustments, pharmacy-driven automatic intravenous to oral antimicrobial changes, empiric antibiotic drug recommendations (including in ICU and sepsis patients), de-escalation

procedures. Formulary reviews were performed and certain antimicrobial use was restricted to certain criteria (Vancomycin, Linezolid, Carbepenems) or restricted to a certain provider, for example intensivist (Tigecycline, Caspofungin), or removed from the formulary alltogether (Ertapenem, Oritavancin).

Results: *Total number of interventions* made in the first 3 years was 648. There were 768 interventions made in the 4th year.

Intravenous to oral changes accounted for 29% (50/172), 16% (42/264), and 11% (24/212) in the 1st, 2nd, and 3rd year, respectively. In the 4th year, these accounted for 8% (61/748).

Number of recommendations to *discontinue/streamline antibiotic* was ~53% (91/172), 46% (122/264) and 61% (129/212) in the 1st, 2nd year, and 3rd year, respectively. In the 4th year, these accounted for 12.5% (96/768).

Conclusion: Initially, a lot of time and effort was spent on antimicrobial prescribing evaluations, policies and procedures, provider education. In the fourth year of the program we made more total number interventions then the first three years combined possibly because of automation of the process, policies/procedures and providers being more familiar and agreeable with our efforts.

B-069

Risk-Based Approach to Ensure Operator and Patient Safety in Point of Care Blood Gas Testing during COVID-19 Outbreak in Hospital Sungai Buloh

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Background: The COVID-19 global pandemic has changed the way hospitals and laboratories approach patient and operator safety worldwide. At the start of the pandemic, Sungai Buloh Hospital was chosen as the COVID-19 center in Malaysia for screening, isolating, and treating those cases. To reduce the spread of the disease among hospital staff and ensure patient safety, the Pathology Department performed a risk-based approach to determine the best test methodology to address the increased demands on blood gas testing. **Methods:** A hazard risk matrix was utilized to identify and categorize the potential risk of contagion caused by following the established practices. Risk value (RPN) was calculated by the product of the Severity (1 to 5) and Probability (1 to 5) factors on the identified activities and hazards. Blood gas testing at the POC site was selected to assess risks and their mitigations compared to the current practices (Figure1). GEM Premier analyzers (8 GEM Premier 3500 and 2 GEM Premier 5000) were the selected for POC blood gas analyzers during the exercise. **Results:** A significant reduction in risk values was observed after the evaluation of the POC approach. All observed high-risk factors were mitigated and a significant reduction in overall risk was observed each of the evaluated factors, where the RPN values dropped from 14-10 to 8-3. The impact of the GEM analyzers in the risk analysis results was significant, especially in to reducing biohazard exposure, low maintenance, internal management approach (iQM) which includes the ability to self-diagnose and self-correct system issues, and the capability to remotely manage through GEMweb Plus. **Conclusions:** Overall, the new framework enabled a reduction in lab staff exposure, reduced turn-around-time, improved sample results integrity, and improved safety during the testing processes.

Figure1: Risk Assessment for Blood Gas Testing

Risk Identification		**Risk assessment pre-Implementation**	**Risk Evaluation**	**Risk assessment post-implementation**
Work activity/ Process step	**Hazard**	**Risk Priority Number (RPN)***	**Risk-controlling measures**	**Risk Priority Number (RPN)***
Blood Gas analysis on GEM Premier 3500 and GEM Premier 5000	Specimen exposure	12	Proper PPE; self-contained PAK; no maintenance/ troubleshooting or tubing exposure, isolated environment	6
	Specimen disposal	10	Clinical waste bin is placed next to the analyzer in an isolated environment	6
	Pre-analytical, clotted specimen	14	iQM/iQM2 detects/flags micro-clots and performs corrective action without biohazard exposure to operators	4
	tHb correlation to DxH Unicel 800	12	Lab quality tHb in POCT, no exposure to lab and quick TAT	3
	Delayed lactate analysis	12	Lab quality Lactate at POC to reduce TAT	3
	Unauthorized patient data access	14	GEMweb Plus for remote access and analyzer user lock-out if not trained	4
	PPE disposal	12	Clinical waste bin is placed next to the analyzer (4) in isolated environment	8
Wrong test ordered in the LIS system	Priority sample delayed in lab queue	12	Place designated personnel in receiving counter	8
Unmanaged samples (incomplete sample electronic transaction)	samples cannot be processed	12	i-record and discard after 4 hours; ii-phone call to clinician, POC offered quick TAT	4
Delayed specimen send to lab (> 4 hours) for electrolytes testing	samples cannot be processed - samples quality is questionable	12	i-record and discard after 4 hours; ii-phone call to clinician, POC offered quick TAT	4
Test cartridge requires replacement	Operator exposure to isolation ward	12	Surveillance remotely via GEMweb Plus and replacement of cartridges by operators in isolation ward	6
Equipment malfunction	samples cannot be processed	14	simple troubleshooting done by user/ officer with online/phone assist	6

*RPN >12=high risk, between 12-6=medium risk and <6=low risk

B-070

Two cases of incorrect manual preparations of formula contributing to severe hypernatremia in tube-fed children

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Background: Like breast milk, infant formula has an osmolality similar to serum. This minimizes osmotic movement of water between intestinal contents and blood, thereby preventing electrolyte disturbances. To achieve the correct osmolality in manual preparation of formula, care must be taken to mix the appropriate volume of formula powder with water. Mixing errors can produce hyper- or hypo- osmolal formula, which could have a significant impact on patients who cannot control their intake. We describe two cases of children provided overly concentrated manually-prepared formula via feeding tubes who later experienced severe hypernatremia. **Methods:** Both cases were identified after the laboratory reported critically high plasma sodium, chloride or osmolality, or was asked to run the same tests on formula samples to determine if any were prepared incorrectly. Cases and results were reviewed with attending physicians, nursing staff and clinical safety teams to determine if incorrectly prepared formula was the cause of any electrolyte disorders. **Results:** Case 1: the laboratory was sent a formula sample for a 5 month old patient (at birth: 1500 g, 29 week gestation) in the pediatric intensive care unit (PICU) with a nasojejunal tube (NJ). The patient recently had a pancreatectomy due to congenital hyperinsulinism, and experienced profound hypernatremia (> 180 mmol/L) and hypovolemia after feeding of manually-prepared formula via NJ tube. The formula sample had an osmolality of >2000 mOsm/kg. After discussion with attending physicians and the clinical safety team, it was determined that formula was not mixed by unit staff and that its osmolality was responsible for the patient's profound hypernatremia and hypovolemia. Case 2: the laboratory was contacted about a 1 year old patient with spinal muscular atrophy and a nasojejunal tube (NJ). After a NJ feed of manually-mixed formula, the patient became lethargic and later presented in the emergency department with septic shock, a Glasgow coma score of 8, plasma sodium of 174 mmol/L, watery stools and hypovolemia. The patient's hypernatremia was gradually resolved through intravenous fluids and NJ feeds. Two formula samples were provided to the laboratory - one manually-prepared by the family had an osmolality of 523 mOsm/kg whereas a sample of pre-mixed formula had an osmolality of 306 mOsm/kg. After discussion with attending physicians and the clinical safety team, it was determined the manually-prepared formula from the family was very likely the cause of the patient's hypernatremia and hypovolemia. **Conclusion:** Manual preparation of formula must be done carefully in patients with feeding tubes. Pre-mixed formula should ideally be provided to high-risk patients.

B-071

Large Biases in Sodium Measurement Between the ABL90 Flex and ABL800 Blood Gas Analysers in the Severe Hypernatremic Range

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Background: We recently reported clinically significant differences in sodium results between routine plasma and whole blood assays in the severe hypernatremic range, where the ABL90 Flex blood gas analyser (Radiometer) was found to produce values as much as 9 mmol/L greater against other methods evaluated. In the present study, we sought to test whether this phenomenon was similarly exhibited on the widely used ABL800 blood gas analyser.

Methods: Whole blood was collected from a healthy donor into lithium heparin vacutainers (BD), a portion of which was spiked with sodium chloride solution to generate a high sodium pool. 8 samples with target sodium concentrations ranging from 140 to 185 mmol/L were obtained via admixture. Total protein and triglycerides were verified to be within their respective reference intervals. Whole blood sodium was measured in duplicate on ABL90 Flex and ABL800. The samples were immediately centrifuged and plasma sodium was measured in duplicate on cobas c702 (Roche).

Results: Figure 1 shows sodium values obtained by each method against expected sodium in the spiked samples. The magnitude and pattern of positive bias in ABL90 sodium results were consistent with our previous work. Whereas differences of 5-6 mmol/L were found on the ABL90 compared to cobas c702 in samples with sodium above 160 mmol/L, results on the ABL800 were always between 1-3 mmol/L of plasma results.

Conclusion: In severe hypernatremia, sodium results produced by the ABL90 Flex analyser can differ significantly from a corresponding plasma measurement. The extent of bias is not seen with the ABL800 analyser produced by the same manufacturer, suggesting the issue may be based in differences in methodology or design. Given accurate and consistent measurement of sodium is critical for the management of hypernatremic patients, laboratories and clinicians that utilize the ABL90 Flex instrument must be alert to such potential discrepancies.

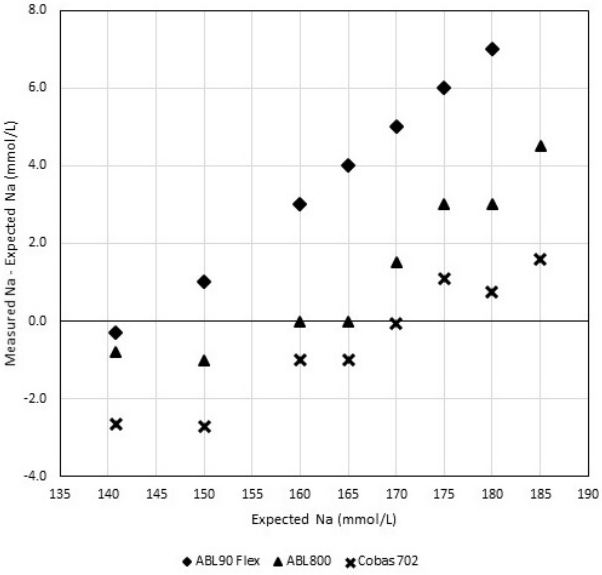

Figure 1 Observed bias between ABL90 Flex (whole blood), ABL800 (whole blood) and Cobas 702 (plasma) relative to expected values

B-072

The importance of clinically appropriate acceptance criteria for creatinine reagent lot validations

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Background: Reagent lot-to-lot validations ensure changes to reagent lots are both analytically and clinically acceptable. An important aspect of lot-to-lot validations is the use of appropriate acceptance criteria. This case study describes a creatinine lot change that resulted in clinically significant changes despite having been found analytically acceptable by both laboratory and manufacturer criteria. This experience helped us refine our internal acceptance criteria to ensure future lots are appropriately assessed. **Methods:** Reagent lot validation compared creatinine results from QC material (Bio-Rad Liquid Unassayed Multiqual) and 10 de-identified patient serum samples across the analytical measuring range of the assay. Acceptance criteria were based on proficiency testing guidelines from the Institute of Quality Management in Healthcare (IQMH) ([Creatinine] <100 µmol/L: ±9 µmol/L; [Creatinine] ≥100 µmol/L: ± 9%; Precision limits: CV <3 %). Investigation of patient means before and after new reagent lot implementation was performed through an anonymized database linked to the lab information system (LIS). **Results:** For the initial reagent lot validation, a total error associated with the patient correlation was a maximum of 6.8 µmol/L for creatinine <100 µmol/L and 6 % for creatinine >100 µmol/L. QC results for all levels were within our acceptable criterion of <3 % bias and CV <2 %. Nevertheless, repeated inquiries by physicians about clinically significant changes in eGFR results prompted further investigations. The manufacturer indicated an expected bias of 6 - 8 µmol/L (manufacturer acceptable limit: 8 µmol/L) with the new reagent lot. Further investigation of patient means pre and post reagent implementation indicated a positive shift of 7.2 µmol/L. Physicians in the community were concerned with these changes, and descriptions of deviations >10 µmol/L and more significant changes to eGFR were reported. Similar observations were made by other institutions around the world. A matrix-dependent effect was observed with this reagent lot that affected serum and EDTA plasma, but not heparinized plasma samples. Our lot-to-lot validation data confirmed the impact on patient serum samples, but not on QC material. **Conclu-**

sion: This case study clearly demonstrated the need to critically evaluate commonly used assay performance acceptance criteria. For assays with stringent clinical performance requirements, an analytically acceptable level of change may still translate into a clinically significant deviation in assay performance. Monitoring patient means during the lot transition period may help early identification of matrix-dependent reagent lot changes. In addition, all clinical uses of an assay, such as calculation of eGFR, should be considered when assessing acceptance of a new reagent lot.

B-073

Validation study of neutralizing antibodies and IGG-RBD SARS CoV 2

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Background: Since the emergence of COVID 19 in December 2019, many tests for detection of SARS-CoV-2 have been developed, as well as methods for immunological study in response to contact. Most serological tests were authorized for use on an emergency basis by government institutions, and the clinical laboratory had a great responsibility of assessing assay performances and stablishing the application in the laboratory routine. Understanding the performance of a method is of great importance to evaluate its impact in clinical applicability, results, and patient safety. In the year 2020, clinical laboratories, as well as manufacturers, were subjected to a continuous learning curve in the face of the short term of study of development, validation and availability in the laboratory market and great demand. Immune responses observed in COVID-19 frequently differ from the antibody dynamics of appearance that is usually observed in other infectious diseases, making it difficult to interpret the results of validation studies. In 2021 new methodologies emerged, such as neutralizing antibodies immunoassay (NAbs) and anti-RBD-IgG, showing greater performance than those observed in conventional serological assays used previously. The aim of this study was to evaluate the correlation between NAbs (c-pass Genscript™), chemiluminescent immunoassay (CLIA) anti-RBD-IgG (Snibe™) and sVNT (viral neutralization test).**Methods**The study was conducted in 120 serum samples. Expected positive were 93 samples collected at least 40 days after symptoms onset from patients diagnosed with COVID-19 by RT-PCR; and expected negative samples were 7 sVNT negative samples plus 20 samples from the 2019 archive of the laboratory. The samples were assayed by c-pass Genscript™ ELISA, anti-RBD-IgG CLIA (Snibe™) and sVNT. The correlation was evaluated for NAbs (c-pass Genscript™) and anti-RBD-IgG (Snibe™) compared to sVNT. The sVNT was performed employing pseudoviruses containing the receptor-binding domain from the original SARS-CoV-2 Wuhan 1 strain. Inhibition of luciferase activity by patient`s sera in susceptible cells expressing ACE2 was measured and the geometrical mean titers compared to a negative standard.For the study of the correlation between neutralizing antibodies and IgG-RBD, we used a total of 80 samples, with 20 samples from 2019 being used as negative samples, and 60 samples for COVID-19 diagnosed patients (by RT-PCR) distributed according to time from the onset of symptoms: 7-14 days (n=20), 15-21 days (n=20) and >21 days (n=20).**Results**Compared to sVNT, NAbs showed 100% sensitivity (CI: 96-100%) and 100% specificity (CI 87.5-100%); and IgG-RBD showed 95% sensitivity (CI: 88-97.7%) and 93% specificity (CI 76.6-97.9%).Using NAbs as the reference method, IgG-RBD showed 91% sensitivity (CI: 80.4-96.1%) and 96% specificity (CI 80.5-99.3%). Divergent results (n=5) were submitted to sVNT; 4 of them were positive for NAbs and negative for IgG-RBD – of these, 3 were negative by sVNT, and 1 was positive; 1 sample was negative for neutralizing antibodies (19%), positive for IgG-RBD (1.30AU/mL) and negative by sVNT.**Conclusion**Our data show excellent correlation between NAbs immunoassay and IgG-RBD with sVNT, as well as between CLIA IgG-RBD and NAbs. IgG-RBD, although not a functional test, can be considered to be used as a correlate.

Microbiology and Infectious Diseases

B-074

Designer probiotics: pH-sensitive Bacillus subtilis for disrupting the growth & quorum sense signaling of pathogenic bacteria in the gut microbiome

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Background: Commensal bacteria shape and regulate the micro-environment of the gastrointestinal tract. Commensals produce short chain fatty acids (SCFAs) to regulate and maintain the gut microbiome. Diet or excessive antibiotic use can cause dysbiosis or an imbalance within the gut microbiome where more pathogenic bacteria proliferate. "Healthy" bacteria of the microbiome prevent and remove the presence of pathogenic bacteria through its release of a specific SCFA termed lactate or lactic acid in solution. The lactic acid produced by the probiotic called *Lactobacillus acidophilus* eradicates many pathogenic bacteria because most harmful bacteria are neutrophiles that prefer neutral pH conditions. **Objective:** According to Miller's study called "*Probiotics as medical therapies",* there is a lack of determining the pharmacodynamic properties and viability of probiotic organisms when commercially preparing probiotics. For this reason, the method proposed attempts to design probiotics that are viable, are pH sensitive, can suppress quorum sensing, and release "death proteins" for the program cell death of pathogenic bacteria. **Methods:** The proposed method will add a glutamate decarboxylase system such as a PgadA promoter into plasmids. The *gadAB* genes, translated into GAD enzymes, are produced by most pathogenic strains of *Escherichia coli* to survive high levels of acidity. PgadA can detect GAD and can detect low pH values indicative of inflammatory bowel diseases. Plasmids will be engineered to release cell "death proteins" induced by the addition of a mazEF system. Additional plasmids will produce siRNAs or Cas9 nucleases of *accA* , which is a gene found to disrupt the expression of *luxS* that is required for quorum sense (QS) signaling. A probiotic strain of *Bacillus subtilis* will be transformed with each plasmid to recognize GAD (PgadA), a low pH (PgadA or Pasr), generate a programmed cell death (mazEF), and disrupt the QS of pathogenic bacteria (siRNAs or Cas9 of *accA*). **Results:** The expected outcome for the proposed probiotic design will reduce the growth of pathogenic bacteria in the lower gastrointestinal tract by inhibiting *accA* and *luxS* expression while including a system of programmed cell death. In *E. coli* cells, the silencing of the *accA* gene significantly reduced *luxS* levels and decreased antibiotic resistance ($P < 0.05$). The gene copy number of *luxS* , for samples with and without *accA* inhibition, were quantified via real-time PCR. *E. coli* samples with asRNA inhibition of *accA* produced a lowered *luxS* gene copy number of 199 (n=3, 277 ± 37) versus 1 x10^6 *luxS* gene copies for the control (n=3, 658,114 ± 483,499). **Conclusion:** Designing pH-sensitive probiotics to disrupt QS communication between pathogenic bacteria combined with inducing program cell death can limit the growth of harmful bacteria in the lower GI tract. Reducing dysbiosis through probiotic use can restore the normal microflora of the gut and may control symptoms of inflammatory bowel disease. Re-designing probiotics may offer alternative therapies for IBDs and rapidly improve the clinical laboratory results of processed specimens from IBD patients.

B-075

Label-Free Serological Assays for SARS-CoV-2 Antibody Characteristics and Longitudinal Study of COVID-19 Patients

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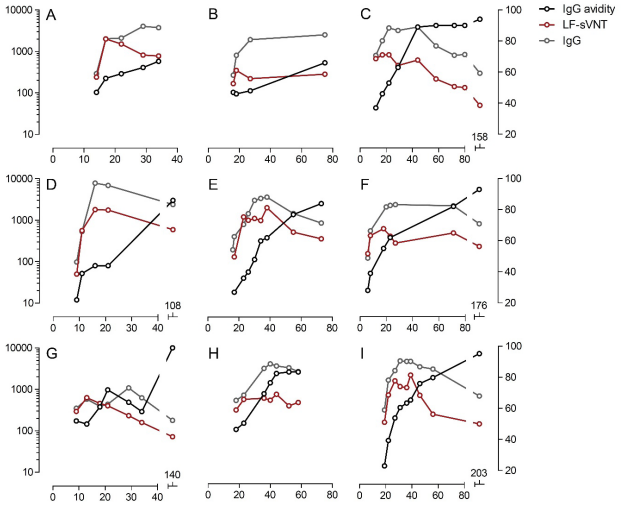
Background: Since the start of the COVID-19 pandemic, much research has focused on the kinetics and magnitude of the immune response. Laboratory-based methods used to measure the SARS-CoV-2 humoral response include quantitative assessment of antibody characteristics, i.e. antibody concentration, avidity, and neutralization activity. **Methods:** Using a label-free technology, we have established a label-free IgG avidity assay and a label-free surrogate virus neutralization test (LF-sVNT). To carry out the serological assays, the sensing probes were coated with the receptor-binding domain (RBD) of SARS-CoV-2 spike protein, and applied to serum samples containing anti- SARS-CoV-2 antibodies. The label-free IgG avidity assay measures the binding strength between RBD and IgG under urea dissociation, and the LF-sVNT analyzes the binding ability of RBD to ACE2 after neutralizing RBD with antibodies. **Results:** The IgG avidity indices and neutralizing antibody titers (IC50) were determined from serum samples (n=246) from COVID-19 patients (n=113), and the IgG concentrations were measured with a fluorescent immunoassay. As the kinetics of antibody characteristics for 9 patients shown in the graph, the IgG concentrations and neutralizing antibody titers presented an initial rise, plateau and then in some cases a gradual decline after 40 days post-symptom onset. The IgG avidity indices, in the same cases, plateaued after the initial rise and did not show a decline. **Conclusion:** The label-free serological assays can be valuable tools in clinical laboratories for assessment of antibody characteristics in COVID-19 patients. This study is the first to provide longitudinal neutralizing antibody titers beyond 200 days post-symptom onset. Despite the decline of IgG concentration and neutralizing antibody titer, IgG avidity index increases, reaches a plateau and then remains constant up to 8 months post-infection. The decline of antibody neutralization activity can be attributed to the reduction in antibody quantity rather than the deterioration of antibody quality, as measured by antibody avidity.



are used to identify active infection, transmission risk, and immune response but have limitations. This study investigated the diagnostic utility of SARS-CoV-2 nucleocapsid protein (N-Ag) in serum.**Methods:** We retrospectively studied 208 randomly-selected cases with PCR-confirmed SARS-CoV-2 infection. N-Ag concentrations were measured by enzyme-linked immunosorbent assay in remnant serum samples, compared to PCR or Ab results, and correlated to electronic health records for clinical value evaluation. **Results**: During active infection, serum N-Ag increased quickly in the first few days after symptom onset, peaked around day 3-7 with the median value > 1000 pg/mL, and declined in week 2-3 (Fig A). Serum N-Ag was detected as early as day2 with a sensitivity of 81.5%. Within one week of symptom onset, the sensitivity and specificity reached 90.9% (95% CI:85.1–94.6%) and 98.3% (95% CI: 91.1–99.9%), respectively. Area under the receiver operating characteristic curves were 0.961, 0.925, and 0.782 for samples collected within Day 1-7, 8-14, and 15-21 respectively (Fig B). Moreover, serum N-Ag concentration is closely correlated to disease severity, reflected by highest level of care, medical interventions, chest imaging, and the length of hospital stays (Fig C-E). Longitudinal analysis revealed the simultaneous increase of Abs and decline of serum N-Ag (Fig F and G). **Conclusions:** Our study validated serum N-Ag as a biomarker for SARS-CoV-2 acute infection with high sensitivity and specificity compared to viral RNA in the respiratory system. We further revealed the correlation between serum N-Ag concentrations and disease severity as well as the inverse relationship of N-Ag and Abs. The diagnostic values and technical and practical advantages of serum N-Ag test may meet unsatisfied diagnostic and prognostic needs during the COVID-19 pandemic.

B-077

Evaluation of a Paragonimus IgG ELISA

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Background:*Paragonimiasis*, or lung fluke disease, is caused by infection with a number of species of trematodes belonging to the genus *Paragonimus*. *Paragonimus* spp. is a common parasite of crustacean-eating mammals such as tigers, leopards, domestic cats, dogs, mongooses, opossums and monkeys (reservoir final hosts). Humans may be substitute reservoir hosts when they ingest partially cooked or poorly processed crustaceans. In humans, the earliest stages of paragonimiasis may present an elusive clinical picture, and be asymptomatic or scarcely symptomatic. Conversely, when worms reach the lungs, symptoms may be significant and typically include chronic cough with blood-stained sputum; chest pain with dyspnoea and fever; pleural effusion and pneumothorax are possible complications. Symptoms and signs mimic those of tuberculosis, and paragonimiasis should always be suspected in patients with tuberculosis who are non-responsive to treatment. Ectopic paragonimiasis may result from erratic migration of the juvenile worms: the most frequent locations include the abdominal cavity and subcutaneous tissues and, most frequently, the brain: cerebral paragonimiasis is a severe condition that may be associated with headache, visual impairment and epileptic seizures. Serologic tests are commonly used for the diagnosis of paragonimiasis. Here, we report a new ELISA diagnostic testing kit for serodiagnosis of human paragonimiasis spp.**Methods:**Banked human serum samples were run per the manufacturers kit instructions.**Results:Performance Data**

		Reference Method	
		+	-
New Life	+	14	86
	-	1	0

Sensitivity = 93%Specificity = 100%In addition, reactive sera from 8 different parasite infections were tested. All were non-reactive in this assay.**Conclusion:**The New Life Diagnostics LLC Paragonimus Serum IgG ELISA is a simple to use kit and is both sensitive and specific for *Paragonimus* spp infection diagnosis.

B-078

Diagnostic value of Nucleocapsid protein in the blood for SARS-CoV-2 infection

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Background: Biomarkers have been widely explored for COVID-19 diagnosis. Both viral RNA or antigens (Ag) in the respiratory system and antibodies (Ab) in the blood

Figures **Zhang et al.**

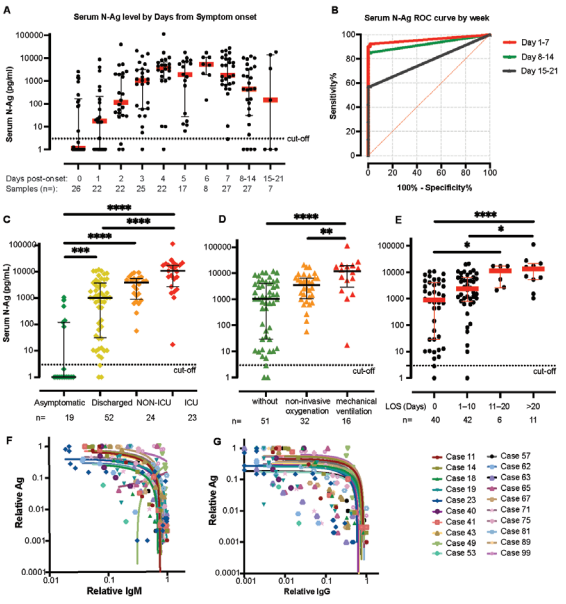

Figure (**A**) Kinetics of serum N-Ag level by days after symptom onset. Each dot indicates one serum sample which was collected on the same day of NP swab collection for RT-PCR; each red line indicates the mean value in each time-period group. Cut-off was set at 2.97 pg/ml. (**B**) The Receiver operating characteristic (ROC) curves for serum N-Ag within the indicated weekly time frames. Area under the ROC Curve (AUC) are 0.961 (day 1–7), 0.925 (day 8–14), and 0.782 (day 15–21). (**C–E**)The correlation of serum N-Ag concentrations with disease severity (**C**) in cases with different highest level of outcomes, including asymptomatic, symptomatic but discharged, hospitalized-to-non-ICU, and hospitalized-to-ICU; (**D**) in cases without medical interventions, with non-invasive oxygenation, or with mechanical ventilation; and (**E**) in cases with different length of hospital stay (LOS), including 0, 1-10, 11-20 and > 20 days. All serum samples were collected during day 3-7 from symptom onset. *, P< 0.05; **, P< 0.01; ***, P< 0.001; ****, P < 0.0001. The case number (n) is indicated under each group. Any Ag concentration under 1 pg/mL was transferred to 1 pg/mL for the data representation on the log-scale. (**F** and **G**) The inverse relationship between N-Ag and IgM (**F**) and IgG (**G**) in the 20 cases. To calculate the relative level in each case, Ag and Ab concentrationss were normalized to the highest one found in each case.

B-079

A Multicenter Evaluation of the Performance of the ADVIA Centaur HBcT2 Assay*

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Background: Hepatitis B virus (HBV) is endemic throughout the world with a global prevalence of 3.9% and over 300 million people worldwide estimated to be chronic

carriers of the virus. HBV infection, particularly in cases of chronic infection, is clearly associated with the development of liver cirrhosis, liver failure, and hepatocellular carcinoma.During viral hepatitis infection, many serological markers appear. IgM and IgG antibodies to Hepatitis B core antigen (HBcAg) can be detected serologically in HBV-infected individuals. Anti-HBc IgM is detectable first and remains detectable for approximately 6 months. Shortly after the IgM response, anti-HBc IgG appears and can remain detectable indefinitely. The presence of anti-HBc IgM is characteristic of acute infection, while the presence of anti-HBc IgG is characteristic of chronic or recovered stages of HBV infection. The presence of antibodies to HBcAg can be used as an aid in diagnosis of HBV infection as well as in determination of clinical status of infected individuals. The ADVIA Centaur® HBc Total (HBcT2) assay is a fully automated, two-step, antigen-bridging, microparticle chemiluminescent immunoassay used for the detection of antibodies to hepatitis B core antigen in human serum or plasma. The ADVIA Centaur HBcT2 assay detects both IgM and IgG anti-HBc responses. The aim of this study was to evaluate the performance of this prototype assay on the ADVIA Centaur® XP, XPT, and CP Immunoassay Systems.

Methods: Samples were collected and tested at three U.S. sites. Individuals met criteria for inclusion by being at risk for hepatitis B or experiencing signs and symptoms of the disease. A total of 1751 samples from subjects in various disease states were characterized. Classification of the samples was confirmed using FDA approved commercial assays. Positive percent agreement (PPA%) and negative percent agreement (NPA%) for the HBcT2 assay were assessed against the Abbott ARCHITECT CORE assay. In addition to method comparison, performance characteristics evaluations included reproducibility, using a six-member panel that was assayed in replicates of three, with two runs per day over 5 days for each lot (n = 270 for each sample), and seroconversion, using seven subjects with bleed-days ranging from 29 to 99 days.

Results: Evaluation of patient samples using the ADVIA Centaur HBcT2 assay on the ADVIA Centaur XP system indicated a PPA% of 98.0% (95% CI: 96.2-98.9%) and an NPA% of 98.5% (95% CI: 97.6-99.0%) compared to the Abbott ARCHITECT CORE assay results. The assay demonstrated good reproducibility, with a CV of 9.4-12.5%. The commercially available HBV patient seroconversion panels were tested using the ADVIA Centaur HBcT2 assay to determine the seroconversion sensitivity. When compared to the results of the reference Abbott ARCHITECT CORE assay, the ADVIA Centaur HBcT2 assay yielded a cumulative net reactivity of +1 for each platform.

Conclusion: Study results demonstrated good performance characteristics of the ADVIA Centaur HBcT2 assay on the ADVIA Centaur XP, XPT, and CP systems.

*Not commercially available in the U.S., future product availability not guaranteed. Product availability may vary from country to country and will be subject to varying regulatory requirements.

B-080

Novel Advancements in Microbial Imaging: The Automated Metafer Mycobacteria Scanner

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Background: Numerous pathogens classified as mycobacterium cause some of the most severe maladies known to mankind, such tuberculosis. Swift detection of mycobacterium or acid-fast bacteria (AFB) is crucial in mitigating the spread of disease and in commencing applicable treatment. Due to its low cost, sputum smear microscopy of auramine-stained AFB is commonly utilized as the first step in mycobacterium detection. However, this manual technique is time-intensive and implements a substantial burden on laboratory personnel. Thus, it is essential to find a cost-effective solution to automate this process within the clinical microbiology laboratory to facilitate faster patient results. To meet this need, we formulated the Metafer Mycobacteria Scanner (MSC), an automated microscope scanning and imaging system. The aim of this study was to evaluate the amount of technician time as well as operating costs that the MSC can save a microbiology lab. We hypothesized that automated scanning and image acquisition of auramine-stained slides would be a faster procedure compared to the standard manual microscopy technique.**Methods:** Auramine-stained respiratory slides were scanned automatically with the MetaSystems MSC as well as evaluated manually through the oculars of a Zeiss AxioImager Z2. For the manual workflow, 100 fields of view (FOVs) of each slide were analyzed under a 100X oil immersion objective. Counting the number of AFB was performed manually and the slides were classified according to CDC guidelines. Slide results were entered into Excel to mimic LIS data entry. For the automated workflow, 100 FOVs of each slide were captured under a 20X objective. Slides were evaluated by viewing the digitized images captured in the Metafer gallery. The quantification of the number of AFB and the proposed slide classifications based on CDC/WHO guidelines were presented to the user in Metafer as well. To determine the financial benefits of the MSC, we developed a return on investment (ROI) economic model based on known average wages and fringe benefit rates for medical technologists, the average number of slides processed daily in microbiology laboratories, and equipment cost including a service contract. **Results:** Our study verified that the MSC diminishes the time as well as the monetary burden for microbiology laboratories. When comparing the time that a technologist is required to physically interact with the microscope or computer (hands-on time), we found that the automated workflow was comprised of only an average of 1.19 minutes of hands-on time in comparison to an average of 6.1 minutes of hands-on time for the manual workflow. In concordance with a previous study we conducted on tuberculosis samples, the MSC provided users with a higher sensitivity for scoring AFB slides in comparison to scoring them manually. Lastly, a ROI analysis of the MSC revealed a breakeven point in 1.4 years, saving laboratories more than $900,000 over 5 years. **Conclusions:** The MSC is an ergonomic investment in full lab automation that decreases laboratory costs, significantly reduces microbiologists' time at the microscope, and increases overall lab efficiency through more cost-effective use of technician time.

B-081

Utility of Real-Time PCR Testing on Whole Blood for the Diagnosis of Early Lyme Disease

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Background: *Borrelia burgdorferi* is the causative agent of Lyme disease in the United States. Molecular testing for *B. burgdorferi* in whole blood has high clinical specificity, but the low clinical sensitivity reduces the clinical value of a negative result; thus, molecular testing has not been widely recommended for diagnosis. However, molecular testing can identify early Lyme disease, which serology testing may miss and could delay treatment. To explore the clinical utility in molecular testing for *B. burgdorferi*, we examined the results of concurrent molecular and serological testing as well as results of sequential serological testing.**Methods:** De-identified and retrospective specimen results from 2017 with both serological and molecular testing performed on the same draw for Lyme disease diagnosis were reviewed. Serological testing was performed by 2 antibody capture EIAs: an IgM and an IgG assay. Molecular testing was done by RT-PCR targeting a sequence within the OspA gene of *B. burgdorferi*. Analysis of this data set identified positive results broken into 3 categories: PCR negative/serology positive, PCR positive/serology negative, and double-positives. A separate analysis included sequential samples from patients initially tested by concurrent molecular and serological methods in 2017 to 2019 and then again by serological methods 5 to 191 days later.**Results:** Of over 33,000 results that met criteria, 1,379 specimens had positive results: 1,179 for serology only, 131 for PCR only, and 69 for both. A separate analysis of 85 pairs of samples (with both an initial and follow-up submission) showed a considerable amount of seroconversion from patients that had whole blood *B. burgdorferi* PCR positivity but initially negative serology (Table 1, Group 2).**Conclusion:** Whole blood *B. burgdorferi* PCR, when combined with concurrent serological testing may identify patients with early Lyme disease that serological testing alone may not.

Table 1. Eighty-five paired samples, initial and follow-up, on a Lyme antibody capture EIA platform.

Group	Concurrent Initial *B. burgdorferi* PCR Result	Initial *B. burgdorferi* Antibody Capture IgM Result	Follow-up *B. burgdorferi* Antibody Capture IgM or IgG Result	Number of sample pairs (N)
1	Negative	Positive	Positive	20
2	Positive	Negative	Positive	21
3	Positive	Positive	Positive	12
4	Negative	Negative	Negative	32

B-082

A Comparison of Lyme Serological Testing Platforms with a Panel of Clinically Characterized Samples from various stages of Lyme Disease

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Background: In 2019, the CDC recommended an alternative algorithm testing for Lyme disease, known as modified two-tiered testing (MTTT). MTTT replaces 2nd

tier immunoblotting in standard two-tiered testing (STTT) with an additional enzyme immunoassay (EIA). This study compares STTT, MTTT, and a laboratory-developed capture EIA, a methodology not compared to current testing algorithms. **Methods:** Ninety-two clinically characterized, blinded serum samples from the CDC (Lyme Research Panel II) were tested using STTT, MTTT, and capture EIA. For STTT, testing was performed on the research panel by the CDC. For MTTT, the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM (Tier 1), *B. burgdorferi* IgM and *B. burgdorferi* IgG (Tier 2) were used. For capture EIA, 2 separate tests were performed for IgM and IgG antibodies against *B. burgdorferi* OspA-free whole-cell lysate. **Results:** Based on CDC-provided results, MTTT performed better than STTT detecting cases of early Lyme (50% MTTT vs. 40% STTT), defined clinically by presence of erythema migrans rash or positive culture or PCR result. However, STTT performed better than MTTT detecting cases of non-early Lyme (58% MTTT vs. 92% STTT). Overall, there was no statistically significant difference between MTTT and STTT. The antibody capture EIA performed best at detecting cases of Lyme disease (81% sensitivity) but suffered a higher rate of false-positive results (77% specificity). The main source of false positives for capture EIA was IgG cross reactivity with Syphilis confirmed patients.**Conclusion:** Antibody capture EIA has higher sensitivity in detecting early Lyme disease (Defined by CDC data in the panel as the time between noticing an EM and collection of sera before 28 days). It suffered from poorer specificity compared to CDC-recommended algorithms. Overall, MTTT performed similarly to STTT when analyzing these clinically characterized samples from the CDC repository and is a simpler and more scalable method.

Table 1. Lyme disease test results of Lyme Research Panel II between MTTT, STTT, and Capture EIA.

Clinical Category	MTTT IgM Results	MTTT IgG Results	STTT IgM/IgG Results	Antibody Capture IgM Results	Antibody Capture IgG Results
Early Lyme	9/20	7/20	8/20	11/20	6/20
Non-Early Lyme	6/12	6/12	11/12	10/12	11/12
# samples positive for IgM or IgG	17/32		19/32	26/32	
Sensitivity	53%		59%	82%	
Fibromyalgia	0/6	0/6	0/6	1/6	0/6
Mono	1/6	0/6	0/6	1/6	0/6
MS	0/6	0/6	0/6	1/6	0/6
RA	1/6	0/6	1/6	0/6	1/6
Periodontitis	0/6	0/6	0/6	0/6	0/6
Syphilis	0/6	0/6	1/6	2/6	5/6
Healthy controls	1/24	0/24	0/24	2/24	3/24
# samples positive for IgM or IgG	3/60		2/60	14/60	
Specificity	95%		97%	77%	

B-083

Tripartite Motif-Containing 22 Genotype is Correlated with the IFN-α Therapy Response in Hepatitis B e Antigen-Positive Chronic Hepatitis B

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Background: Interferon-α (IFN-α) plays an important role in the treatment of chronic hepatitis B (CHB), but its limited response rate and side effects limit its clinical application. We intend to explain the factors affecting the efficacy of IFN-α from the perspective of host SNPs and clarify the relevant mechanism.**Methods:**One hundred and twenty-four patients diagnosed CHB was enrolled in this study. Patients received IFN-α for 48 weeks. Response was defined as an HBV DNA <500 IU/mL at 12 months post-treatment. DNA samples are genotyped by Asian Screening Array (ASA) chip.

Results:CHB patients were genotype into 483500 loci with ASA chip, of which we focused on TRIM family including TRIM15, TRIM22, TRIM26, TRIM31, TRIM38, TRIM39. Among them, we found that the expression of TRIM22 mRNA in patients with rs10838543 CT and CC genotypes was significantly higher than that in patients with TRIM22 rs10838543 TT genotypes, while other TRIM SNPs did not show significant statistical difference. During the 48-week follow-up, there was no significant difference in the distribution of different TRIM22 rs10838543 genotypes in the decrease of HBV DNA and HBsAg. However, the genotype of TRIM22 rs10838543 TT was positively correlated with the negative conversion of HBeAg. In vitro experiments showed that TRIM22 was one of the significantly upregulated TRIM members and HBV inhibits the expression of TRIM22 after IFN-α treatment. The level of HBV-related serological markers in the supernatant of HepAD38 cell lines overexpressing TRIM22 was consistent with that of microarray and in vitro experiments.

Conclusion:TRIM22 involves in the anti-HBV of IFN-α and its polymorphism rs10838543 may affect the therapeutic responses to chronic HBV infection. These findings may provide new insight into choosing the optimal candidates and improving the therapeutic effect for anti-HBV treatment with IFN-α.

B-084

IFIT3 promotes the effect of IFN-α via JAK-STAT signaling pathway in patients with chronic HBV infection

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Background: Increasing evidence indicates that adjuvant interferon-α (IFN-α) therapy is an effective treatment option for a subgroup of patients with chronic hepatitis B (CHB). Moreover, it has been shown that IFN-induced proteins with tetratricopeptide repeats 3 (IFIT3) genes, which were classical IFN-stimulated genes (ISGs), are associated with the regulation of HBV replication. However, whether IFIT3 could develop interferon-α (IFN-α) biotherapy for patients with chronic HBV infection is still unclear. Therefore, the aim of this study is to identify the role and mechanism of IFIT3 on IFN-α antiviral activities against HBV.**Methods:**Peripheral blood was collected from untreated patients with chronic HBV infection or healthy controls. The level of IFIT3 mRNA was examined by real time PCR. Correlations between IFIT3 mRNA expression and clinical features were analyzed. The role of IFIT3 in JAK-STAT signaling and HBV replication was investigated through gain of function and loss of function by real time PCR and western blot simultaneously.

Results: Our results showed that untreated patients with chronic HBV infection exhibited elevated expression of IFIT3 than that in healthy cohort. Further, we observed that individuals under hepatitis B e antigen (HBeAg)-positive phases had higher levels of IFIT3 than those under HBeAg-negative phases of the disease. Consistently, IFIT3 was verified to be induced by HBV via transient transfection with the pHBV1.2 plasmid in HepG2/HL-7702 cells, and IFN-α enhanced the upregulation effect on IFIT3. Mechanistically, knockdown of IFIT3 inhibited the phosphorylation of STAT2, but not STAT1, whereas overexpression of IFIT3 produced an opposite effect in vitro. Meanwhile, overexpression of IFIT3 could enhance IFN-α triggered ISGs expression, including myxovirus protein A (MxA), 2′, 5′-oligoadenylate synthetases (OAS), and double stranded RNA-dependent protein kinase (PKR), while knockdown of IFIT3 suppressed the expression of these genes.

Conclusion: Our findings suggest IFIT3 may serve as a novel therapeutic target for promoting the effect of IFN-α by the JAK-STAT signaling pathway in patients with chronic HBV infection.

B-086

Sars-CoV-2 Immunoglobulin G Antibodies Increase Post Infection and Persist in the Serum.

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Background: Severe acute respiratory syndrome coronavirus 2 (Sars CoV-2) is a novel betacoronavirus virus responsible for the Coronavirus disease 2019 (COVID 19). Diagnosis of infected individuals is by detection of the virus in nasal/nasopharyngeal swabs. Infected individuals usually develop antibodies to the virus that are associated with protection from Sars CoV-2 infection. Antibody response is detectable within days – weeks and may decline in intensity. This study was designed to determine the time of detection and duration of antibodies to Sars CoV-2. Method: Ten male patients diagnosed with COVID-19, with end stage renal disease and requiring thrice weekly dialysis were followed for several months. Specimens were tested weekly initially for one month and subsequently monthly. Total antibody and Immunoglobulin G (IgG) antibody to Sars CoV-2 was determined in the specimens retrieved in the first month and only total antibody determined in the subsequent specimens. A signal/Co (S/Co) ratio >1.0 determined the presence of both total and IgG antibodies. All analyses were performed on the Vitros 5600. Results: Antibody to total Sars CoV-2 was detected in twenty-two percent (22%) of study participants while none had IgG antibodies to Sars CoV-2 eight days after the PCR test. The percentage increased to eighty six percent (86%) and seventy one percent (71%) respectively twenty-three days after the positive PCR test. Two individuals (22%) did not develop measurable IgG antibodies at the end of the initial one-month study period. The rate of increase of measurable total an-

tibody based on the S/Co ratio ranged from 2 – 200-fold. Total antibody increased and persisted at high S/Co ratios until the fifth month of follow-up. The Sars-CoV-2 virus was still detectable in eighty percent of individuals twenty-two days after the initial PCR test although none had symptoms of active infection. Conclusion: Detectable Sars CoV-2 antibody response in infected individuals is variable and antibody levels continue to increase in the majority of individuals studied. Antibody levels persisted at elevated levels in the serum of individuals who have been infected with the virus for the duration of the five-month study period. The significance of the persistence in the antibody levels should be evaluated relative to the vaccination program.

B-087

Antibody Response to COVID-19 Vaccine in Patients with Mature B-cell Neoplasms

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Background: Humoral response to COVID-19 is considered as one of the key protective mechanisms against COVID-19 infection. In mature B-cell neoplasms, regular adaptive antibody response to novel antigens is suppressed either by clonal expansion of neoplastic B-cell populations or by treatment with chemotherapy (chemo) and B-cell/plasma cell depletion therapies. The COVID-19 pandemic has placed such patients under a vulnerable condition. The benefit of COVID-19 vaccine in this patient population is unknown and prior study supports an impaired antibody response to other viral vaccines. In the current series, antibody response to COVID-19 mRNA vaccine was evaluated in a heterogeneous group of lymphoma/myeloma patients.

Methods: A total of 31 vaccinated patients in the study included 10 diffuse large B-cell lymphoma (DLBCL), 6marginal zone lymphoma (MZL), 4 Hodgkin lymphoma (HL), 4 multiple myeloma (MM)/MGUS, 2 follicular lymphoma (FL), 1 primary central nervous system lymphoma (PCNSL), 1 T-cell/histiocytic-rich large cell lymphoma (THRLBCL), 1 mantle cell lymphoma (MCL), 1 chronic lymphocytic leukemia, and 1 low-grade lymphoproliferative disorder. A qualitative antibody assay (Wondfo) detecting IgM and IgG specific to receptor-binding domain (RBD) of COVID-19 Spike protein was used to evaluated all the blood samples from these patients. Positive samples were further tested for nucleocapsid protein IgG (Abbott IgG) to ensure no prior infection.

Results: The average age of the patients was 74.7 years old (range 29-90). Sixteen (51.5%) were female and 15 (48.4%) were male. About 3 quarters (76%) of the vaccine were made by Pfizer-BioTech and the remaining by Moderna. The average time from the first vaccine placement to blood draw was 39 days (range 7-71). Three patients (1 MM, 1 DLBCL and 1 low-grade lymphoproliferative disorder) were tested within 14 days post-vaccination, all with negative results. Among patients tested after second vaccination, 8 out of 9 DLBCL, 5 of 6 MZL, 2 of 2 FL, 1 of 1 MCL, 1 of 1 PCNSL, 2 of 4 HL patients had no antibody response. In contrast, 1 CLL, 2 MGUS, 1 MM and 1 THRLBCL were positive (P = 0.04). Twelve (12) of 13 patients currently on treatment, 2 of 4 treated within 3 months, 3 of 5 treated before 3 months, and 2 of 6 untreated patients tested negative (P = 0.04). Fourteen (14) of 16 patients treated by chemoimmunotherapy (bendamustine [most frequent], anti-CD20, CD19, and CD38 antibodies and anti-CD3:CD20 bispecific antibody), 1 of 2 treated by chemo alone, 1 of 1 treated by anti-CD30 antibody, and 2 treated by targeted therapy (TRAIL receptor agonist and Bruton's tyrosine kinase inhibitor) tested negative. One HL patient treated by anti-PD1 antibody tested positive.

Conclusion: This is the first report on COVID-19 vaccine antibody response in mature B-cell neoplasm patients. The response was significantly suppressed in patients treated by chemo and/or B-cell depletion therapies. In about half of the patients, the suppression persisted in patients more than 3 months after the treatment. Suppression occurred in patients who began the vaccine series prior to treatment as damped antibody response was seen in about a third of patients without any treatment.

B-088

Pediatric SARS-CoV-2 Surveillance in School Children and Adolescents: Unique Application of Rapid Antigen and Antibody Testing

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Background: The role children play in the COVID-19 pandemic remains unclear. As schools remain open, it is essential to understand the infectious burden and potential for transmission within the pediatric population in order to develop short and long-term public health policies to prevent severe childhood disease as well as transmission to higher risk populations. The main objective of this study is to conduct a pediatric surveillance program to monitor SARS-CoV-2 exposure and antibody response in the student population of Toronto, ON, Canada in 2020/2021 academic year. **Methods**: An initial analytical and clinical assay evaluation was completed for the DiaSorin Liaison SARS-CoV-2 S1/S2 IgG assay, including precision, sensitivity and specificity estimates. Asymptomatic children and adolescents (5 to 18 years) were then recruited from schools throughout the Greater Toronto Area and Hamilton regions. Upon informed consent, a nasopharyngeal swab and venous blood sample (SST Vacutainer, BD) were collected. Nasopharyngeal swabs were immediately evaluated using the PanBio Rapid SARS-CoV-2 Ag assay (Abbott Diagnostics) to determine current SARS-CoV-2 exposure. Serum specimens were subsequently evaluated for SARS-CoV-2 IgG antibodies on the DiaSorin Liaison platform. Antigen and antibody prevalence were then calculated and stratified by sociodemographic characteristics (e.g. age, sex, geographic location). **Results**: The DiaSorin Liaison SARS-CoV-2 S1/S2 IgG assay demonstrated acceptable assay performance with a specificity of 100% (n=30, pediatric specimens collected pre-COVID-19) and a sensitivity of 100% (n=11, adult and pediatric specimens collected 10 days post-positive PCR). In our community cohort, all SARS-CoV-2 antigen results as determined by the PanBio Rapid SARS-CoV-2 Ag assay were negative, demonstrating a prevalence rate of 0%. Further, 3% of study participants demonstrated SARS-CoV-2 antibody positivity. All results were correlated to positive SARS-CoV-2 test history. Maximum time since positive PCR result was 3 months. **Conclusion**: Study findings suggest low antigen and antibody prevalence of SARS-CoV-2 exposure in asymptomatic students Toronto, ON, Canada. These data will provide important information towards public health policies regarding asymptomatic pediatric transmission and need for school closures. Further work is needed to characterize SARS-CoV-2 immune response in pediatrics as well as antigen test performance in comparison to gold standard PCR methods.

B-089

A Multicenter Evaluation of the Performance of the Atellica IM HBcT2 Assay*

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Background: Hepatitis B virus (HBV) is endemic throughout the world with a global prevalence of 3.9% and over 300 million people worldwide estimated to be chronic carriers of the virus. HBV infection, particularly in cases of chronic infection, is clearly associated with the development of liver cirrhosis, liver failure, and hepatocellular carcinoma.During viral hepatitis infection, many serological markers appear. IgM and IgG antibodies to Hepatitis B core antigen (HBcAg) can be detected serologically in HBV-infected individuals. Anti-HBc IgM is detectable first and remains detectable for approximately 6 months. Shortly after the IgM response, anti-HBc IgG appears and can remain detectable indefinitely. The presence of anti-HBc IgM is characteristic of acute infection, while the presence of anti-HBc IgG is characteristic of chronic or recovered stages of HBV infection. The presence of antibodies to HBcAg can be used as an aid in diagnosis of HBV infection as well as in determination of clinical status of infected individuals. The Atellica® IM HBc Total (HBcT2) Assay is a fully automated, two-step, antigen-bridging, microparticle chemiluminescent immunoassay used for the detection of antibodies to hepatitis B core antigen in human serum or plasma. The Atellica IM HBcT2 Assay detects both IgM and IgG anti-HBc responses. The aim of this study was to evaluate the performance of this prototype assay on the Atellica® IM Analyzer. **Methods:** Samples were collected and tested at three U.S. sites. Individuals met criteria for inclusion by being at risk for hepatitis B or experiencing signs and symptoms of the disease. A total of 1612 samples from subjects in various disease states were characterized. Classification of the samples was confirmed using FDA approved commercial assays. Positive percent agreement (PPA%) and negative percent agreement (NPA%) for the HBcT2 assay were assessed against the Abbott ARCHITECT CORE assay. In addition to method comparison, performance characteristics evaluations included reproducibility, using a six-member panel that was assayed in replicates of three, with two runs per day over 5 days for each lot (n = 270 for each sample), and seroconversion, using seven subjects with bleed-days ranging from 29 to 99 days.

Results: Evaluation of patient samples using the Atellica IM HBcT2 Assay on the Atellica IM Analyzer indicated a PPA% of 97.7% (95% CI: 95.9-98.8%) and an NPA% of 98.6% (95% CI: 97.8-99.2%) compared to the Abbott ARCHITECT CORE assay results. The assay demonstrated good reproducibility, with a CV of 3.0-10.0%. The commercially available HBV patient seroconversion panels were tested using the

Atellica IM HBcT2 Assay to determine the seroconversion sensitivity. When compared to the results of the reference Abbott ARCHITECT CORE assay, the Atellica IM HBcT2 Assay yielded a cumulative net reactivity of +1.

Conclusion: Study results demonstrated good performance characteristics of the Atellica IM HBcT2 Assay on the Atellica IM Analyzer.* Not commercially available in the U.S., future product availability not guaranteed. Product availability may vary from country to country and will be subject to varying regulatory requirements.

B-090

Clinical performance of the TaqPath™ COVID-19 CE-IVD RT-PCR Kit as compared to Cobas® SARS-CoV-2 Assay

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Background: The TaqPath™ COVID-19 CEIVD RTPCR Kit and Cobas® SARS-CoV-2 Assay are among the most widely used RT-PCR tests for the detection of SARS-CoV-2. The TaqPath™ COVID-19 CEIVD RTPCR Kit targets three viral genes (ORF1ab, N and S), while the Cobas® SARS-CoV-2 Assay targets two viral genes (ORF1ab, E-gene). In this study, we compared the clinical performance of the two above-mentioned RT-PCR diagnostic tests using Next Generation Sequencing (NGS) to resolve discordant results. **Methods:** The retrospective study was performed on 450 (175 positive and 275 negative) upper respiratory tract samples from routine leftover clinical specimens collected in Germany in February 2021 with an attempt to include any SARS-CoV-2 variants. All samples were previously characterized for SARS-CoV-2 status using the Cobas® SARS-CoV-2 Assay. The positive cohort spanned the dynamic range of the Cobas® SARS-CoV-2 Assay with Ct values in equal proportions (Ct<20, 20≤Ct<25, and Ct≥30). The samples were thawed following storage at -80°C and tested using both tests in a blinded, randomized fashion. Inconclusive or invalid results from either test method were excluded from analysis. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were assessed and discordant samples were analyzed using the EasySeq™ SARS-CoV-2 Whole Genome Sequencing kit on the Illumina® NGS sequencing instrument. Sequencing results were analyzed using ncbi-blast-2.11.0+ by alignment to the SARS-CoV-2 reference sequence, NC-045512.2. Clinical Sensitivity and Specificity for both assays were calculated using a combination of concordant results, and discordant results in which the resolver method was used to define true positive and true negative. Discordant results not resolved by NGS were excluded from the analysis for Clinical Sensitivity and Specificity. **Results:** Of the 450 samples, 4 samples yielded inconclusive or invalid results and were excluded from the analysis. Of the 172 samples positive by the Cobas® SARS-CoV-2 Assay, 171 samples yielded a positive result using TaqPath™ COVID-19 CEIVD RTPCR Kit with only 1 sample showing a discordant result, resulting in a PPA of 99.4%. Of the 274 samples negative by the Cobas® SARS-CoV-2 Assay, 261 samples yielded a negative result using TaqPath™ COVID-19 CEIVD RTPCR Kit with 13 samples showing discordant results, resulting in a NPA of 95.3%. Of the 14 discordant samples, NGS did not yield a valid result in 8 samples due to low RNA concentration and need to be resolved by an alternate method. In the remaining 6 discordant samples, NGS results yielded valid results and revealed the presence of SARS-CoV-2 RNA in all 6 samples. All the valid NGS results agreed with the results from the TaqPath™ COVID-19 CEIVD RTPCR Kit. For the TaqPath™ COVID-19 CEIVD RTPCR Kit, the Clinical Sensitivity and Specificity were, both, 100%. For the Cobas® SARS-CoV-2 Assay, the Clinical Sensitivity was 96.61% and the Clinical Specificity was 100%. **Conclusion:** The TaqPath™ COVID-19 CEIVD RTPCR Kit showed good concordance with the Roche Cobas® SARS-CoV-2 Assay (99.4% PPA, 95.3% NPA) and is a highly accurate method for detection of SARS-CoV-2 with a 100% Clinical Sensitivity and Specificity.

B-091

Seroprevalence of SARS-Cov-2 antibodies and risk factors for seroconversion in Healthcare workers at a tertiary Hospital

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Background: Frontline healthcare workers (HCWs) are considered to be at increased risk of acquiring and transmitting COVID-19 while caring for patients. In addition, many health care workers have potential work-related exposure to infected colleagues because of work place areas where physical distancing is not possible. Data on the infection rates of healthcare staff are scanty and often do not include details of exposure risk. The International Council of Nurses (ICN) estimated that 8% of all COVID-19 cases were among healthcare workers, and WHO believes it could be up to 10%. We aimed to determine the seroprevalence and risk factors for a positive SARS-Cov-2 antibody test among HCWs at a tertiary hospital in Kenya.**Methods:** This was a census conducted at the Aga khan University hospital Nairobi (AKUHN) from November 2020 through February 2021, before the roll out of mass vaccination of HCWs. The study involved both hospital employees as well as contracted staff working in the hospital. In total, 1711 participants enrolled. Participation was voluntary and the Staff provided personal and work details in a structured questionnaire.The Abbott SARS-Cov-2 IgG assay was employed for covid-19 serology status.**Results:** AKUHN employees comprised 82.5% of total staff tested. There were nearly equivalent number of male and female participants. The median age was 35 and 38 years for males and females respectively. The overall seroprevalence was 17.8 %, and was higher among AKU staff (18.9%) compared to contracted workers (12.5%) (p value= 0.01). Notable cadres with the highest proportions of exposed staff included: pharmacy (25.6%), outreach clinics (24%) and nursing (22.2%).*Risk factors*: Having previously tested for Covid-19 (PCR) irrespective of test result increased the odds of testing positive with the antibody test; OR 1.76 (95% CI: 1.4-2.3). The odds were even higher in those who had had a positive PCR result; OR 12.2 (8.1-18.5).The risk was also higher among those who had experienced flu-like symptoms in the past 3 months leading to enrolment in the study. Univariate analysis showed significant association with cough, headache, fever, coryza and loss of smell. However, multivariate analysis only showed significant association for cough OR 1.67 (1.2-2.4), fever OR 1.7 (1.1-2.8) and loss of smell OR 8.7 (5.3-14.4).Other significant risk factors included interacting with household contacts or workmates with Covid-19 infection as well as working (daily) or having close interactions with Covid-19 patients. Age, sex, diabetes, hypertension and other comorbidities were not a risk factor for a positive Covid-19 IgG test. **Conclusion:** The seroprevalence in our study was higher than what has been reported elsewhere. A meta-analysis of 49 studies published late last year put the estimated overall seroprevalence of SARS-CoV-2 antibodies among HCWs as 8.7% (95% confidence interval 6.7–10.9%). Seroprevalence was higher in studies conducted in North America (12.7%) compared with those conducted in Europe (8.5%), Africa (8.2%) and Asia (4%). There is need therefore to mitigate the risk factors identified in our study to minimise exposure of HCWs at AKUHN.

B-092

A rapidly measurable host assay comprising TRAIL, IP-10 and CRP has potential to reduce antibiotic overuse without increasing underuse in adults with suspected lower respiratory tract infection

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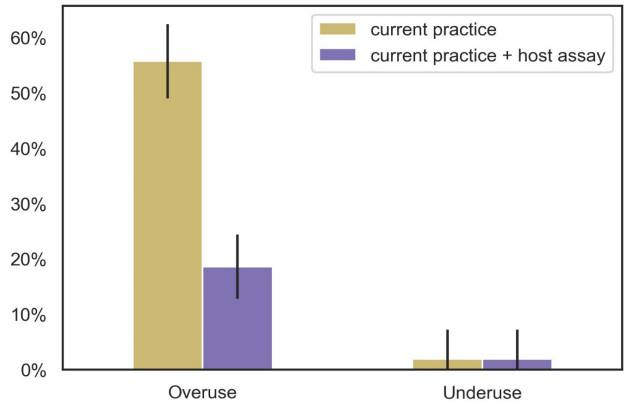
Background Identifying infectious disease etiology is oftentimes challenging, yet essential for appropriate patient management, including antibiotic use. Studies have shown that a host assay comprising TNF-related apoptosis induced ligand (TRAIL), interferon gamma induced protein-10 (IP-10) and C-reactive protein (CRP) accurately differentiates bacterial from viral infections with negative predictive value >98%. Here we estimate the potential impact of the host assay to complement current practice for adults with suspected lower respiratory tract infection. **Methods** Adults aged >18 years with suspected lower respiratory tract infection were prospectively recruited at three medical sites (OBSERVER; grant #684589; NCT003011515). Infection etiology was adjudicated by three independent experts based on clinical, laboratory, microbiological, radiological and follow-up data. The host assay was conducted retrospectively, giving three possible outcomes, viral, bacterial or equivocal. **Results** Out of 583 adults recruited, 422 met infectious inclusion criteria; of these, 314 were assigned etiological labels, 210 viral and 104 bacterial infections. Patients with bacterial infections were older (mean 61 years (SD 21) vs. 51 (SD 20; p<0.001), had higher fever (mean 39.0°C (SD 0.7) vs. 38.6 (SD 0.6); p<0.001), and were more likely to be admitted (77.0% vs. 31.0%); p<0.001). To estimate the assay's possible impact on antibiotic misuse, the treatment documented in the medical record was considered the 'current practice', and it was assumed that a timely contraindicative assay result would have triggered a change in practice ('current practice + host assay'), with current practice occurring in cases of equivocal results. Based on this estimation, the host assay has potential to reduce antibiotic prescription to virally infected patients by 3.0-fold (p< 0.001), from 55.7% (95% confidence interval, CI 49.0-62.3) to 18.6% (95%CI: 13.9-24.4), without impacting antibiotic underuse (Figure). **Conclusions** Timely provision of TRAIL/IP-10/CRP assay results has the potential to complement current practice and contribute to improved antibiotic stewardship practices.



B-093

Portrait of measles Immunization in Brazil in 5 years compared to 2020 - relative data on numbers of measles tests from a large laboratory in Sao Paulo (Brazil)

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BACKGROUND: Data released by Fundação Oswaldo Cruz, diseases considered eliminated or eradicated in Brazil, such as measles, again represent a serious public health problem. In Brazil, the vaccine coverage rates of the main vaccines offered by the Sistema Unico de Saude (SUS) have been dropping significantly. Data from the Pan American Health Organization (PAHO / WHO) point to a 300% increase in reported cases of measles in the world in the first three months of this year (2021), compared to the same period in 2018 and 2019. With the pandemic installed in 2020 vaccinations suffered a greater fall. In this context, measles is a disease to be, with risk of complications and death, and the disease with higher transmissibility known. **OBJECTIVE:** The authors of this study evaluated data from a clinical laboratory five years, their number of positive cases for measles immunization and relationship. The authors compared the behavior of the 5 years from 2017 to 2019 with 2020 year of Covid -19. **METHODS:** Analyzed data of test results for measles from 2015 to 2019 database of a large laboratory that meets 60% of public health units in São Paulo. The methods for implementing these tests were -Quimioluminescência (POSITIVE- IgG> and IgM 16.5> 1.1) and Enzyme immunoassay (POSITIVE - IgG> 200, and IgM> 15). **RESULTS**: From 2015 to 2019 there were 5003 requests for measles with a gradual increase in immunizations and positive cases. In 2020 it presented the same number of requests with a drop in relation to 2019 of immunizations but with an important percentage of positivity. (Table) **CONCLUSION:** With the Covid -19 pandemic, the scenario for other contagious infectious diseases, together with the vaccination program, had significant changes. This situation underscores the need for campaigns without interruption followed many enlightening information for all types of disease.

Laboratory results overall						
Years	2015	2016	2017	2018	2019	2020
Tests	762	522	889	1229	1601	1636
Immunized IgG +	253 (33%)	150 (29%)	412 (46%)	790 (64%)	1019 (64%)	461 (28%)
IgG + and IgM +	1 (0.13%)	1 (0.19%)	4 (0.44%)	3 (0.24%)	104 (6.49%)	65 (4,0%)

B-094

Development of SARS-CoV-2 Serology QC designed for a broad range of commercial assays and methodologies for the detection of antibodies against COVID-19.

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Background: COVID-19, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), first emerged at the end of 2019, and despite global containment and quarantine efforts has resulted in an unprecedented pandemic leading to over 120 million confirmed cases and 2.6 million deaths worldwide. Testing generally falls into two categories: molecular diagnostic and serological tests. Diagnostic tests show an active SARS-CoV-2 infection enabling positive individuals to isolate, whereas antibody tests determine previous infection by the virus. The ability to sustain natural or vaccine-induced immunity to COVID-19 has yet to be established, and the need for reliable antibody tests and quality controls for monitoring and screening is paramount. With 70 EUA certified COVID-19 commercial serological tests distributed, the need for quality controls to ensure confidence of reported patient results is increasingly important. To this end, Bio-Rad Laboratories has developed VIROTROL SARS-CoV-2 and VIROCLEAR SARS-CoV-2 positive and negative quality controls for the qualitative determination of antibodies to SARS-CoV-2 and assessed their performance on a variety of commercial assays. These controls have been formulated and rigorously tested to demonstrate functionality, repeatability, and reliability of results.

Methods: Convalescent human plasma from previously SARS-CoV-2 nucleic acid tested positive individuals was sourced, heat pasteurized, processed (defibrinated), and subsequently pooled as raw material stocks for VIROTROL SARS-CoV-2 positive control. VIROCLEAR SARS-CoV-2 was derived from pooled, processed normal human plasma and confirmed negative for antibodies to SARS-CoV-2. Evaluation of the Bio-Rad Laboratories VIROTROL SARS-CoV-2 and VIROCLEAR SARS-CoV-2 quality control was conducted over a 20-week period at ten (10) sites consisting of clinical laboratories and/or test kit manufacturers located either in the US or EU. VIROTROL SARS-CoV-2 and VIROCLEAR SARS-CoV-2 are designed to mimic patient samples and are used following specimen handling and result interpretation instructions provided by the test kit manufacturer for qualitative methods of detection for antibodies to SARS-CoV-2. Testing was conducted over a five-day period, consistent with CLSI EP-15 A with results collected and examined for precision.

Results: VIROTROL SARS-CoV-2 and VIROCLEAR SARS-CoV-2 were found to be 100% in agreement with the expected "positive/reactive" or "negative/non-reactive" result, depending on the intended use of the quality control used. Within-run imprecision (%CV), between-run imprecision (%CV) and total imprecision (%CV) results reflected those claimed by the test kit manufacturer. All calculated %CV results were <10% with the majority being <5% for reactive specimens. Further, VIROTROL SARS-CoV-2 has been qualified for use as a reference standard on the Bio-Rad Bio-Plex Pro Human IgG SARS-CoV-2 N/RBD/S1/S2 Serology Assay (RUO) for semi-quantitative detection of SARS-CoV-2 antibodies providing an additional feature of this control product.

Conclusion: Bio-Rad Laboratories VIROTROL SARS-CoV-2 and VIROCLEAR SARS-CoV-2 controls are optimally suited for use as independent quality control materials on a wide variety of commercial test methods for the detection of antibodies to SARS-CoV-2, ensuring confidence in overall test performance and reported patient test results.

B-095

Pooled Surveillance Testing for Detection of SARS-CoV-2 in Asymptomatic and Symptomatic populations at an Institute of Higher Education

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Background: Efforts to mitigate the reproductive number (R0) for COVID-19, a respiratory infection caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has been obfuscated by the potential for sustained transmission through pre-symptomatic, paucisymptomatic (subclinical), and asymptomatic carriers. RT-PCR is considered the gold standard for the detection of SARS-CoV-2 RNA from upper respiratory specimens. However, increased demand for testing has created shortages in materials and technical personnel. Sample pooling, whereby the ratio of specimens to result is greater than one, has encouraged protocol optimization that would allow an increase in testing capacity without requiring additional resources. Caveats include a linear loss of sensitivity with sample dilution, and, in hierarchical systems, disease prevalence may determine efficiency as positive pools are deconvo-

luted to retest each patient individually. Most pooling studies have been conducted in symptomatic patients and pooling data on asymptomatic populations is limited. In this study, we assessed the performance of pooling samples in, both, symptomatic and asymptomatic cohorts to demonstrate a feasible mechanism for population testing.

Methods: The Life Science Testing Center (LSTC) provides COVID-19 testing for Northeastern University students, faculty, and staff. Anterior nare swabs are collected and categorized as either symptomatic or asymptomatic based on the collection site. About 22% of positive samples are symptomatic while the remainder come from asymptomatic sites. To validate intended workflow, 80 µL of a previously reported positive sample was diluted in 320 µL of negative sample material to generate a 1:5 positive sample pool. Seventy pools were generated for 30 asymptomatic, 30 symptomatic, and 10 negative samples with 280 negative samples and RT-qPCR was performed using the SARS-CoV-2 Pooling Assay (Thermo Fisher Scientific, Waltham, MA). Positive pools were deconvoluted and each component was tested individually using the TaqPath™ COVID-19 Combo Kit. Both kits detect the open reading frame (ORF) 1ab, nucleocapsid (N), and spike (S) gene and threshold cycle (Ct) values for each gene were extracted using the Applied Biosystems™ COVID-19 Interpretive software V1.5.

Results: Each positive sample from both, asymptomatic and symptomatic, cohorts was detected in its pool and subsequently confirmed upon deconvolution testing. Notwithstanding a distinct frequency of variants identified by the S-gene target failure proxy, the pooled average Ct difference between the symptomatic and asymptomatic cohorts was minimal at 1.34% (ORF1ab), 2.83% (N), and 1.01% (S). Average Ct shifts calculated were 1.536±1.072 (Orf1ab), 1.366±0.772 (N), and 2.344±1.676 (S) for the asymptomatic cohort and 1.818±1.007(Orf1ab), 1.615±0.475 (N), and 3.103±1.368 (S) for the symptomatic cohort. Independent t-test performed on Ct shifts confirmed an insignificant difference with p-values of 0.306 (Orf1ab), 0.147 (N), and 0.052 (S). All negative pools were correctly reported as negative.

Conclusion: The SARS-CoV-2 Pooling Assay accurately detects the presence of SARS-CoV-2 in up to 1:5 pooled samples from Asymptomatic and Symptomatic populations with minimal impact on sensitivity. Moreover, the false negative rate was unaffected. Sample pooling is a viable testing option that allows for a scalable solution for back-to-school COVID-19 monitoring at an Institute of Higher Education.

B-096

Reagent Lot Consistency of Three SARS-CoV-2 Diagnostic Assays on Two Abbott Platforms

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the novel coronavirus responsible for the global COVID-19 pandemic, has thrust medicine and laboratory science into uncharted territory. The past 12 months have seen a widespread health crisis, but also unprecedented innovation. Drug manufacturers raced to find treatments and cures while diagnostic companies rose to the challenge of developing tests that could detect infection and immune response. Clinical laboratories have done their part by rapidly integrating testing into their programs to give physicians the information needed to treat and advise patients. Assays were developed in a shortened timeframe and received accelerated FDA emergency use authorization to provide testing options as quickly as possible. In the months that followed, data have been carefully analyzed and used to support the accuracy and increasing utility of antibody testing for diagnosing and tracking the spread of this disease as well as the ongoing vaccination campaign. **Objective:** The goal of this study was to evaluate the reagent lot-to-lot performance of three SARS-CoV-2 antibody assays (SARS-CoV-2 IgG, SARS-CoV-2 IgM, and SARS-CoV-2 IgG II Quant) on the Abbott Architect and Alinity platforms. **Methods**: Quality controls (QC) were tested and tracked during reagent lot manufacturing. Data represent multiple reagent lots manufactured from product launch until March 2021 and include 8 - 37 lots on Architect and 4 - 19 lots on Alinity, depending on the analyte. For all assays, QC was tested in multiple replicates on multiple runs on both the Abbott Architect and Alinity platforms. Imprecision (percent coefficient of variation (%CV)) was calculated across all reagent lots on all positive control level means for each assay. For the SARS-CoV-2 IgG Quant assay, mean calibrator concentrations for each lot were also tracked and %CV was calculated across lots for calibrators B through F, representing the positive range of the assay. **Results**: The within assay lot-to-lot imprecision for each positive control level for all assays on both platforms was less than 3.5%CV. The SARS-CoV-2 IgG Quant B through F calibrator lot-to-lot imprecision was less than 1.1%CV. **Conclusions**: The results show that each of the Abbott SARS-CoV-2 assays evaluated are consistent from lot-to-lot, which aids physicians in accurately diagnosing disease, tracking the spread of the pandemic, and monitoring high titer levels on convalescent plasma and vaccine recipients.

B-097

Two dose, or not two dose, that is the question facing many countries implementing mRNA-based COVID-19 vaccination programs

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Background: The COVID-19 pandemic is a public health emergency effecting every country in the world. Vaccine development for SARS-CoV-2 was prioritized to end this pandemic. 2 messenger RNA (mRNA)-based vaccines that encode the spike (SP) protein of SARS-CoV-2 resulting in generation of neutralizing antibodies (NAbs) (Pfizer-BioNTech and Moderna) were granted FDA EUA after successful clinical trials. Both require 2 doses administered 3 and 4 weeks apart, respectively. Given the current shortage of vaccines some countries have delayed administering the 2nd dose by as much as 4 months in order to prioritize delivering initial doses. However, little is known about the humoral response generated after primary dose (D1), or the booster dose (D2) in naive and previously SARS-CoV-2 infected individuals. **Methods:** In this work, 611 samples from 569 unique individuals tested in an outpatient setting with COVID-19 vaccination information available. In order to accurately evaluate the serological response following vaccination, only outpatients with information concerning their vaccine status were included. Two new serological assays designed to quantitatively evaluate the presence of SARS-CoV-2 spike protein specific IgM (IgM_{SP}) and IgG (Abbott SARS CoV-2 IgG II; IgG_{SP}) antibodies and an assay which detects IgG specific to the nucleocapsid protein (IgG_{NC}) assay (Abbott) in SARS-CoV-2 vaccinated groups were included in order to evaluate vaccine response and previous infection. Individuals with IgG_{NC} values above positivity threshold (>=1.4 AU/mL) were deemed previously infected (ExCOVID-19 group); those below were considered naïve. **Results:** SARS-CoV-2 vaccination results in robust SARS-COV-2 spike protein-specific Ab responses among those vaccinated, 91% received the Pfizer-BioNTech formulation (n=132) and 8% obtained the Moderna formulation (n=13). Ab responses in the vaccinated group (n=145) were compared to that of the unvaccinated group (n=424). Following the first dose in the naïve vaccinated group, we observed IgG_{SP} positivity with a median of 2217 AU/mL (95% CI, 0-44182), which was drastically increased by 8.2-fold following the booster to a median of 18272 AU/mL (at 98% CI, 11724-21750) ($p<0.001$). The median IgG_{SP} level following D1 and D2 in the exCOVID-19 group was found to be 17519 AU/mL and 20760 AU/mL, respectively. However, unlike the naïve vaccinated group, the booster dose in the exCOVID-19 group displayed only a dampened response. A similar response to IgG_{SP} was noted for the IgM_{SP} in both the naïve vaccinated and exCOVID-19 vaccinated groups.**Conclusions:** SARS-CoV-2 spike specific IgG response elicited by a single dose of mRNA vaccine in exCOVID-19 group was comparable to two dose response seen in naïve group. The dramatic single dose immune response seen in exCOVID recovered individuals could be ascribed to the natural SARS-COV-2 infection functioning in a similar manner as a priming dose. Vaccination in this group can be delayed or limited to a single dose to extend the coverage of limited COVID-19 vaccine supply.

B-098

Validation of the targeted TaqMan™ SARS-CoV-2 Mutation Panel for identification of SARS-CoV-2 variants of concern or interest

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Background: Since the SARS-CoV-2 pandemic started, multiple clinically relevant mutations in the S gene (D614G (start of the pandemic), followed by N501Y and E484K) emerged, all of which substantially increased the virus's transmissibility. Since the beginning of 2021, we observed that the SARS-CoV-2 strain B.1.258 was gradually replaced by the N501Y containing B1.1.7 strain in the alpine region of Austria and Italy. The pandemic became aggravated by the appearance of E484K present in strains with (B.1.351) and without (B.1.525) N501Y, since data indicate that E484K undermines public health recommendations against SARS-CoV-2 spread. To unambiguously assign a specific SARS-CoV-2 strain, sequencing of its genome is required. However, the turnaround time (TAT) of ≥ 3 days needed for whole genome sequencing of SARS-CoV-2 is prohibitive for swift public health decision making. Therefore, we evaluated a workflow using a six SARS-CoV-2 mutation panel ran in a 384-well

format for which the results can be made available in < 24 hours and include key mutations of concern for increased infectivity (N501Y) and vaccine escape (E484K, K417N, K417T) as well as mutations that discriminate between major VOCs.

Methods: The strategy was to implement a mutation panel that could i) reliably identify the four strains imposing the highest threat at the moment in the region (B.1.1.7, B.1.351, B.1.525 and P.1) and ii) flag samples possibly representing novel strains of concern. The mutation panel was comprised of the following 6 assays: E484K, N501Y, L242_244Del, K417N, K417T and P681H (the mutation panel can be adapted by choosing from 22 assays depending on SARS-CoV-2 variants of concern or interest). A set of 96 samples positive for SARS-CoV-2 (using TaqPath™ COVID-19 CE-IVD RT-PCR kit interrogating Orf1ab, N-gene and S-gene with Ct values for the N-gene between 19 and 34) were completely sequenced using the Ion Torrent S5 Next Generation Sequencing (NGS) method and subsequently analyzed by the mutation panel.

Results: Comparison of the results showed that the strain predicted from the mutation panel results was in complete agreement with that determined by sequencing. 32 samples showed a wild-type call for all 6 mutation assays and sequencing allocated 4 to the strain B.1.177, 27 to the strain B.1.258 and 1 to strain B.1.1.127. The mutation panel was able to accurately assign 64 samples to three SARS-CoV-2 strains; B.1.1.7 (59 samples), B.1.351 (2 samples) and B.1.525 (3 samples).

Conclusion: By using the bipartite workflow with (i) testing samples with the high-sensitive TaqPath COVID-19 CE-IVD assay with the S-gene Target Failure as a proxy for H69_V70Del, followed by (ii) analyzing positive samples with the 6 mutation panel described above, every single of the 4 relevant SARS-CoV-2 strains (B.1.1.7, B.1.351, B.1.525 and P.1) is covered with 2 to 4 assays. This workflow allows the correct assignment of the SARS-CoV-2 positive samples to the respective variants of concern/interest for genomic surveillance within a TAT (<24 hr) that allows a swift reaction by the health care system to implement public health measures.

B-099

Limited correlation between SARS-CoV-2 serologic assays for identification of high-titer COVID-19 convalescent plasma

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Background: COVID-19 convalescent plasma (CCP) has received Food and Drug Administration (FDA) Emergency Use Authorization (EUA) for the treatment of hospitalized patients with SARS-CoV-2 infection. As of March 9th, 2021, eleven tests have been granted EUA for use in the manufacture and identification of high-titer CCP. Here we compared the overall agreement and concordance of three serologic assays with EUA for CCP assessment and a laboratory developed (LDT), recombinant, live cell neutralization assay at both manufacturer recommended qualitative cut-off thresholds and at the FDA-indicated thresholds for high-titer CCP.

Methods: 1005 serum samples from CCP donors collected at least 14 days post-symptom resolution, were tested using the Elecsys semi-quantitative anti-spike (S) and qualitative anti-nucleocapsid (NC) SARS-CoV-2 electrochemiluminescent immunoassays (ECLIAs) from Roche Diagnostics (Indianapolis, IN). A 210 sample subset from this cohort was also evaluated by the GenScript cPASS SARS-CoV-2 Neutralization Antibody test (Piscataway, NJ) and the Mayo Clinic Neutralizing Antibody (nAb) LDT. All assays with EUA were performed without deviation from manufacturer instructions. The manufacturer established qualitative cut-offs for the anti-NC ECLIA, anti-S ECLIA, cPASS nAb, and Mayo nAb assays are ≥1.1 COI, ≥0.8U/mL, ≥30% inhibition, and ≥1:80 titer, respectively. Per the FDA-EUA, high-titer CCP using the anti-NC, anti-S and cPASS nAb assays are ≥109 COI, ≥132 U/mL, and ≥68% inhibition, respectively. A potential high-titer cutoff of ≥1:320 for the Mayo nAb assay was evaluated against the EUA-authorized cutoffs.

Results: Among the 1005 sample set, 91.8% (923/1005) and 93.8% (943/1005) of samples were positive using the manufacturer established thresholds for the Roche anti-NC and anti-S ECLIAs, respectively. Notably, 19.0% (191/1005) and 44.7% (453/1005) of samples would meet the FDA EUA criteria for high-titer CCP using the anti-NC and anti-S assays, respectively. The Roche Diagnostics anti-NC and anti-S ECLIAs demonstrated a positive percent agreement of 91.7% (922/1005) using manufacturer established qualitative thresholds, however only agreed for high-titer CCP in 13.4% (135/1005) of samples. Among the 210 sample subset, 85.7% (180/210) of the samples showed qualitative positive agreement across all four assays. Compared to the cPASS nAb assay, the Roche anti-NC and anti-S ECLIAs showed 14.8% (31/120) and 68.3% (82/120) percent-positive-agreement (PPA), respectively, for high-titer CCP. The three EUA-approved assays showed high-titer agreement in 11.9% (25/210) of samples. Using the potential high-titer CCP cutoff of the Mayo nAb assay of ≥1:320 titer, the four assays had high-titer agreement in 9.0% (19/210) of samples.

Conclusion: The Roche Diagnostics anti-NC and anti-S ECLIAs show excellent qualitative agreement using the manufacturer recommended thresholds. However, using the FDA EUA thresholds to identify high-titer CCP, a significantly lower proportion of donated CCP meet the high-titer qualification criteria using the Roche Diagnostics anti-NC ELCLIA versus the anti-S ECLIA. This may lead to a significant number of CCP donations unused for therapeutic purposes due to characterization as low-titer by the Roche Diagnostics anti-NC ECLIA. Further studies are needed to better define optimal thresholds for high-titer CCP. Additionally, these data further demonstrate the need for standardization across SARS-CoV-2 serologic assays.

B-100

Vitamin profile of the elderly in previous years compared to the years 2020 and early 2021.

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Background Recent clinical trials have reported that vitamin supplementation can reduce the incidence of acute respiratory infections and the severity of respiratory tract diseases in adults and children. In this context, several studies associate vitamins with better immunological conditions for coping with Sars-CoV-2. Many recommendations have emerged for the administration of vitamins are the way to improve the population in the face of this enemy. The authors of this study aimed to analyze the levels of concentrations of these vitamins in elderly people aged 60 or over, between the years 2018 and 2019 compared to 2020 and 2021 for vitamins 25(OH)D, B6, B1 and B12 which were the most requested. **Methods:** the authors analyzed the database of a large laboratory in São Paulo - Brazil. They extracted data on the results of the most requested vitamins in the periods from 2018 to the beginning of 2021. Results of averages of vitamins 25(OH)D and the most requested of the B complex (B1, B6 and B12) were analyzed in the group of highest risk above 60 years. **Results:** The data presented showed that the average levels of B complex vitamins for the elderly, remained within the reference values. No significant differences were seen between the years studied for all vitamins studied (p=0.607). Only the averages of Vitamins 25(0H)D were at lower levels for this risk range. The recommended values for the risk groups are: 30 to 60ng/mL (Table). **Conclusion:** The data suggest that the recommendations for the administration of vitamins, in particular Vitamin D, may be an important ally in the fight against coronavirus infections. The age group at risk had lower levels of Vitamin D. Studies suggest that people with low levels of vitamin D were 4.6 times more likely to be affected by Covid-19 than those with normal levels.

		TOTAL ELDERLY		VB6 ug/L	VB1 mcg/L	VB12 pg/mL	VD325OH ng/mL
2018	Pacientes 60+	283660	n	541	536	151971	232135
			Média	28,37	33.46	440.94	28.45*
2019	Pacientes 60+	241280	n	535	427	115224	202162
			Média	63.70	29.75	447.87	28.05*
2020	Pacientes 60+	231066	n	458	421	127979	192855
			Média	30.33	44.11	444.29	28.15*
2021	Pacientes 60+	72913	n	126	88	41478	63350
			Média	28.36	44.75	430.55	28.07*

* level s below the reference (30 to 60 ng/mL)

B-101

Leveraging the immune response to improve outbreak management: derivation of a rapidly measurable host-protein signature for stratifying severity of COVID-19 patients

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Background: In most SARS-CoV-2-positive patients, a localized, short-lasting immune response is sufficient to clear virus from the lungs. However, in up to 14% of patients, a dysregulated immune response ensues, triggering a hyperinflammatory state that can lead to acute lung injury, acute respiratory distress syndrome, organ failure, and death. Despite multiple evaluation tools, early and objective detection of progression to severe COVID-19 disease remains a major challenge. We describe derivation of a rapidly measurable immune-signature that indicates the likelihood of severe COVID-19 outcome based on TNF-related apoptosis-induced ligand (TRAIL), interferon gamma-induced protein-10 (IP-10), and C-reactive protein (CRP), host-immune proteins that change expression levels in response to infection severity. **Methods**: SARS-CoV-2-positive hospital admitted adult patients were recruited in USA, Germany, and Israel. Severe outcome was defined as mortality or ICU-level care (i.e., ICU admission, high flow nasal cannula, continuous positive airway pressure, bi-level airway pressure, intubation with mechanical ventilation). TRAIL, IP-10 and CRP were measured using an analyzer that provides values from serum in 15 minutes. To integrate TRAIL, IP-10 and CRP levels into a single score (ranging from 0-100) we chose regularized logistic regression due to its relative simplicity. The final model (COVID-19 Severity) was trained on the entire derivation cohort and showed comparable results to 10-fold cross validation indicating it was not over-fitted. To render the model clinically intuitive, four score bins were developed where each patient is assigned to a bin based on their score: bin 1, $0 \leq$ score ≤ 20, very low likelihood for severe outcome; bin 2, $20 <$ score < 40, low likelihood for severe outcome; bin 3, $40 \leq$ score ≤ 80, moderate likelihood for severe outcome; and bin 4, $80 <$ score ≤ 100, high likelihood for severe outcome. **Results**: Between March and November 2020, 518 COVID-19 patients were recruited. 394 were eligible for the derivation cohort; 113 (28%) patients met the composite severe outcome. In area under the receiver operating curve (AUC) analysis, COVID-19 Severity score with AUC 0.86 (95% confidence interval, CI: 0.81-0.91) outperformed other biomarkers reported as candidate severity stratification tools, including IL-6 (n= 139; AUC 0.77 (95%CI: 0.67-0.87); p=0.03). Additionally, the tool's performance was assessed by demonstrating a significant trend in likelihood ratio going from low to high score bins, showing that the likelihood of severe outcome significantly increases with increasing score ($p < 0.01$). This trend was significant ($p < 0.01$) also when only severe patients meeting outcome on day of/after blood sampling were included (n=339; 58 severe and 281 non-severe). The probability for 14-day mortality was assessed with Kaplan-Meier survival estimator. Survival distribution was significantly different across the bins ($p < 0.01$). **Conclusion**: The COVID-19 Severity score has potential to serve as an accurate risk stratification tool, facilitating timely escalation of care across acute settings. A multinational multicenter validation study is ongoing. Capability to perform the test rapidly and easily using the newly developed analyzer can support better triage decisions, leading to improved patient outcomes and objective resource allocation, thereby relieving burden on the healthcare system.

B-102

Vaccination against Covid 19 leads to a very efficient immune response with strong neutralizing antibody occurance

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BackgroundIn late 2019 an outbreak of a novel coronavirus SARS-CoV-2 was reported in the Chinese province ofHubei in the city of Wuhan. In March 2020, the spread was declared a pandemic. In February 2020,the WHO named the disease caused by SARS-CoV-2 "COVID-19" (Coronavirus disease 19). To forminfectious virions SARS-CoV-2 produces four proteins: spike protein (S; consisting of S1 and S2),envelope (E), membrane (M), and nucleocapsid (N). The trimeric S (TriS) protein comprises monomersconsisting of S1 and S2 subunits, and it facilitates the entry of the virus into the host cell. Herein, thereceptor binding domain (RBD) in the S1 subunit is responsible for the recognition and binding to theangiotensin-converting enzyme 2 (ACE2) on a host cell, blocking of which inhibits virus entry. Thereare currently several vaccines in the market that are directed against the spike protein. Widely in usein Estonia and other European countries are the vaccines by Pfizer-BionTech and AstraZeneca. In thepresent study we turned our focus to the vaccine by Pfizer-BionTech.**Methods**Using an in-house ELISA assay that determines separately IgG, IgM and IgA against S1, S1-RBD, S2 andN proteins, we analyzed seroconversion of a cohort of 18 medical workers and volunteers that werevaccinated with the vaccine by Pfizer-BionTech. Among those 18, 4 individuals went through Covid-19in 2020. We compared our result to 14 patients that had suffered from Covid 19 in spring 2020. Wefurther used a commercial IVD-CE kit ("SARS-CoV2 Neutralizing Antibody ELISA kit", Icosagen, Estonia).The assay makes use of the binding between ACE2 and TriS. If neutralizing antibodies in theindividual´s serum are present, they will bind to TriS and thereby block the interaction with ACE2. Thetest is an inverted ELISA in which lowest readings (OD450) present the strongest blocking of ACE2-TriSbinding. We compared our results to a pseudovirus assay that mimics infection of a cell-culture andallows for estimating the efficiency of blockage of viral entry into a cell.**Results**We found that for vaccinated individuals IgG-type of antibodies against the spike proteins of SARS-Cov2 are detected at much higher ratios, at least after the second dose of vaccine, than for individualsthat suffered from a Covid19 infection. We further showed that antibodies stemming from vaccinatedindividuals to a great extent efficiently blocked the binding of ACE2 to the RBD of TriS. This was truenot only for the wild-type strain ("Wuhan") but also for the mutations originating from the UK, SouthAfrica, and Brazil.**Conclusion**Compared to patients, vaccination with the Pfizer-BionTech vaccine causes a more intense immunereaction latest after the second dose of vaccine. The capacity to block ACE2 binding to TriS suggeststhe presence of efficient neutralizing antibodies in vaccinated individuals. We show that vaccinationis efficient against the mutations originating from the UK, South Africa, and Brazil. We also observedthat antibodies from patients who had recovered from Covid 19 showed much lower efficiency toneutralize those mutants than those from vaccinated individuals.

B-103

UPLC-MS/MS Analysis of SARS-CoV-2 using Peptide Enrichment for Clinical Research

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Background: Detection of SARS-CoV-2 is critically important in clinical research in understanding the impact of SARS-CoV-2 infection. As part of this process, the detection of low levels of viral proteins, that would indicate viral replication and infection, could enable investigation of long-term impacts of the virus in longitudinal research studies. Polymerase Chain Reaction (PCR) is widely used to indirectly measure the virus through amplification of the viral mRNA, whereas LC-MS/MS technologies can be used to directly detect the viral proteins through selective and analytical sensitive detection of a range of peptides liberated from the viral proteins following tryptic digestion. SISCAPA® (Stable Isotope Standards with Capture of Anti-Peptide Antibodies) is a technique used in proteomics to improve the detection limits of LC-MS/MS peptide methods. In this instance, the analytical sensitivity of viral proteins is greatly improved, expanding the applicability of the analytical method for detecting the presence of SARS-CoV-2.

Methods: SARS-CoV-2 NCAP protein purchased from R&D Systems (Abingdon, UK) was used to create calibrators and quality controls (QCs) in Viral Transport Medium (VTM) purchased from VWR (Poole, UK). Sample preparation was performed using the Andrew Alliance™ Andrew+ pipetting robot. VTM samples were added to 96-well plates. Rapigest™ in ammonium bicarbonate was added to denature the samples. Trypsin was added to digest the viral proteins for 45 minutes. Internal standard was added mixed prior to the addition of SISCAPA Antibody (Ab) beads that target the AYN, ADE, NPA and DGI peptides from the NCAP protein. Capture was performed for 60 minutes at room temperature. The beads were washed using CHAPS and a magnetic array plate. The wash was repeated prior to elution of the peptides using CHAPS in formic acid and the eluate was transferred to a QuanRecovery 96-well plate. Using an ACQUITY UPLC™ I-Class system, samples were injected onto a 2.1mm x 30mm ACQUITY™ PREMIER C18 1.7µm column with an in-line filter using a water/acetonitrile/formic acid gradient and quantified with a Xevo™ TQ-XS mass spectrometer.

Results: The method was shown to be linear from 3 - 4000 amol/µL for AYN and 16-4000 amol/µL for ADE, NPA and DGI. AYN, ADE, NPA and DGI were chromato-

graphically separated. Investigations indicate the analytical sensitivity of this method would allow precise quantification (<20%) at 3 amol/µL for AYN and 16 amol/µL for ADE, NPA and DGI. Coefficients of variation (CV) for total precision and repeatability on 5 occasions for low (20 amol/µL), mid (200 amol/µL) and high (2000 amol/µL) concentrations in VTM were ≤15% (n = 25) for AYN and ≤20% (n=25) for ADE, NPA and DGI. The method was used to analyze 50 SARS-CoV-2positive and 50 negative VTM samples.

Conclusion: We have successfully quantified SARS-CoV-2 NCAP protein in VTM using a tryptic digestion and SISCAPA enrichment sample preparation followed by LC-MS/MS analysis, for clinical research purposes.

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

B-104

Performance of Persepsin, IL-6, and Procalcitonin for Length of Stay Risk Stratification of COVID-19 Patients Presenting to the Emergency Department

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Background: Currently, tools for risk stratification of SARS-CoV-2 positive patients at presentation to the Emergency Department (ED) are lacking. Reports indicate associations between high levels of inflammatory markers, specifically IL-6, and morbidity. Recent studies have also shown that Presepsin (sCD14-ST) is elevated in severe SARS-CoV-2 infections. In bacterial sepsis, Presepsin is formed due to the activation of TLR-4. SARS-CoV-2 has been shown to activate the MAPK signaling pathway via TLR-4 in a similar manner to LPS. Finally, studies have shown that Procalcitonin levels are elevated in severe SARS-CoV-2 infections, presumably due to secondary bacterial infections. Taken together, it was hypothesized that elevation of these biomarkers could aid in risk stratification for COVID-19 patients at ED presentation. **Methods:** Seventy-two individuals presenting to the University of Maryland Medical Center ED and determined to be RT-PCR+ for SARS-CoV-2 were enrolled. Plasma samples were collected at presentation. Presepsin levels were measured on the PATH-FAST. Il-6 and Procalcitonin levels were measured using ELISAs (R&D Systems). Presepsin, IL-6, and Procalcitonin levels were evaluated to determine if results could be used stratify individuals by hospital length of stay (LOS) (≤3 or >3 days). **Results:** Twenty-six individuals had LOS of >3 days, while 46 individuals had LOS <3 days. Persepsin, IL-6, and Procalcitonin showed significantly higher levels (p=0.004, 0.008 and 0.025, respectively) in those with LOS ≤3 days compared to >3 days (see Figure1). The AUC for Persepsin, IL-6, and Procalcitonin were 0.822, 0.697, and 0.719, respectively, for LOS ≤3 days versus >3 days. **Conclusions:** Presepsin, IL-6, and Procalcitonin appear useful for LOS risk stratification for individuals presenting to the ED with confirmed SARS-CoV-2, however, Presepsin appears to be a better predictor. While this preliminary data is promising, further work is needed to determine the role IL-6, Procalcitonin, and Presepsin can play in overall risk stratification for COVID-19 patients.

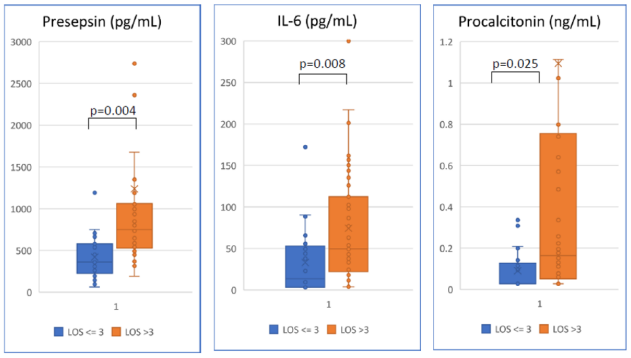

Figure 1: Box plots and p-values of t-test comparisons of Presepsin, IL-6, and Procalcitonin levels at presentation for patients categorized by hospital length of stay (LOS) ≤ 3 days and > 3 days.

B-105

Development of a PCR-based Mutation Panel for Rapid SARS-CoV-2 Variant of Concern (VOC) Discrimination

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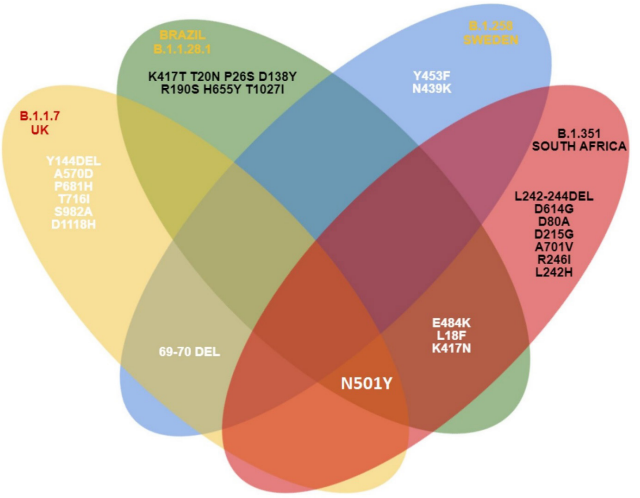
Background: During the SARS-CoV-2 pandemic, ABC Labs were testing samples from 13 out of 21 regions in Sweden on behalf of The Public Health Agency for identification of COVID-19 carriers using the Taq-Path™ COVID-19 CE IVD RT PCR kit containing three viral target genes (ORF-1ab, N, S). In November 2020, an increasing frequency of S gene target failures were noted in cases simultaneously positive for the other viral targets. This was at a time when the UK variant (PANGO lineage B.1.1.7) was not widely spread in the Swedish population. Therefore, we set out to develop a PCR-based mutation panel for variant determination in clinical swab samples.

Methods: A set of 22 TaqMan™ primer-probe sets were designed to specifically target three major variants of concern (VOC): UK (B.1.1.7), South African (B.1.351), and Brazilian (P.1) variant, and additionally a Scandinavian strain (B.1.258) carrying the S gene 69-70 deletion shared with the UK variant. Dual probes labeled with fluorophores AM and VIC, targeting wild type and mutant strain, were evaluated for discrimination using known positive samples and genotyping functionality.



Results: Mutation PCR was performed in a blind test of 33 samples and correctly identified all samples when compared to gold standard whole genome sequencing (WGS). By using primer-probe sets targeting three S-gene mutations; Y144del (B.1.1.7), N439K (B.1.258) and N501Y (shared mutation in VOC), we were able to differentiate the UK and the Scandinavian variants by single mutation (N439K) positive identification whereas the N501Y positive Y144del negative samples identified variants of either the Brazilian or South African VOC, to be further discriminated by WGS.

Conclusion: A panel of three PCR-based mutation assays can rapidly discriminate between three SARS-CoV-2 VOC, enabling tracing and early isolation of carriers, benefitting infection control.

B-106

Clinical and Analytical Assessment of a Spike-specific Quantitative IgG Assay for SARS-CoV-2

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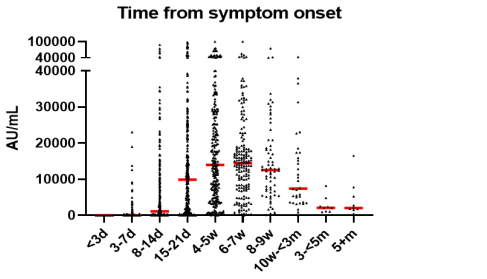
Introduction: Serological testing is an important tool to assist with determining immune defense to SARS-CoV-2, the causative agent of COVID-19. The Abbott AdviseDx SARS-CoV-2 IgG II assay, a quantitative assay that measures antibodies

against the receptor binding domain of the spike protein, was recently developed. Here we evaluated the clinical and analytical performance of the assay, and characterized longitudinal dynamics of IgG response in 402 infected individuals up to 322 days post symptom onset.**Methods:** To assess test sensitivity, 1257 specimens derived from 402 patients who tested positive for SARS-CoV-2 by RT-PCR were analyzed on the Abbott Alinity platform. To evaluate test specificity, we tested 394 specimens from patients who were symptomatic but PCR negative, as well as 305 archived samples collected pre-pandemic. This work was approved by the Beaumont Research Institute (IRB#2020-233).**Results:** The Abbott AdviseDx SARS-CoV-2 IgG II assay exhibited diagnostic specificity of 99.02% using 305 pre−COVID-19 serum specimens and 98.73% using 394 PCR negative specimens. Using 1257 sequential serum samples collected from PCR-confirmed individuals, clinical sensitivity of the assay was 39.7 % at 3-7 days, 75.9 % at 8−14 days, 95.6 % at 15−21 days, and 98.7 % at 4−5 weeks post symptom onset. The assay is linear across the analytical measurement range (22-25000 AU/mL), and exhibited good analytical precision. The median concentration of IgG increased steadily from <22 AU/mL 3-7 days after symptom onset to reach the peak of 14421 AU/mL at 6-7 weeks. Although antibody titer started to decline from 8-9 weeks following symptom onset, all patients remained seropositive during the observation period.**Conclusion:** The Abbott AdviseDx SARS-CoV-2 IgG II assay offers good linearity, high test specificity and sensitivity. We anticipate this assay will be a useful method for determination of prevalence of COVID-19 infection and to evaluate immune response after vaccination.

Figure1: Seropositivity in 1257 specimens from 402 patients with positive PCR results relative to days from symptom onset.



Time after symptom onset	<3 d	3-7d	8-14d	15-21d	4-5w	6-7w	8-9w	10w-<3m	3m-<5m	5+m
Median IgG concentration (AU/mL)	<22	<22	1142	9895	13942	14421	12558	7431	2101	1955

B-107

Antibody Signature of the Protective and Pathologic Responses to SARS-COV-2 Infection

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Background: The outbreak of 2019 novel coronavirus disease (Covid-19) caused by SARS-CoV-2 has spread rapidly, leading to life-threatening multi-organ dysfunction. SARS-CoV-2 is transmitted via respiratory droplets or the airborne route by both asymptomatic carriers and symptomatic individuals. Clinical manifestations of COVID-19 range from mild flu-like illness to respiratory distress and multi-organ dysfunction. Antibodies are key immune effectors that confer protection against pathogenic threats. However, the level and nature of the antibodies response to SARS-CoV-2 infection and its correlation with symptoms progression during the course of disease is poorly understood. Understanding humoral immune responses to SARS-CoV-2 infection will play a critical role in monitoring and improving vaccines and antibody-based interventions.

Methods: In this study, we assessed humoral IgG and IgA antibody responses in serum samples from SARS-COV-2 infected individuals (n=29) who are either asymptomatic or exhibit mild or severe and life-threatening manifestations of COVID-19. Serum samples were collected approximately 14 days after the onset of symptoms and/or positive PCR test. The titers (quantity) of IgG and IgA antibodies as well as level of neutralizing antibodies in serum samples were assessed using Euroimmune IgG and IgA assays and GenScript neutralization assay, respectively. The IgG and IgA assays detect antibodies against the S1 domain of the spike protein, while the neutralizing assay measure the total neutralizing antibodies that block the binding of the receptor binding domain (RBD) of the spike protein to the ACE2 receptor.

Results: Our data show that higher IgG titer levels are associated with disease severity, where patient with severe disease had a higher titer (≥1:2000) of IgG antibodies compared to asymptomatic individuals or patients with mild disease. Interestingly, patients with severe disease exhibited the lowest levels of IgA antibodies (≤ 1: 500), while a more robust IgA response was found in those who are asymptomatic or have mild disease. Surprisingly, assessment of neutralization function of antibodies revealed greater total neutralization responses in the serum from patients with severe disease compared to patient with mild disease.

Conclusion: The correlation between high IgG antibody levels, and higher titer of neutralizing antibodies in sera from patients with disease severity suggests that higher concentrations of IgG antibodies may act in a detrimental or non-protective manner. Furthermore, our data suggest that the antibody response may not be the only component of immune responses required to provide protection against SARS-COV-2 infection. Future characterization of the kinetics and functions of humoral and cell-mediated immune responses is critical for the development of new diagnostic tests and identification of prognostic biomarkers of COVID-19 during the course of infection, and future novel improvements to vaccination and therapeutic strategies that target both antibody and cell-mediated immune responses.

B-108

SARS-CoV-2 Antibody Response and Adverse Reactions Following Administration of either Moderna or Pfizer Vaccines

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Background: SARS-CoV-2 is a novel coronavirus responsible for the coronavirus disease 2019 (COVID-19) global pandemic. In late 2020, Pfizer and Moderna vaccines were given emergency use authorization by the FDA for distribution in the United States. The vaccines consist of SARS-CoV-2 spike protein mRNA encapsulated in lipid nanoparticles. When inoculated, individuals synthesize antibodies to the spike protein. The vaccines require two doses either three (Pfizer) or four (Moderna) weeks apart, and the CDC considers individuals to be fully vaccinated 14 days following the second dose. However, the immunization response to the vaccine is not well understood. **Methods:** Serum samples from 148 recipients of the COVID-19 vaccine were collected. 78 individuals were given the Moderna vaccine and 70 received Pfizer. Samples from Moderna recipients were collected at 24-30 days (pre-dose 2), 39-48 days (14 days post-dose 2), and 57-62 days (30 days post-dose 2) following the first dose. Samples from Pfizer recipients were collected at 17-25 days (pre-dose 2), 31-42 days (14 days post-dose 2), and 48-57 days (30 days post-dose 2) following the first dose. An additional serum sample from 14 individuals was collected prior to the first dose of vaccine. Nucleocapsid and spike antibodies were measured using the qualitative Elecys® SARS-CoV-2 and semi-quantitative Elecys® SARS-CoV-2 S assays respectively (Roche Cobas® e801 module). Additional samples will be collected at 90 and 180 days following the second dose of vaccine. Additionally, a survey was administered which invited participants to self-report and rate the severity of any symptoms following vaccination. **Results:** All 14 samples collected prior to the first dose of vaccine had undetectable spike protein antibodies (1/14 was positive for nucleocapsid antibodies). The median spike protein antibody signal for those given the Pfizer vaccine was determined at 17-25 days (64.6 U/mL), 31-42 days (2865 U/mL), and 48-57 days (1986 U/mL). Median antibody signal was measured for individuals given Moderna at 24-30 days (225.5 U/mL), 39-48 (5637 U/mL), and 57-62 days (4244 U/mL). Spike antibody signal was significantly higher ($p < 0.001$) in serum from Moderna recipients, relative to Pfizer recipients, prior to dose 2, as well as 14 and 30 days following vaccination. In general, participants who received the Pfizer vaccine also reported milder symptoms overall. **Conclusion:** Individuals given the Moderna vaccine have a higher spike antibody response relative to those given Pfizer. One contributing factor may be the additional week for seroconversion to occur in individuals who received Moderna. A second potential factor is the difference in dosage between Moderna (100 µg) and Pfizer (30 µg). However, the number of mRNA copies per dose may not be comparable between the Moderna and Pfizer vaccines. A drop in antibody response is observed for most participants, regardless of vaccine given, at the 30-day time point relative to the 14-day collection. This drop suggests that antibody response has peaked prior to the 30-day time point.

B-109

Surface plasmon resonance imaging (SPRi)-based immunobiosensor for the detection and comprehensive analysis of SARS CoV-2 antibodies

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Background: To develop a highly sensitive and specific serological assay for antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is paramount to determine previous exposure, response to vaccination and to able to assess patterns of immunity to wild type and its variants.

Methods: The SARS CoV-2 immunoassay uses surface plasmon resonance imaging (SPR)i technology to detect antibodies against both the receptor binding domain (RBD) and Nucleocapsid (NC) proteins. As well as several of the current SARS CoV2 variants (B.1.1.7, B.1.1.28, B 1.1.33, B.1.351). SPRi is a cutting-edge nanotechnology and microfluidic biosensor technology that detects biomolecular interactions in real time without the need for detection labels. Additionally, as it does not involve washing steps, SPRi can detect both high and low affinity antibodies. Sensorgrams provide information on concentration, affinity and equilibrium constants, stoichiometry and binding specificity.

We analysed 76 PCR-confirmed SARS CoV-2 - infected and 738 non-SARS CoV-2 (pre-pandemic) patient sera, and 12 sera from patients with antibodies against SARS, MERS, Haemophilus Influenza, Parainfluenza, Influenza, Human metapneumovirus, Rhinovirus, Adenovirus, Enterovirus, HCV, HBV, ANA. We also assessed and compared immunoreactivity of antibodies to wild type RBD and RBD from the UK, South African and Brazilian variants in 79 sera from post vaccinated subjects. We also analysed 79 sera from subjects who had received 1 or 2 doses of the SARS CoV2 vaccine.

Results: Seventy-six out of 76 PCR-confirmed SARS CoV2-infected Patient Sera tested positive for either anti-RBD or anti-NC antibodies, with a combined diagnostic specificity of 100% (93.4% and 81.6% testing positive for RBD and NC antibodies respectively). Seven hundred and thirty-three of the 738 un-infected patient sera tested negative for anti-RBD and anti-NC antibodies with a combined diagnostic specificity of 99.3%, with 99.7% and 99.5% testing negative for anti-RBD and anti-NC antibodies, respectively. Sera from patients infected with other viruses did not cross-react with either RBD or NC. Patients with antibodies against RBD had higher levels of antibodies against the endemic coronavirus strains. In serial samples of patients, the avidity of the antibodies increased over time as evidenced by a decrease in dissociation rate of the antibody-RBD interaction. Amongst the subjects who received a first dose of the vaccine, younger individuals mounted a vigorous antibody response than older individuals whose antibody levels were comparatively low even after 6 weeks. However, after the second dose, the antibody response was boosted dramatically. Increases in antibody levels were accompanied by a concomitant increase in inhibitory activity of the antibodies against ACE2 binding to RBD. We also observed extensive cross-neutralizing activity between WT and mutant RBDs.

Conclusion: We have developed a highly accurate, sensitive and specific Covid-19 antibody test that will serve as a critical tool in the global battle against the disease. With billions of people expected to receive Covid-19 vaccines in the coming years, our best-in-class SPRi-based immunoassay could potentially be used for this rapidly emerging unmet need. Measuring avidity and titer towards the wild type and variants will help our understanding of the immunity to this virus, post vaccination.

B-110

Expanding COVID-19 vaccine availability: Role for combined orthogonal serology testing

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Background: Persisting Coronavirus Disease 2019 (COVID-19) pandemic and limited vaccine supply compels a shift in global health priorities to expand vaccine coverage. Relying on Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) molecular testing alone cannot inform the infection proportion, which could play a critical role in vaccination prioritization. We evaluated the utility of a combination orthogonal serological testing (COST) algorithm alongside RT-PCR to quantify prevalence with the aim of identifying candidate patient clusters to receive single and/or delayed vaccination.

Methods: We utilized 108505 patients with suspected COVID-19 in retrospective analysis of SARS-CoV-2 RT-PCR vs IgG-nucleocapsid (IgG_{NC}) antibody testing coverage in routine practice for estimation of prevalence. Prospectively, an independent cohort of 21388 subjects were tested by SARS-CoV-2 RT-PCR and IgG_{NC} simultaneously to determine the prevalence. We used 614 prospective study subjects (after exclusions, n=70) to assess the utility of COST (IgG_{NC}, IgM-spike (IgM_{SP}), and IgG-spike (IgG_{SP})) in establishing CI to identify a single-dose vaccination cohort.

Results: Retrospectively, we observed 6.3% (6871/108505) positivity for SARS-CoV-2 RT-PCR, and only 2.3% (2533/108505) of cases having paired IgG_{NC} serology performed. Prospectively, IgG_{NC} serology identified twice the number of COVID-positive cases in relation to RT-PCR alone. COST further increased the number of positive cases detected: IgG_{NC}+ or IgM_{SP}+ (18.0%); IgG_{NC}+ or IgG_{SP}+ (23.5%); IgM_{SP}+ or IgG_{SP}+ (23.8%); and IgG_{NC}+ or IgM_{SP}+ or IgG_{SP}+ (141/584=24.1%).

Conclusion: COST may be an effective tool for evaluation of infection proportion, and thus, could define a cohort for a single dose and/or delayed vaccination.

B-111

Development and Characterization of Novel MAS™ anti-SARS-CoV-2 Antibody Quality Controls for Clinical Serology Tests

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Background: The Serological assays have revolutionized infectious disease testing and therapeutic monitoring for determination of the prevalence of these infections, led to better diagnosis and treatment plans for patients. COVID-19 serology testing allows for the determination of immunity against SARS-CoV-2 viruses via identification of antibodies and antigens in a patient's specimen, to timely diagnose an acute infection or post-infection. The Thermo Scientific™ MAS™ SARS-CoV-2 IgG Controls containing anti-SARS-CoV-2 IgG antibodies in human plasma based matrix are quality controls mimicking patient samples to monitor serological assay performance, therefore providing additional confidence in routine test results, test methods troubleshooting and system errors identification. We report here the development and performance evaluation of the MAS™ SARS-CoV-2 IgG Controls.

Methods: The MAS™ SARS-CoV-2 IgG Controls development including the developing of controls with (Positive) or without (Negative) SARS-CoV-2 IgG antibodies in the diluent human plasma based matrix. Analyte response was determined by testing on IVD assay platforms. Performance evaluation was performed on multiple commonly used and most up-to-date clinical diagnostic platforms including Abbott™ ARCHITECT™ and Roche™ Elecsys™, etc. Shelf life of the controls was determined by accelerated and real-time stability monitoring studies. The products were verified and validated with many function performance aspects such as Freeze Thaw allowance, Open Vial stability, Precision, Shipping validation and Sterility Testing. Each study was initiated with a completely approved protocol with all data then analyzed and summarized in written reports.

Results:MAS™ SARS-CoV-2 IgG Controls were successfully developed and evaluated with IVD assays, showing lot to lot consistency with CV% under 3% for MAS™ SARS-CoV-2 IgG Positive Control and CV% under 6% for MAS™ SARS-CoV-2 IgG Negative Control when testing with three different lots. All results demonstrated compatible performance across different clinical diagnostic platforms. Stability studies showed that minimal of 12 months shelf life and 60 days of open-vial stability is guaranteed when store at 2-8 °C. The Thermo Scientific™ MAS™ SARS-CoV-2 IgG controls are authorized for IVD/CE-IVD use.

Conclusion: The MAS™ SARS-CoV-2 IgG Controls that mimic patient samples have been developed as true third party controls for the assessment of assay performance in clinical laboratories for COVID-19 diagnostics. This is the first time we report the performance evaluation and characterization of the MAS™ SARS-CoV-2 IgG Controls using different assay platforms. The controls were confirmed can be serve as reference materials/quality controls for COVID-19 assay or test method development, validation and verification, and routine assessment of COVID-19 related diagnostic assays.

B-112

5-Minute Point-of-Care COVID-19 Antibody Test

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Background: We developed the COVID-19 Total Antibody test for detecting SARS-CoV-2 antibodies in blood using the point-of-care LightDeck platform. Validation included a retrospective study to establish performance characteristics of the LightDeck COVID-19 Total Antibody Test using the LightDeck Analyzer.

Methods: The Clinical Agreement Study was designed to determine the sensitivity, or Positive Percent Agreement (PPA), to detect antibodies against the SARS-CoV-2 virus in 95 de-identified serum or plasma samples from RT-PCR positive COVID-19 subjects. Specificity was determined in a Cross Reactivity Study using 547 samples that were collected prior to the COVID-19 pandemic. Matrix equivalency studies compared serum, plasma, venous whole blood and fingerstick whole blood.

Test procedure: 50 µL of serum, plasma or whole blood is added to the single-use cartridge and inserted into the LightDeck Analyzer. The rest of the procedure is automatic. The analyzer mixes the sample with a lyophilized pellet that contains fluorescently labeled RBD antigen. Mixing/incubation is complete in 2 minutes, then the analyzer punctures the seal to allow sample to flow over the microarray. Printed on the microarray are positive and negative controls, and detection zones for measuring total anti-RBD antibody levels via the fluorescent signal of the labeled RBD bound to anti-RBD antibodies in the sample. Fluorescence signal of the labeled RBD from the sample bound to anti-RBD antibodies on the waveguide is detected using laser excitation and detection by a CCD camera. Results are generated in five minutes after the cartridge is inserted in the analyzer. A cut-off index (COI) result is reported along with POSITIVE (COI ≥ 1.0) or NEGATIVE (COI < 1.0) interpretation.

Results: Sensitivity (PPA) was 97.9% (93/95 PCR+ samples, 95% CI 92.6% - 99.4%). Specificity (NPA) was 99.6% (545/547 PCR- samples, 95% CI 98.7% - 99.9%). Positive Predictive Value was 92.8% (95% CI 71.6% - 100.0%) and Negative Predictive Value was 99.9% (95% CI 99.8% - 99.9%). NPV and PPV are calculated at prevalence of 5%. Correlation coefficient R^2 of COI values from venous whole blood samples to plasma samples was 0.93, and correlation coefficient R^2 of COI values from venous whole blood samples to fingerstick samples was 0.98.

Conclusion: The 5-minute point-of-care LightDeck COVID-19 Total Antibody Test demonstrated performance characteristics similar to those of assays designed for a central lab. Additional studies with non-laboratory users are underway.

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B-113

Increase Blood Culture orders in Long-Term Care Facilities During COVID-19 Pandemic: Overutilization or Necessity!

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

Background: Covid-19 is pandemic infection caused by new strain of coronavirus, SARS-CoV-2 virus, with over 30 million cases and over 500,000 deaths in the USA. Long-term Care Facilities (LTCF) residents were the first to get affected because of their setting, fragility and comorbidity. Covid-19 had tremendous impact on the laboratories not only with SARS-CoV-2 molecular/antigen/antibody testing but it had major impact other sections of the laboratory. Fever is the most common symptoms which led to massive increase in blood culture orders during the peak of the pandemic.

Method: We analyzed data collected from 7,700 blood culture sets collected from residents in LTCF from January-December 2020. Every set included two vials (aerobic and anaerobic) which were incubated in a Bactec FX instrument. Positive cultures were subcultured and then identified using Microscan96 Walkaway conventional panels. They were segregated further by organism isolates .Statistical analysis was done using Analyse-it.

Results: the percentage positive culture in 2020 was 9.8%. The highest blood culture orders were in the second half of March (753 sets) and the first half of April (514 sets); however, they had the lowest positivity rate throughout 2020.

	Total blood culture	% positive
1/1/20-1/15/20	348	8.0%
1/16/20-1/31/20	452	9.5%
2/1/20-2/15/20	344	8.1%
2/16/20-2/29/20	333	10.2%
3/1/20-3/15/20	359	9.5%
3/16/20-3/31/20	731	6.0%
4/1/20-4/15/20	514	5.3%
4/16/20-4/30/20	274	5.8%

Conclusion: LTCF had the highest prevalence and death from COVID-19 in the first few months of the pandemic. The increase prevalence of COVID-19 in geriatric population causes increase in blood culture orders but it did not increase positive cultures. The low percentage of positive blood culture in March and April is most likely due to increase in unnecessary cultures which were negative and had biased the results. COVID-19 had a major impact not only on SARS-CoV-2 virus testing but extended the overutilization of other tests, an open dialogue between physicians and laboratorians is very important during pandemic crisis to help concentrating the effort on diagnosing and treating.

B-114

Performance Evaluation of the BioGX SARS-CoV-2 Reagents for BD MAX™ System in Geriatric Population.

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

Background: Covid-19 is pandemic infection caused by new strain of coronavirus, SARS-CoV-2 virus. Identifying the infected patient is the most important step to fight the pandemic and stop the spread of the disease; however, there was no available test on the market since it was new strain. Several manufacturers have developed test to detect the virus and applied for Emergency Use Authorization from FDA.

Methodology: The BioGX SARS-CoV-2 Reagents for BD MAX™ system is RT-PCR EUA approved assay; the primer and probe sets are based on the CDC assay by amplifying two unique regions of the N gene (N1 and N2).it takes about 3 hours to complete the run. The assay was evaluated for: precision, reproducibility, limit of detection, and accuracy. Over 12,000 specimens collected from residents and employees in Long-Term Care Facilities from April-July 2020 and ran using BD Max; the percentage positivity of N1, N2 and both N1 and N2 targets were calculated. Statistical analyses were done using Analyse-it.

Results: The percentage agreement for precision, reproducibility, and accuracy were 100%. The limit of detection was 1000 copies/sample. The patients' correlation was 100%. N1 was more prevalent than N2 throughout the time tested; having the both N1 and N2 positive was higher in April and decreased with the time.

	Total Positive	Both N1 &N2 Positive	N1 Positive	N2 Positive
April	1509	91.5%	96.8%	94.7%
May	765	67.8%	88.2%	79.9%
June	283	60.8%	89.0%	72.1%
July	81	38.3%	71.6%	66.7%

Conclusion: The BioGX SARS-CoV-2 Reagents for BD MAX™ System gave the benefit of a fully automated, high precision, accuracy and acceptable sensitivity assay; however, more validation needed to use it for asymptomatic patient. Although the assay is available, however, there is a limited supply of reagents and more work needed to increase the production to be able to make the test available to more patients. More studies are needed to check the reason for the decrease in having both N1 and N2 targets positive and N1 more prevalent than N2.

B-115

Comparative study of the positivity of human papillomavirus in male samples by pathological anatomy and molecular technique of hybrid capture

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Background: The human papillomavirus (HPV) are small, non-enveloped epitheliotropic viruses, with double circular DNA. They may be responsible for subclinical disease, associated with pre-malignant lesions and even with some intra-epithelial neoplasms. HPV infection in men is usually incipient, without significant clinical manifestations. This asymptomatic carrier state impairs a prompt diagnosis, in which male individuals may potentially become active disseminators of the virus. This study evaluated the frequency of high (HR) and low-risk (LR) HPV in samples processed by hybrid capture, from a large laboratory in São Paulo, Brazil. **Methods:** The study consisted of an analytical and retrospective review, including samples from male patients submitted to HPV detection by hybrid capture and complementary biopsy (upon request). Topographies and biopsy results were evaluated. Samples were processed by Hybrid Capture II (CHII), in the Rapid Capture System (RCS) equipment - USA, with Digene HC2 HPV DNA test kit - QIAGEN®. Biopsy material was processed by automation and manual histotechnics followed by hematoxylin-eosin staining, and analyzed by a medical pathologist. The results were stratified into four groups considering: (HR+/LR+), (HR+/LR-), (HR-/LR+) and (HR-/LR-). **Results:** A total of 2,256 samples were analyzed by CHII, from which 14.5% were also submitted to biopsy analysis. CHII analysis resulted on 29.66% positivity for LR, HR or both, as follows: HR-/LR+ (n=250), HR+/LR+ (n=190) and HR+/LR- (n=229). Samples collected from penile glands, body and shaft were responsible the largest number of requests (n=1585), from which 33.12% were HR+. One-way ANOVA testing was performed between groups, with no significant difference between them ($p>0.05$). Individuals ranging from 21-40 years represented most samples analyzed (n=1,714), in which 30.74% had a positive result. Of those, 34.34% and 35.10% were considered, respectivelly, HR+ and LR+. When compared with biopsy results, a total of 35.27% (n=236) of HR+ samples had a scaly cell papiloma diagnosis. **Conclusion:** The current study showed elevated HPV rates in young men, detected by CHII and/or biopsy. Since HPV infection in men is usually asymptomatic, this report highligths the importance of using molecular methods as major allies in the detection of HPV before oncogenic evolution.

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Clinical Validation of the Abbott Alinity s to Improve Organ and Tissue Donor Screening

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Background: The testing methods utilized for the screening of organ and tissue donors are highly regulated by the FDA and designed to adequately and appropriately reduce the risk of transmission of relevant communicable disease agents and diseases. Testing for Hepatitis C Virus Antibodies (HCV), HIV I/II antibodies (HIV), Hepatitis B Surface Antigen (HBsAg), Hepatitis B Core Antibodies (HBc) and HTLV I/II (HTLV) is traditionally performed using EIA/ELISA technologies requiring sample batching, turn around times between 24-36 hours, and manual manipulation resulting in variation due to testing personnel technique. Manual EIA/ELISA testing requires 150-180 minutes with batched testing due to the number of calibrators, controls, and individual wells required for testing. An evaluation of the Abbott Alinity s for organ and tissue donor screening was performed to verify performance. The Alinity s system is a high-volume, automated, blood-screening analyzer utilized to determine the presence of assay specific antigens and antibodies using chemiluminescent microparticle immunoassay (CMIA) detection technology. The Abbott Alinity s performs testing in a random access, “on-demand” environment with a 29-minute turnaround time. **Methods:** The Abbott Alinity S assays for HCV, HIV, HBsAg, HBc, and HTLV were compared to manual EIA/ELISA tests on 75 pre-mortem samples, 247 post-mortem samples, and 8 previously tested proficiency samples. Additionally, commercially available qualification panels for each assay with S/CO values that span the assay analytical measuring range were also tested with 100% agreement. Quality control is performed once every 24 hours with a separate release control performed with each sample(s). **Results:**

Assay	Total Agreement	Positive Agreement	Negative Agreement	Total SD	Total CV%
HBc	98.2%	88.9%	99.3%	0.061	2.3
HBsAg	97.9%	81.0%	99.0%	0.474	6.2
HCV	98.8%	94.6%	99.3%	0.089	3.4
HIV	100%	100%	100%	0.057	4.1
HTLV	100%	100%	100%	0.651	2.8

Conclusion: The Abbott Alinity s allows for automated, rapid, on-demand screening of infectious diseases in the Organ & Tissue Donor populations.

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Seroprevalence of Anti SARS CoV-2 (Total) Antibody; A single Centre Study

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Background: Transmission of COVID-19 (Coronavirus Disease 2019) epidemic has been converted in pandemic soon after its appearance from Wuhan in late 2019. In Pakistan, this pandemic is still increasing day by day. This is causing a serious challenge for government authorities and healthcare workers as well

Methods: This cross-sectional study was done at Makhdoom Laboratories & Blood Bank (MLBB), Mandi Bahauldin, from October 2020 to March 2021. Total 1242 subjects were included in study. 03 CC blood sample was collected in lithium heparinized vacutainer and Anti SARS CoV-2 Antibody was measured on Cobas e411, using electrochemiluminescence immunoassay.

Results: Total 1242 subjects were included with mean age of 39±20 years. 857 (69%) were male and remaining 385 (31%) were female. From total 599 (48%) participants were found seronegative and remaining 643 (52%) were found positive Anti SARS CpV-2 Antibody. All participants were divided in 04 age groups and highest prevalence 251 (39%) was seen in age group 4. This was followed by age group 2, 3, 1 with 179 (27.9%), 165 (25.6%) and 48 (7.5%) seroprevalence, respectively.

Conclusion: The estimated population seroprevalence of SARS-CoV-2 antibodies in this study depicts that infection may be much more widespread. More studies are needed to improve precision of seroprevalence determination. The large scale seroprevalence would be beneficial for government authority for the prevention of this pandemic.

Age wise distribution of seropositive cases

Age group number	**1**	**2**	**3**	**4**
Age group	15-25	26-35	35-45	46-55
Cases	48	179	165	251

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Development and Characterization of a Dual Antigen SARS CoV-2 IgG ELISA Capable of Detecting Antibodies Derived from Natural Infection and Vaccination

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Background: The emergence of SARS CoV-2 sparked the development of numerous serology tests, with clinical utilities still being explored. For example, detecting the presence and duration of an immune response from SARS CoV-2 infection or vaccination is of much interest. Two viral antigens are widely utilized for capturing SARS CoV-2-specific antibodies within serology assays: Spike and Nucleoprotein. Many serology assays incorporate one of these antigens; and those that utilize only Nucleoprotein will theoretically not detect antibodies derived from currently available Spike protein-based vaccines. Therefore, we chose to develop a SARS CoV-2 IgG ELISA using both antigens and set out to validate its ability to detect antibodies derived from natural infection and vaccination. **Methods:** Antibody specificity - Five IgG-positive samples were diluted in standard assay diluent, diluent containing 10% PBS, and diluent containing 10% Goat anti-human IgG (GAHG), then assayed by ELISA. Matrix - Serum, Li-Heparin plasma, Sodium Citrate plasma, and K2-EDTA plasma was collected from 4 healthy donors, and 1 convalescent COVID-19 donor.

A strong IgG-positive sample was spiked into the 4 healthy-donor samples, then all spiked and non-spiked samples were assayed by ELISA. Repeatability - 4 positive samples, and 1 negative sample, were assayed in triplicate, twice per day, for 5 days. Cross Reactivity - 180 pre-pandemic samples were obtained (90 healthy donors and 90 febrile donors) were assayed by ELISA. Sensitivity - 35 samples from SARS CoV-2 RT-PCR positive donors and 50 serology-positive samples, were assayed by ELISA. Specificity - 34 samples derived from SARS CoV-2 RT-PCR negative donors and 77 serology-negative samples, were assayed by ELISA. Automation - Positive and negative samples were assayed by ELISA manually and on 3 Dynex instruments (DS2®, DSX®, Agility®). Vaccine Study - Serum samples were collected prior to vaccination, just prior to a second dose, and after a second dose, from multiple SARS CoV-2 vaccines/donors, then assayed by ELISA.**Results:** Antibody specificity - 100% of samples from 10% GAHG assay diluent were negative, 100% of samples from standard and 10% PBS assay diluents remained positive. Matrix Equivalence - Spiked and non-spiked donor-matched samples yielded 100% qualitative agreement between sample matrices. Repeatability - Negative (CV = 10.37%), Borderline (CV = 8.45%), Low positive (CV = 8.76%), Medium Positive (CV = 8.68%), High Positive (CV = 7.51%). Cross Reactivity - 89/90 healthy donors were negative (98.9%. (95% CI = 96.7% to 100%) and 89/90 febrile donors were negative (98.9%. (95% CI = 96.7% to 100%). Sensitivity -82/85 = 96.5%. (95% CI = 92.5% to 100%). Specificity - 111/111 = 100%. (95% CI = 100% to 100%). Automation - Manual vs. DS2 (R^2 = 0.9828), Manual vs. DSX (R^2 = 0.9865), Manual vs. Agility (R^2 = 0.9829) Vaccine Study - 100 % of pre-vaccination samples were negative, 100 % of the post vaccination samples (pre and post a second dose) were positive. **Conclusion:** These studies demonstrate that the newly developed dual antigen ELISA is highly accurate, reproducible, automatable, and effective at detecting IgG antibodies derived from natural infections, as well as multiple SARS CoV-2 vaccines.

B-119

Development and Characterization of a Dual Antigen SARS CoV-2 IgM/IgG/IgA ELISA Capable of Detecting Antibodies Derived from Natural Infection and Vaccination

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Background: The emergence of SARS CoV-2 sparked the development of numerous serology tests, with a spectrum of clinical utilities still being explored. For example, detecting the presence and duration of an immune response from SARS CoV-2 infection or vaccination is of much interest. Two viral antigens are widely utilized for capturing SARS CoV-2-specific antibodies within serology assays: Spike and Nucleoprotein. Many serology assays incorporate one of these antigens; and those that utilize only Nucleoprotein will theoretically not detect antibodies derived from currently available Spike protein-based vaccines. Therefore, we chose to develop a SARS CoV-2 IgM/IgG/IgA ELISA using both antigens and set out to validate its ability to detect antibodies derived from natural infection and vaccination. **Methods:** Matrix Equivalence - Serum, Li-Heparin plasma, Sodium Citrate plasma, and K2-EDTA plasma was collected from 5 healthy donors. A strong positive sample was spiked into each of the healthy-donor samples, then all spiked and non-spiked samples were assayed by ELISA. Repeatability - 3 positive samples spanning the assay's detection range, as well as 2 negative samples, were assayed by ELISA in triplicate, twice per day, for 5 days. Cross Reactivity - 180 pre-pandemic serum samples were obtained (90 from apparently healthy donors and 90 from febrile donors (suspected of various respiratory or other illnesses) were assayed by ELISA. Sensitivity - 50 samples derived from SARS CoV-2 RT-PCR positive donors were assayed by ELISA. Specificity - 84 samples derived from SARS CoV-2 RT-PCR negative donors were assayed by ELISA. Automation - A set of positive and negative samples were assayed by ELISA manually and in parallel on 3 Dynex instruments (DS2®, DSX®, Agility®). Vaccine Study - Serum samples were collected from consented donors prior to vaccination, just prior to a second dose, and various times after a second dose, from multiple SARS CoV-2 vaccines, then assayed by ELISA.**Results:** Matrix Equivalence - Spiked and non-spiked donor-matched samples were in 100% qualitative agreement between sample matrices. Repeatability - Low negative (CV = 8.35%), High negative (CV = 8.23%), Low positive (CV = 6.56%), Medium Positive (CV = 5.53%), High Positive (CV = 5.38%). Cross Reactivity - 90/90 apparently healthy donors yielded negative results (100% - 95% CI = 95.9% to 100%) and 90/90 febrile donors yielded negative results (100% -95% CI = 95.9% to 100%). Sensitivity - 48/50 = 96.0% (95% CI of 86.3% to 99.5%). Specificity - 81/84 = 96.4% (95% CI of 90.0% to 98.8%). Automation - Manual vs. DS2 (R^2 = 0.9887), Manual vs. DSX (R^2 = 0.9837), Manual vs. Agility (R^2 = 0.9812) Vaccine Study - 100 % of pre-vaccination samples yielded negative results, 100 % of the post vaccination samples (pre and post a second dose) yielded positive results. **Conclusion:** These studies demonstrate that the newly developed dual antigen ELISA is highly accurate, reproducible, automatable, and effective at detecting antibodies derived from natural infections, as well as multiple SARS CoV-2 vaccines.

B-120

Clinical Utility of an Automated IL-6 Immunoassay for COVID19 Prognosis and Follow-Up

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Background: The acute and longterm outcomes of COVID19 are highly variable and difficult to predict. Whereas some COVID19 patients are asymptomatic others experience a severe response to infection that requires intensive hospital care and mechanical ventilation. Understanding the pathophysiology of COVID19 will be key to developing targeted treatment and rehabilitation. Severe cases of COVID19 are characterized by a hyperinflammatory response that is associated with organ failure and respiratory complications. The cytokine Interleukin6 (IL6) is a key marker of the hyperinflammatory response in COVID19 and is significantly elevated in COVID19 patients that require mechanical ventilation. Despite the recent development of automated IL-6 assays for clinical use, IL6 measurement for the prognosis of COVID19 patients has yet to be widely adopted. The objective of this study was to determine the utility of an automated IL-6 assay in assessing the duration and severity of the hyperinflammatory response in COVID19 patients with variable clinical courses. **Methods:** Analysis of remnant serum samples from COVID19 patients admitted to San Francisco General Hospital was approved by the UCSF Institutional Review Board. Serum samples were collected at the time of hospital admission from PCRconfirmed COVID19 patients for routine clinical testing and remnant samples were stored at 20°C prior to IL6 measurement with the Siemens ADVIA Centaur IL6 immunoassay. **Results:** Of the 173 patients that were hospitalized for COVID19 in this study, 45 were mechanically ventilated patient with severe COVID19. 128 control COVID19 patients did not require mechanical ventilation. Based on a cutoff value of 35 pg/mL, admission levels of IL6 were effective at predicting the need for mechanical ventilation in this cohort. ROC curve analysis demonstrated that the IL6 level during hospital admission is a significant predictor of ICU admission and mechanical ventilation in COVID19 patients (AUC = 0.85, $p<0.0001$ and AUC = 0.85, $p<0.0001$, respectively). **Conclusion:** Admission levels of IL6 measured with the Siemens ADVIA Centaur platform can inform clinicians of which COVID19 patients have a high risk for progressing to mechanical ventilation.

B-123

SARS-CoV-2 IgG levels after 1st and 2nd dose of Comirnaty vaccination measured on Beckman Coulter UniCel DxI800

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Background: COVID-19 vaccination is an important tool to help stop the pandemic situation. SARS-CoV-2 vaccination in Czech Republic started on December 27th, 2020. First vaccination was provided by mRNA Comirnaty vaccine. This study is evaluating level of IgG antibodies after vaccination in during the time. **Method:** Samples were measured in Beckman Coulter Unicel 800 DxI analyzer using Access SARS-CoV-2 IgGII assay. That assay is semiquantitative targeting RBD of the S1 protein. Many studies indicated that an antibody response from this region may be neutralizing to SARS-CoV-2. The initial cohort was 37 people, 25 of them provided all data. Comirnaty vaccine was administered according to guideline in two doses, 3-4 weeks apart. Blood samples were taken on day 14 (ie 14 days after the 1st dose), then on day 7 after the 2nd dose and then 3 weeks after the 2nd dose of the vaccine. The obtained data were processed by statistical software SigmaPlot (Systat, USA) and MS Excel. Results were presented as box plot graph and as a MS excel (each proband in separate line). The hypothesis of the dependence of antibody production on age was verified by Spearman correlation for 37 probands 14 days after 1st dose. Site effects of vaccine was evaluated after 1st and 2nd dose. **Results:** The median of IgG level 14 days after the first dose of vaccine was 32.3 AU/ml. This value is above the cut off value specified by the manufacturer (10 AU/ml). The initial response to the vaccine corresponds to the primary response after the first exposure to the virus. Another dose of vaccine causes a fold increase in this component of the immune response. This increase was found in all probands in the test group 1 week after the 2nd dose of vaccine. Three weeks after the 2nd dose, a decrease in antibody production was found in 84% of probands, 16% of people had an increase in production even after this time.

This increase was verified in these individuals by repeated sampling in the next week, when a decrease in antibodies was found. **Conclusion:** In our study group an effect on B cells in terms of antibody production was found after mRNA vaccination. The Beckman Coulter SARS-CoV-2 IgGII semiquantitative assay is suitable for monitoring antibody levels after vaccination. The study is still on going to monitor long term IgG antibodies levels.

B-129

The Accula SARS-CoV-2 test

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Background: Testing pre-procedure patients for SARS-CoV-2 via a satellite or drive-thru location has shown to have logistical challenges in our pediatric healthcare system. Asymptomatic patients scheduled to have one of the following procedures are required to have SARS-CoV-2 testing completed prior to the procedure: Nasal/Sinus Procedures, Tonsillectomy, Adenoidectomy, Mastoidectomy, Dental Procedures, Bronchoscopy, Cleft Lip and Palate, Oral Surgery, Upper GI Procedures. To improve the patient experience, as well as patient and staff safety, our facility sought to perform SARS-CoV-2 testing on these patients at the point of care immediately prior to surgery. The Accula SARS-CoV-2 test is a Nucleic Acid Amplification Test (NAAT) for detection of SARS-CoV-2 viral RNA in approximately 30 minutes. negative test result. The Accula SARS-CoV-2 Test is intended for use by trained operators who are proficient in performing tests on the Accula Dock. The Accula SARS-CoV-2 Test is only for use under the Food and Drug Administration's Emergency Use Authorization. Some challenges that we faced when implementing this point of care test was proper pipetting technique, which could contribute to invalid test results. The nurse had to revamp their work flow in order for this test to be beneficial to their patient and improve turnaround time. Objective:Our goal was to provide same day point of care testing that is reliable, waived, and rapid. Due to wide-ranging symptoms associated with COVID-19 infection and the frequency with which children are likely to display these symptoms, rapid point of care tests may be useful diagnostic tools for testing persons in the early stages of infection with SARS-CoV-2. Screening testing for SARS-CoV-2 is intended to identify infected persons who are asymptomatic and without known or suspected exposure to SARS-CoV-2. Screening testing is performed to identify persons who may be contagious so that measures can be taken to prevent further transmission.Methodology: The following parameters were used and measured to validate the Accula SARS-Cov-2 RNA test: 20 known patient samples that compared to an established in-house reference method. Three level of controls were run over 3 days. Validation covered simple precision, complex precision, and accuracy. Results and Conclusions: The point of care Accula test can provide results in 30 minutes and can be performed at the bedside compared to alternative methods which require up to five hours, complex equipment, and a more complex validation protocol. The turnaround time was a huge advantage as well. The Accula also makes it easier for patients to get tested the day of the procedure rather than get tested elsewhere a few days before their procedure. Rapid tests and results can provide a physician and other clinical personnel with answers that can help determine a course of treatment for a patient. From rapid blood testing to COVID 19 antibody testing, the shift from the lab to POC testing is becoming more important than ever. The COVID-19 pandemic has transformed the healthcare industry by directly improving the patient experience with more options for where and when they get testing.

B-130

Multicenter Evaluation of Gentamicin MIC Results for Gram Negative Bacteria Using CLSI Breakpoints on MicroScan Dried Gram Negative MIC Panels

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Background: A multicenter study was performed to evaluate the accuracy of gentamicin on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to a frozen CLSI/ISO broth microdilution reference panel. **Materials/Methods:** A total of 429 clinical isolates were tested using the turbidity and Prompt® methods of inoculation during the efficacy phase at three external sites. An evaluation was conducted by comparing MIC values obtained using the MSDGN panels to MICs utilizing a CLSI/ISO broth microdilution reference panel. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually at 16-20 hours. Frozen reference panels were prepared according to CLSI/ISO methodology, incubated for 16-20 hours and read visually. CLSI breakpoints (µg/mL) used for interpretation of MIC results were: ≤ 4 S, 8 I, ≥ 16 R for *Enterobacterales*, Other Non-*Enterobacterales*, *Acinetobacter* spp., *P. aeruginosa*, and *Aeromonas* spp. **Results:** Essential, categorical agreement and categorical errors were calculated compared to MIC results from frozen reference panels for all isolates tested in efficacy and found in the following table.

Read Method	Essential Agreement (EA) %		Categorical Agreement (CA) %		Very Major Error (VMJ) %		Major Error(MAJ) %		Minor Error(MIN) %	
	P	T	P	T	P	T	P	T	P	T
Walk-Away	94.6 (402/425)	98.6 (423/429)	97.4 (414/425)	97.4 (418/429)	0.0 (0/29)	0.0 (0/29)	0.0 (0/389)	0.0 (0/393)	2.6 (11/425)	2.6 (11/429)
auto-SCAN-4	96.7 (411/425)	99.1 (425/429)	97.9 (416/425)	98.1 (421/429)	0.0 (0/29)	0.0 (0/29)	0.0 (0/389)	0.0 (0/393)	2.1 (9/425)	1.9 (8/429)
Visually	95.8 (407/425)	98.4 (422/429)	97.7 (415/425)	97.9 (420/429)	0.0 (0/29)	0.0 (0/29)	0.0 (0/389)	0.0 (0/393)	2.4 (10/425)	2.1 (9/429)
T = Turbidity inoculation method, P = Prompt inoculation method										

Conclusion: This multicenter study showed that gentamicin MIC results for Gram Negative bacteria obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using CLSI interpretive criteria.

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CLSI Tobramycin Breakpoints for MicroScan Dried Gram Negative MIC Panels from a Multicenter Assessment of Gram Negative Bacteria

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Objectives: CLSI tobramycin breakpoints were evaluated against data from a multicenter clinical study with *Acinetobacter* spp., *Enterobacterales,* Other Non-*Enterobacterales* 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:** MSDGN panels were evaluated at three clinical sites by comparing MIC values obtained using the MSDGN panels to MICs utilizing a CLSI/ISO broth microdilution reference panel. The study included 419 clinical isolates tested using the turbidity and Prompt® methods of inoculation during efficacy. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and visually. Read times for the MSDGN panels were at 16-20 hours. Frozen reference panels were prepared and read according to CLSI/ISO methodology. CLSI breakpoints (µg/mL) used for interpretation of MIC results were: ≤ 4 S, 8 I, ≥ 16 R for *Acinetobacter* spp., Other Non-*Enterobacterales*, *Enterobacterales*, and *P. aeruginosa*.**Results:** Essential and categorical agreement was calculated compared to frozen reference panel results. Results for isolates tested during efficacy are as follows:

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
Walk-Away	94.2 (391/415)	98.1 (411/419)	95.7 (397/415)	95.5 (400/419)	0.0 (0/24)	0.0 (0/24)	0.5 (2/379)	0.0 (0/383)	3.9 (16/415)	4.5 (19/419)
auto-SCAN-4	95.9 (398/415)	98.1 (411/419)	95.9 (398/415)	96.2 (403/419)	0.0 (0/24)	0.0 (0/24)	0.5 (2/379)	0.0 (0/383)	3.6 (15/415)	3.8 (16/419)
Visually	95.9 (398/415)	98.3 (412/419)	95.7 (397/415)	96.2 (403/419)	0.0 (0/24)	0.0 (0/24)	0.5 (2/379)	0.0 (0/383)	3.9 (16/415)	3.8 (16/419)
T = Turbidity inoculation method, P = Prompt inoculation method										

Conclusion: Tobramycin MIC results for Gram Negative bacteria obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using CLSI interpretive criteria in this multicenter study.

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Analytical Performance of a Highly-Sensitive HBsAg Assay and Its Early Detection of HBsAg in Seroconversion Panels

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Background and Objective: Hepatitis B surface antigen (HBsAg) is the primary marker for screening and diagnosis of hepatitis B infection. A highly sensitive HBsAg assay can help reduce the window period and detect occult HBV carriage (OBI). The objective of this study was to evaluate the key performance characteristics of the highly sensitive ARCHITECT HBsAg Next Qualitative assay (referred to as HBsAg Next).

Methods: The assay precision, sample carryover and seroconversion sensitivity of the HBsAg Next were evaluated at the Queen Mary Hospital, Hong Kong. The precision evaluation per the CLSI EP5A guideline was performed using a five-member precision panel consisting of three patient specimen pools and two Quality Controls. Sample carryover was evaluated by testing a high positive specimen (concentration at approximately 290,000 IU/mL) followed by 4 replicates of a negative specimen at ~0.4 S/CO. For the seroconversion sensitivity analysis, 9 hepatitis B seroconversion panels (Zeptometrix) were tested, among which the panels 6272 and 11000 were previously identified as probable vaccine breakthrough infections. Roche Elecsys HBsAg II (referred to as Elecsys) was used as the comparator assay in the testing and the seroconversion results of ARCHITECT HBsAg Qual II assay (referred to as Qual II) published previously were also compared.

Results: The within-laboratory coefficients of variation (CV) was 3.46% for the positive quality control (mean S/CO 3.07), and 4.95% and 5.69% for the two positive patient pools (mean S/CO 1.41 and 4.52), respectively. Sample to sample carryover was not observed. In the seroconversion sensitivity evaluation, out of 115 specimens from the 9 seroconversion panels, 69 specimens were confirmed positive. Among these 69 specimens with detectable HBsAg by the HBsAg Next assay, 40 (58%) and 44 (64%) had HBsAg detectable by the Elecsys and Qual II assays, respectively. All specimens with undetectable HBsAg result by the HBsAg Next assay were also tested negative by the other two HBsAg assays. In all the 9 seroconversion panels tested, the number of days to the first repeatedly reactive and confirmed result was less for the HBsAg Next assay (mean: 38.8 days) as compared to the Elecsys (mean: 54.2 days) and Qual II assays (mean: 52.0 days), indicating an earlier detection of HBsAg by the HBsAg Next assay than the two comparators (mean: 15.4 and 13.2 days earlier, respectively). In particular, the HBsAg Next assay could detect HBsAg starting on day 46 for the panel 6272 and day 0 (first blood draw) for the panel 11000, which reduced the window period by 48 days and 26 days, respectively, as compared to both the Elecsys and Qual II assays.

Conclusions: The ARCHITECT HBsAg Next Qualitative assay demonstrated excellent analytical performance. Its sensitive detection of HBsAg using clinical specimens from seroconverting donors demonstrated that it is potentially useful for the detection of early acute HBV infection, OBI, and vaccine breakthrough cases.

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Performance of High Sensitivity Hepatitis B Surface Antigen Quantitative Assay on Fully-automated Chemiluminescent Immunoassay Analyzer

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Background: Quantitative hepatitis B surface antigen (HBsAg) assay is widely usedfor a monitoring of hepatitis B virus (HBV) infection. Many clinical studies indicate theclinical significance of the quantitative HBsAg assay. In 2013, a high sensitivityHBsAg quantitative assay has been launched in Japan, which can detect 0.005IU/mL of the HBsAg and achieve about 10-fold higher sensitivity than existingARCHITECT® and Alinity *i*® HBsAg quantitative assays. It might be expected toimprove the monitoring of the course of HBV infection, HBsAg sero-clearanceundergoing a nucleotide analogs treatment and the monitoring of HBV reactivationundergoing immunosuppression or chemotherapy compared to the conventionalquantitative HBsAg assays.**Objective:** To evaluate the analytical performance of the newly developed highsensitivity HBsAg quantitative assay (prototype Alinity *i* HBsAg Next Quantitativeassay) on the fully-automated chemiluminescent immunoassay analyzer.**Material and Methods**: The prototype Alinity *i* HBsAg Next Quantitative assay is amodified two-step assay for the fully automated chemiluminescent Alinity *i* andARCHITECT Systems analyzer. The assay has achieved a wide measuring intervalwhich covers between 0.0050 IU/mL to 500.00 IU/mL with two assay modes (Highlysensitive mode and Normal mode) and has a 1:500 auto-dilution mode, which is ableto measure up to 250,000 IU/mL. The key analytical performance testing includingprecision, limit of quantitation (LoQ), linearity, and method comparison with existingHBsAg quantitative assay were assessed per CLSI protocols. The seroconversionpanels, mutant panels and specificity were evaluated.**Results:** The LoQ for two assay modes were less than or equal to 0.0016 IU/mL andless than or equal to 0.02 IU/mL, respectively. The detectability against theseroconversion panels and mutant panels also showed better sensitivity than theexisting quantitative HBsAg assay. The specificities (repeat reactive rate over 0.0050IU/mL) were 99.97% (2996/2997). Besides, the standard deviations of totalimprecision in the range of less than 0.01 IU/mL were less than 0.001 IU/mL.The %CVs of total imprecision were less than 7.5%CV within the range of 0.01 to500.00 IU/mL. This assay was also shown linear up to greater than 500.00 IU/mLwithout auto-dilution.**Conclusion:** The prototype Alinity *i* HBsAg Next Quantitative assay demonstratedgood precision, sensitivity, and linearity. Moreover, the assay showed excellentspecificity, mutant detectability and faster detectability of seroconversion panels. Asa conclusion, we have successfully developed the high sensitivity HBsAgquantitative assay as a fully automated assay with high throughput (200 tests/hour).

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Three-Year HPV-16/18 Vaccine Responses in Adolescent Girls Using an Indirect ELISA Method: A Proof-of-Concept

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Introduction: Vaccination of girls against oncogenic human papilloma viruses (HPV) is an important strategy towards cervical cancer elimination. Clinical laboratories bolster public confidence in vaccines by monitoring the long-term immunogenicity of currently available and future vaccines. We set out to optimize an assay to be used in evaluating the long-term durability of HPV 16/18 antibody titers in previously immunized Ghanaian girls. **Methods:** A rapid, high-throughput, indirect enzyme-linked immunosorbent assay (ELISA) was evaluated for the detection and quantitation of anti-HPV L1 (late expression protein: types 16 and 18) immunoglobulin G (IgG) in human serum (n= 89). The performance of the assay was evaluated using serum collected from a cohort of pre-adolescent girls (n= 49) previously vaccinated with a quadrivalent vaccine and non-immune serum obtained from age-matched controls (n= 40). **Results:** Samples with OD greater than 1 were considered as positive and borderline values were defined for samples within ±0.1 of the reference. For both HPV -16 (63.3% vs. 12.5%; p<0.001) and HPV -18 (34.7% vs. 20.0%; p=0.042), the seroprevalence of anti-HPV IgG antibodies was significantly higher among vaccinated than unvaccinated girls, respectively. Among vaccinated girls, significantly higher mean anti–HPV-16 (0.618, 95% CI= 0.559-0.677 vs. 0.145, 95% CI= 0.048-0.242) and anti–HPV-18 (0.323, 95% CI= 0.250-0.396 vs. 0.309, 95% CI= 0.111-0.507) antibody levels were measured 36 months after receiving the third dose of vaccine compared

to unvaccinated girls ($p < 0.05$). A correlation between optical density and antibody activity indicated assay sensitivity to increasing levels of antibody activity. **Conclusion:** Higher anti-HPV seropositivity among vaccinated girls may be indicative of vaccine efficacy 3 years after administration. We have successfully developed and implemented a robust and sensitive assay for the evaluation of antibody responses among immunized adolescent girls. This demonstrated capacity will be useful for future large-scale HPV vaccination studies in low-income settings.

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Evaluation of the Roche and Snibe spike antibody assays compared to prior nucleocapsid and spike antibody assays

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Introduction: We evaluated the Roche Elecsys Anti-SARS-CoV-2 S assay and the Snibe SARS-CoV-2 S-RBD IgG chemiluminescent immunoassays, comparing them to the prior Roche Elecsys/Abbott IgG nucleocapsid assays and the Abbott IgM spike assay.

Methods: We enrolled 184 SARS-CoV-2 RT-PCR positive samples and 215 controls (172 pre-pandemic samples and 43 current samples for cross-reactivity) to evaluate the Roche spike assay. For the Snibe evaluation, we included 119 RT-PCR positive samples and 249 controls (200 pre-pandemic samples and 49 current samples for cross-reactivity). 98 cases had been tested on all three spike assay platforms (Roche total antibody, Snibe IgG and Abbott IgM).

Results: The Roche spike assay had a CV of 0.5%/2.3% (at 0.82/8.72) and was linear from 1.16-240U/mL. The Snibe assay was linear from 6.43-77.7AU/mL and had a CV of 5.5%/8.8% (at 0.43/0.18AU/mL). The Roche spike assay was 100% specific (95% CI 98.3 to 100), but the Snibe spike assay had a specificity of 92.0% (95% CI 87.9 to 95.0) with cross-reactivity with 7 other antibody positive test samples (HBeAg 2/ Dengue 4/ANA 1). The Snibe spike assay was significantly more sensitive than the Abbott IgG assay at 0-6 days POS (difference 29.6%, 95% CI 17.5 to 41.8, $p<0.0001$), and was also slightly more sensitive than the Roche total antibody/Abbott IgM anti-SARS-CoV-2-S assays. We generated lower optimized LORs for all assays (average of ROC associated criterion + 99th percentile of negative controls) which significantly improved the sensitivity of the Roche spike assay (difference 8.7%, 95%CI 3.3-14.1, $p=0.004$), both nucleocapsid assays (Roche difference 20.2%, 95%CI 12.5-27.9, $p<0.0001$, Abbott difference 14.8%, 95%CI 5.3-24.3, $p=0.008$), and the Abbott IgM assay (difference 15.6%, 95%CI 5.0-26.2, $p=0.02$) in early disease (see Table 1).

Conclusion: Both spike assays show higher sensitivity than nucleocapsid assays in early disease, although optimized LORs provided the most significant improvements to assay sensitivity.

TABLE 1: Sensitivity of SARS-CoV-2-Ab assays by days post-first positive SARS-CoV-2 RT-PCR - recommended limits of reactivity versus optimized limits of reactivity

Days POS	Roche Nucleocapsid		Roche Spike		Abbott IgG (nucleocapsid)		Snibe IgG (spike)		Abbott IgM (spike)	
	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)
Recommended limits of reactivity										
0 – 6	45/59	43.3 (33.6-53.3)	50/54	48.1 (38.2- 58.1)	2/52	3.6 (0.4 -12.3)	19/35	35.2 (22.7-49.4)	7/38	15.6 (6.5-29.5)
7 – 13	29/10	74.4 (57.9-87.0)	31/8	79.5 (63.5 -90.7)	23/8	74.2 (55.4-88.1)	25/6	80.6 (62.5-92.5)	17/7	70.8 (48.9-87.4)
≥ 14	39/2	95.1 (83.5-99.4)	39/2	95.1 (83.5-99.4)	33/1	97.1 (84.7-99.9)	34/0	100 (89.7-100)	23/6	79.3 (60.3-92.0)
Optimized limits of reactivity										
0 – 6	66/38	63.5 (53.4-72.7)	59/45	56.7 (46.7-66.4)	10/44	18.5 (9.3-31.4)	22/32	40.7 (27.6-55.0)	14/31	31.1 (18.2-46.6)
7 – 13	36/3	92.3 (79.1-98.4)	32/7	82.1 (66.5-92.5)	23/8	74.2 (55.4-88.1)	25/6	80.6 (62.5-92.5)	22/2	91.7 (73.0-99.0)
≥ 14	40/1	97.6 (87.1-99.9)	39/2	95.1 (83.5-99.4)	34/0	100 (89.7-100)	34/0	100 (89.7-100)	29/0	100 (88.1-100)

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Clinical Validation of GenScript cPassSARS-CoV-2 Neutralization Antibody Assay

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Background: Identification of high-affinity neutralizing antibodies to SARS-CoV-2 is essential to fully understand immunity after natural infection or vaccination. Widely used serological assays measure antibody binding to SARS-CoV-2 spike protein, however, not all spike-binding antibodies can block viral infection. GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit is the first assay granted FDA_ EUA for the qualitative determination of neutralizing anti-SARS-Cov-2 antibodies. The assay is independent of the use virus and cell cultures allowing for high-throughput, automation and fast turnaround times. Previously published extensive validation study (Meyer, B., et al., 2020) demonstrated GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit is useful as an additional assay in determination of COVID19 immune status of infected or vaccinated individuals. Here, we performed clinical validation of the GenScript cPass Assay and for the first time we show correlation between GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit and Vitros SARS-Cov2 IgG for the assessment of post-immunity in infected and vaccinated individuals. **Methods:** We performed validation of GenScript cPass SARS-CoV-2 Neutralization Antibody Detection assay for the detection of neutralizing antibodies at our institution. Intra- and inter-assay CV, linearity, and method comparison studies were carried out. Interference for hemolysis, lipemia, and icterus was evaluated. Specimens from vaccinated individuals (n=130) and convalescent plasma (n=15) were analyzed. Concordance and Cohen's *Kappa coefficient* (ƙ) between the Vitros Anti-SARS-Cov2-IgG assay and GenScript cPass SARS-CoV-2 Neutralization Antibody Detection assay was calculated at signal to noise cutoff of ≥9.5 for the Vitros SARS-Cov2-IgG. GenScript cPass assay was performed according to manufacturer's instructions and ≥30% of signal reduction at 1:160 antibody titer was used to determine positive samples.

Results: Intra- and inter-assay CV of the GenScript cPass SARS-CoV-2 Neutralization Antibody Detection assay was < 10%. The assay showed no significant interference from hemolysis, lipemia, conjugated bilirubin and did not exhibit cross reactivity with specimens positive for common respiratory viruses. For specimens from vaccinated individuals we recorded perfect agreement (100%; Cohen's Kappa 1), all samples with cutoff values ≥9.5 on Vitors SARS-Cov2-IgG had ≥30% of signal reduction at 1:160 antibody titer measured by GenScript cPass Assay. Slight agreement (53%; Cohen's Kappa 0.16 CI 95% 0.223 - 0.543) between Vitros IgG and GenScript cPass assay at 1:160 antibody titer for convalescent plasma specimens, however at 1:80 antibody titer we observed good correlation (93%; Cohen's Kappa 0.7 CI 95% 0.315 - 1) between the assays. **Conclusion:** Our results indicate that GenScript cPass SARS-CoV-2 Neutralization Antibody Detection assay is robust with good analytical performance suitable for clinical offering, it offers for short turnaround times and low sample volume requirements. The neutralizing assay displays excellent correlation to Vitors Anti-SARS-CoV-IgG assay for specimens from vaccinated individuals. We concluded that GenScript cPass SARS-CoV-2 Neutralization Antibody Detection assay can be used to help determine the immune serostatus of COVID-19 infected of vaccinated individuals.

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Evaluation of a Novel Multiplex Platform and an ELISA-based Neutralizing Antibody Assay for the Monitoring of Infection and Vaccine Immunity

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Background Numerous serological methods to detect SARS-CoV-2 antibodies have been developed but they are often directed against one antigen and do not distinguish binding from neutralizing antibodies (Nabs). Simultaneous detection of antibodies against the different targets of SARS-CoV-2 [spike (S), nucleocapsid (N), receptor-binding domain (RBD) or N-terminal domain (NTD)] may help distinguish natural and vaccine-acquired immunity. Additionally, emerging evidence correlates the presence of Nabs with COVID-19 protective immunity. Here, we evaluated the diagnostic characteristics of an FDA-authorized ELISA-based kit, Genscript cPass™ Nab (GcN) assay (RBD) and of a novel multiplex assay from Meso Scale Discovery (MSD), that is capable of detecting total IgG antibodies against all four SARS-CoV-2 antigens

simultaneously. The results were compared and correlated with three commercially available serology platforms: Abbott (N), EUROIMMUN (S), and Siemens (RBD). **Methods** SARS-CoV-2 RT-PCR positive samples (n=124) were used to assess clinical sensitivity and samples (n=100) from healthy donors collected pre-pandemic were used to evaluate clinical specificity. Analytical specificity was evaluated using samples (n=100) from individuals positive for non-COVID respiratory infections. Random PCR-positive samples (n=28) were used to compare GcN assay against a lab-developed viral neutralization test (VNT). All other assays were performed according to manufacturer's instructions. **Results** GcN assay displayed a positive correlation (R^2=0.76) and an agreement of 97.4% (95% confidence interval, 96.2%-98.5%) with the VNT. Clinical sensitivity and specificity of the GcN and MSD assays, their correlation with the commercial platforms and the sensitivity of commercial assays to predict the Nab presence, as detected by GcN and VNT assays are presented in the **Table**. **Conclusion** The combined data support the use of MSD assay for the accurate and simultaneous detection of SARS-CoV-2 antibodies. GcN assay may serve as an excellent substitute to the laborious VNT for the detection of Nabs. Both assays displayed comparable performance to the commercial serology platforms.

Table: Assay characteristics of Genscript cPass™ neutralizing antibody (GcN) assay and Meso Scale Discovery (MSD) total IgG assay.

Assays	Clinical sensitivity % (95% CI)	Clinical specificity % (95% CI)	Analytical specificity % (95% CI)	R^2 (P value) by simple linear regression	Sensitivity % (95% CI) to predict Nab presence
MSD S	100 (98.0-100)	100 (98.0-100)	99.0 (98.6-99.4)		
MSD N	94.1 (92.6-95.6)	99.0 (98.6-99.4)	100 (98.0-100)		
MSD RBD	98.0 (97.2-98.9)	99.0 (98.6-99.4)	100 (98.0-100)		
MSD NTD	98.2 (97.2-98.9)	100 (98.0-100)	99.0 (98.6-99.4)		
GcN (RBD)	96.1 (94.9-97.3)	100 (98.0-100)	100 (98.0-100)		
MSD N vs. Abbott (N)				0.73 (<0.0001)	
MSD S vs. EUROIMMUN (S)				0.83 (<0.0001)	
MSD RBD vs. Siemens (RBD)				0.92 (<0.0001)	
GcN (RBD) vs. Siemens RBD)				0.89 (<0.0001)	
Abbott (N): GcN as reference VNT as reference					94.3 (93.2-95.4) 88.5 (88.5-91.4)
EUROIMMUN (S): GcN as reference VNT as reference					93.3 (94.1-95.3) 88.9 (86.1-91.7)
Siemens (RBD): GcN as reference VNT as reference					97.4 (96.6-98.2) 96.0 (94.2-97.8)

S, Spike; N, Nucleocapsid, RBD, Receptor-binding domain; NTD, N-terminal domain; VNT, Viral neutralization test; CI, Confidence interval; P<0.0001 considered significant.

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Use of the Abbott Architect SARS-CoV-2-IgG Method in Identifying Potential Donors of COVID-19 Convalescent Plasma

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Background Coronavirus Disease 2019 (COVID-19) convalescent plasma (CCP) is human plasma collected from individuals whose plasma contains SARS-CoV-2 antibodies, and who meet donor eligibility requirements. On August 23, 2020, the United States Food and Drug Administration (FDA) issued an Emergency Use Authorization (EUA) for the use of CCP to treat hospitalized patients with COVID-19. Despite the availability of monoclonal antibody treatments and vaccines, the use of CCP remains a viable option to treat patients with COVID-19 infections. On February 4, 2021, the FDA clarified its guidance that the EUA is limited to use of only high titer CCP in hospitalized patients early in the course of disease and those hospitalized with impaired humoral immunity. [1]

Methods Our laboratory employs the Abbott Architect SARS-CoV-2-Immunoglobulin G (IgG) immunoassay (Abbott Diagnostics, Abbott Park, Illinois) which detects immunoglobulin specific for the viral nucleocapsid antigens indicating natural exposure to of COVID-19. Specified in the revised FDA guidance is a signal-to-cutoff (S/C) ≥4.5 using this method as the threshold for identifying donors for high-titer CCP donation.

Results We performed a retrospective analysis of all patients tested for SARS-CoV-2-IgG in our laboratory between June 2020 and March 2021. Of the 1022 patients determined to be positive for SARS-CoV-2-IgG based on S/C ≥1.4 per the manufacturer's instructions for use, 512 (50.1%) had documentation of positive real-time polymerase chain reactivity (rt-PCR) result for SARS-CoV-2 viral RNA. Of these serologically positive for which a diagnosis date was documented, 315 (61.5%) were found to have S/C ≥4.5 which would make for suitable CCP donors. Linear regression analysis was performed between S/C and days from diagnosis date on all patients with S/C ≥1.4. The time between diagnosis and serologic testing dates ranged from 1 to 340 days in patients with a positive serologic response. The correlation coefficient between S/C and time was determined to be 0.1228, indicating that time accounts for approximately 35% of the relationship with S/C, with a negative slope of approximately 0.013 S/C per day. Despite the relatively poor correlation between time and S/C, data were stratified in 10-day increments. In patients tested for SARS-CoV-2-IgG between 10-19 days from diagnosis date, 54% were identified as suitable CCP donors with S/C ≥4.5. For patients between 20-29 days from diagnosis, 84% were identified as suitable CCP donors. For patients between 30-39, 40-49, 50-59, 60-69, 70-79, and 80-89 days from diagnosis, the percentage of suitable CCP donors was found to be 73%, 72%, 71%, 72%, 71%, and 70%, respectively. After 90 days from diagnosis date, the percentage of suitable CCP donors with S/C ≥4.5 dropped to 44%, and after 200 days from diagnosis date dropped to 20%.

Conclusion Despite time demonstrating a relatively poor correlation with SARS-CoV-2-IgG S/C, we devised a simple scheme to identify CCP donors. Targeting those between 30-89 days since SARS-CoV-2 rt-PCR diagnosis is likely to yield a 70% success of CCP donor suitability.

Reference 1. U.S. Food and Drug Administration. 2020. Investigational COVID-19 convalescent plasma: guidance for industry (FDA-2020-D-1825).

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Performance Evaluation of the Elecsys Anti-HBe Immunoassay and a Commercially Available Comparator in Patients at Increased Risk for Hepatitis B

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Background: This was a US multicenter performance evaluation of the Elecsys® Anti-HBe immunoassay (cobas e 602 analyzer; Roche Diagnostics International Ltd) versus an FDA-approved comparator Anti-HBe immunoassay.

Methods: The Elecsys Anti-HBe was evaluated at three centers (California, Iowa, and Indianapolis) and FDA-approved comparator at a fourth site (Florida) between June 2017-April 2018 using 1800 serum samples from patients at increased risk for hepatitis B (HBV; n=1500) and vendor-purchased (n=300). Cut-off sensitivity was determined using the WHO International Standard for Anti-HBe (129095/12). To resolve discrepancies between Elecsys Anti-HBe and the FDA-approved comparator, an in-house Anti-HBe neutralization assay was developed (Roche Diagnostics GmbH). All Elecsys Anti-HBe reactive and one-third of concordant non-reactive results were tested using the Anti-HBe neutralization assay. A composite comparator method (Anti-HBe neutralization assay and FDA-approved immunoassay) was recommended following FDA review. For concordance analyses, positive (PPA) and negative percent agreement (NPA) and 95% confidence intervals (CIs), using Wilson's method, were calculated.

Results: Elecsys Anti-HBe demonstrated higher sensitivity relative to the FDA-approved comparator (cut-off sensitivity: 0.127 vs 0.194 IU/mL). Agreement between Elecsys Anti-HBe and the FDA-approved comparator was: PPA 98.0% (95.4-99.4); NPA 89.0% (87.3-90.5). The Elecsys Anti-HBe performed with higher accuracy in detecting anti-HBe reactivity (based on Anti-HBe neutralization assay results) versus the FDA-approved comparator: PPA 87.5% (84.1-90.2); NPA 98.7% (97.9-99.2) versus PPA 37.3% (33.7-41.1); NPA 99.6% (99.1-99.9). In all Elecsys Anti-HBe reactive and one-third of concordant non-reactive results, 91.7-98.4% were supported by the outcome of the neutralization assay for all samples (**Table**). Agreement between the Elecsys Anti-HBe and the composite comparator was: PPA 98.8% (97.1-99.5); NPA 98.9% (98.1-99.3).

Conclusion: Elecsys Anti-HBe is a sensitive and accurate immunoassay, supporting routine use as an aid in diagnosis of HBV infection in symptomatic patients or individuals at increased risk for HBV.

Elecsys Anti-HBe immunoassay reactive and one-third of concordant non-reactive results supported by

		Outcome of in-house Anti-HBe neutralization assay		
Elecsys AntiHBe immunoassay	**FDA-approved comparator Anti-HBe immunoassay**	**Reactive, n**	**Nonreactive, n**	**Elecsys AntiHBe immunoassay results supported by outcome of Anti-HBe neutralization assay, %**
Non-reactive	Non-reactive	20	464	95.9
Reactive	Non-reactive	155	14	91.7
Reactive	Reactive	240	4	98.4

Anti-HBe, antibody to hepatitis B e antigen; FDA, US Food and Drug Administration

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Performance of the VITROS® Immunodiagnostic Products SARS-CoV-2 Antigen Assay

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Objective: This study was designed to assess the clinical and analytical performance of the VITROS Immunodiagnostic Products SARS-CoV-2 Antigen assay (VITROS SARS-CoV-2 Antigen) on the VITROS 3600 Immunodiagnostic System and the VITROS 5600/ XT 7600 Integrated Systems.

Methods: Detection of SARS-CoV-2 nucleocapsid protein in the VITROS SARS-CoV-2 Antigen assay is achieved using monoclonal anti-SARS-CoV-2 nucleocapsid antibodies coated onto the well. Sample is added to the coated well in the first stage of the reaction, and SARS-CoV-2 nucleocapsid antigen from the sample is captured. After washing, HRP conjugated monoclonal anti-SARS-CoV-2 nucleocapsid antibodies are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent. The assay cut-off for VITROS SARS-CoV-2 Antigen is 1.00; values above the cut-off are Reactive for SARS-CoV-2 antigen and values below 1.00 are Non-reactive. All VITROS testing was performed at the Ortho Clinical Diagnostics R&D lab, located in Rochester, NY, USA. RT-PCR testing of clinical specimen was performed at an external clinical laboratory. Clinical performance was evaluated using 152 paired nasopharyngeal and nasal specimen that were collected in the United States between September and November 2020. Samples were stored frozen between the time of collection and testing and were from patients suspected of having contracted SARS-CoV-2 within seven days of symptom onset. Data were analyzed to calculate the positive percent agreement (PPA) and negative percent agreement to RT-PCR result. Analytical specificity was assessed by testing patient matrix spiked with inactivated organisms known to cause other respiratory infections. Exogenous compounds with potential to be present in upper respiratory specimen collected from patients suffering from upper respiratory infection were also tested for potential interference with the VITROS assay. **Results**: PPA for the VITROS assay in nasopharyngeal specimen was 86.2% overall and 94.8% in samples with RT-PCR cycle threshold (Ct) less than 30. PPA for nasal specimen was 83.1% overall and 92.3% in samples with RT-PCR Ct less than 30. Other respiratory organisms and potentially interfering substances were shown to not impact test results. **Conclusion**: The VITROS SARS-CoV-2 Antigen assay demonstrates excellent clinical agreement with RT-PCR and can be used as an aid in identifying individuals with active SARS-CoV-2 infection.

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Detection of SARS-CoV-2 neutralizing antibodies using a novel semi-quantitative SARS-CoV-2 IgG chemiluminescent immunoassay

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Background SARS-CoV-2 infection elicits a robust immune response primarily directed against the receptor binding domain (RBD) of the viral spike surface glycoprotein. This response can block infection through neutralizing antibodies (NAbs), which bind the virus that prevents host cell infection. SARS-CoV-2 specific NAbs can be measured by conventional virus neutralization assays using live SARS-CoV-2 virus and recently by a surrogate virus neutralization test (sVNT). In contrast to these specialized tests, almost all SARS-CoV-2 infected individuals are tested for anti-SARS-CoV-2 antibodies. If commercial SARS-CoV-2 serology assays could be used to detect NAbs, these assays could serve the dual purpose of documenting past viral exposure and indicating NAbs presence. This study assessed the correlation of anti-SARS-CoV-2 IgG measured by a novel SARS-CoV-2 chemiluminescent immunoassay (CIA) with the results of a sVNT for the detection of NAbs using samples from COVID-19 patients and healthy/diseased controls.

Methods Ninety-one (n=91) serum samples including 50 from PCR confirmed COVID-19 patients, 20 from non-PCR confirmed COVID-19 donors, and 21 individuals with infectious (n=5), autoimmune (n=7) and respiratory diseases (n=4) as well as apparently healthy volunteers (n=5), were tested by QUANTA Flash SARS-CoV-2 CIA (Inova Diagnostics, USA) and cPass™ SARS-CoV-2 NAb Detection ELISA (FDA-EUA approved, Genscript, USA). Clinical performance and correlation analysis were performed.

Results The QUANTA Flash SARS-CoV-2 CIA showed excellent clinical performance when using NAb qualitative results as binary classifier (Table1). Between the two assays, a total agreement of 92.3% (95%CI 85.0-96.2%) and *kappa*=0.82 (95%CI 0.70-0.95) were found. High correlation was observed between the %signal inhibition [ELISA] and chemiluminescent units (CU) values [CIA] as characterized by a *rho* value of 0.79 (95%CI 0.70-0.86, $p<0.0001$).

Conclusion Antibodies detected by QUANTA Flash SARS-CoV-2 IgG CIA show strong correlation with sVNT and offer the potential to estimate the content of NAbs in addition to demonstrating exposure to SARS-CoV-2.

Table 1. Performance characteristics of the QUANTA Flash (QF) SARS-CoV-2 IgG chemiluminescent assay (CIA) against the neutralizing antibody ELISA test results. Notes: LR+ = positive likelihood ratio, LR-= negative likelihood ratio, AUC=area under the curve.

	PCR-confirmed COVID-19 as target group		PCR-confirmed + non-PCR- confirmed COVID-19 as target group	
Parameter	≥30% signal inhibition ELISA cutoff	≥50% signal inhibition ELISA cutoff	≥30% signal inhibition ELISA cutoff	≥50% signal inhibition ELISA cutoff
Sensitivity, % (95% CI)	90.9 (78.8-96.4)	97.4 (86.8-99.5)	93.5 (84.6-97.5)	98.2 (90.6-1.00)
Specificity, % (95% CI)	92.6 (76.6-97.9)	87.5 (71.9-95.0)	89.7 (73.6-96.4)	82.9 (67.3-91.9)
LR+ (95% CI)	12.3 (3.9-44.3)	7.8 (3.7-19.6)	9.0 (3.5-26.2)	5.7 (3.0-12.1)
LR- (95% CI)	0.10 (0.03-0.23)	0.03 (0.01-0.15)	0.07 (0.03-0.17)	0.02 (0.004-0.12)
Odds Ratio (95% CI)	125.0 (22.5-669.7)	266.0 (33.1-1921.5)	125.7 (27.1-580.1)	265.8 (36.6-1779.8)
AUC (95% CI)	0.95 (0.89-1.00)	0.98 (0.96-1.00)	0.95 (0.91-1.00)	0.98 (0.95-1.00)

B-143

Evaluation of epidemiological, clinical and laboratory data in patients under investigation for SARS-CoV-2 infection: Big Data analysis in laboratory medicine

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Background: In December 2019, health officials in China reported presence of an unknown etiology pneumonia outbreak in Wuhan city. At January 2020, a new type of coronavirus, later called SARS-CoV-2, was identified as etiological agent of this disease, called COVID-19. It can presents a wide spectrum of clinical manifestations. Currently, available laboratory tests are divided into two groups: those that detect presence of viral structures and, by the oher hand, tests that detect the production of antibodies by immunological assays. RT-PCR assay consists in gold standard methodology. Commercialized immunoassays detect organism's response to specific virus structures. DASA has one of the largest Data and Analytics areas in Diagnostic Medicine The use of laboratory data can represent an important tool in broader knowledge of this new disease. This study aimed to evaluate epidemiological, clinical and laboratory data on patients under investigation for SARS-CoV-2 infection, using laboratory Big DATA. **Methods:** Database from abril to dezembro 2020 was evaluated. All patients who digitally scheduled their RT-PCR and serology exams on DASA network had access to a digital questionnaire about clinical and epidemiological data, to be filled in voluntarily. **Results:** Over nine months of study, 22,189 questionnaires were inserted in the study. These patients underwent tests for current or previous diagnosis of COVID-19 or evaluation of contact with the virus, totaling 22,509 analyzed tests: 6,842 PCR exams and 15,667 serological tests. About results, 85% of PCR exams and 81% of serology assays were performed in adults, between 20 and 59 years old. Although there was no difference in positivity between genders, most tests were performed on women. There were no statistical differences in the prevalence of positive tests according to age group for PCR. In serological exams, a higher prevalence of positivity in individuals over 60 years of age (23.6%) was observed. Presentation of the first symptom was quite variable among patients, but the initial presentation of myalgia, fever or anosmia and ageusia was associated with greater positivity for PCR and serology. Presence of flu-like symptoms and/or contact with infected people are associated with a higher prevalence of PCR and sorology positivity. From clinical presentation, anosmia alone is associated with a high prevalence of positivity in PCR (64%) and serology (40.4%). Of all reported symptoms, the triad cough/myalgia/

anosmia was associated with a higher prevalence of PCR positivity (69.2%). Arterial hypertension, diabetes mellitus, obesity, asthma, cardiovascular, kidney and hematological diseases, pregnancy were the comorbidities most reported. PCR and serology positive tests prevalence curves are inverted over the days since onset of symptoms, with a clear inversion around the second week. **Conclusion:** Covid-19 pandemic is a worldwide problem. We are learning about the disease while we are treating patients affected by it. The information contained in this study reflects the reality of a robust database. These data contribute to greater knowledge about the disease, enabling better diagnostic accuracy, epidemiological policies and even therapeutic conduct.

B-144

Real-life performance of the Roche/SD Biosensor SARS-CoV-2 rapid antigen test in a maximum care hospital

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Background: In hospitals, it is of particular importance to prevent the unnoticed entry of SARS-CoV-2 infections. Detection using RT-PCR is highly specific and sensitive, but time-consuming. Pending PCR results represent a challenge for optimal patient flow management. Rapid antigen tests generating results within 15-30 minutes could improve hospital admission management.

Methods: Within a 3-months period (October to December 2020), the diagnostic value of the Roche/SD Biosensor SARS-CoV-2 rapid antigen test taking on-site swabs (n=2.941) was determined in comparison to RT-PCR. Cycle thresholds (Ct) for various reactive genes were recorded and the mean Ct was used as an indicative measure of viral load.

Results: Out of 2.941 samples, 82 tested positive by RT-PCR and rapid antigen test and 2.791 negative by both methods. Discordant results were found in 68 samples (false-negative n=58; false-positive n=10). The agreement between results of both methods was good (Cohen's κ, 0.70; 95% CI, 0.63-0.76). Overall specificity and sensitivity of the rapid antigen test was 99.6% (95% CI, 99.34%-99.83%) and 58.6% (95% CI, 49.95%-66.83%), respectively, compared with RT-PCR. The mean Ct values were significantly lower for concordant RT-PCR-positive/rapid antigen test-positive samples (22.6; 95% CI, 21.79-23.48), than that in discordant RT-PCR-positive/rapid antigen test-negative samples (32.8; 95% CI, 31.59-33.93) (p-value <0.0001). The detection rate of the rapid antigen test, grouped according to Ct classes, was 89.2% (Ct <25), 62.1% (Ct 25-30), 25.0% (Ct 30-35) and 4.3% (Ct >35), respectively.

Conclusion: The Roche/SD Biosensor SARS-CoV-2 rapid antigen test showed very high specificity with a false-positive rate of only 0.4%. The lower sensitivity compared to RT-PCR correlates with the Ct value, which can serve as an indicator for the viral load. Several working groups have derived Ct "cut-off" values, which are mostly between 31 and 34, as a proxy for a limit value for the virus RNA load associated with reduced virus growth. All in all, in patient admission management rapid antigen tests can be helpful to promptly differentiate highly infectious patients from currently presumably non-infectious patients. However, at times of high incidence, negative rapid antigen test results should be considered preliminary and should either confirmed by RT-PCR or verified by a close follow-up of the rapid antigen test.

B-145

Evaluation of Three Commercial SARS-CoV-2 Serology Assays in A tertiary Care Hospital in the United Arab Emirates

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Background: Serological assays are useful for supporting the diagnosis of SARS-CoV-2 infections. Regulatory agencies have been supporting the use of various assays to manage the global pandemic of SARS-CoV2 through emergency use authorization assays. However, there is considerable uncertainty regarding the accuracy and clinical performance of these tests due to the limited verification and validation data required for authorization. We have studied three commercially available SARS-CoV2 antibody assays for their diagnostic accuracy.

Methods: We tested serum specimens from 93 patients of which 63 were SARS-CoV2 RT-PCR confirmed and 30 RT-PCR negative patients. All were admitted to our tertiary care hospital. The specimens were analysed using three assays cleared under FDA and CE emergency use authorization. The assays used were the Roche total nucleocapsid antibody assay, Abbott nucleocapsid IgG assay and Euroimmun nucleocapsid IgG assay. Sensitivity, specificity, precision and time of seroconversion were evaluated for each assay.

Results: The sensitivity of Roche, Abbott and Euroimmun assays were 38.7%, 35.5% and 20% respectively for specimens collected < 10 days and 84.4%, 84.4% and 70 respectively for specimens collected ≥ 10 days after the first positive RT-PCR. The specificity of all the three assays in this study was 100%.

Conclusion: The assays evaluated in this study have different sensitivities for detecting antibodies in SARS-CoV-2 infection. The sensitivity for detecting antibodies for all three assays was higher for specimens collected ≥10 days after first positive PCR compared with specimens collected <10 days. The apparent time of seroconversion is variable and assay dependent.

B-147

Manganese Superoxide Dismutase Ala16Val gene polymorphism (rs4880) and levels in Tuberculosis patients

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Background: Oxidative stress mediated by host cells, especially macrophages, plays a pivotal role in fighting *M. tuberculosis* infection. This is often accompanied by increased production of reactive oxygen species and free radicals which, if not quenched, causes redox imbalance damaging DNA and proteins. Antioxidants such as MnSOD (Manganese-Superoxide Dismutase) reduce this exacerbated oxidative stress response. However, in TB, there is no commensurate and proportional increase in production of antioxidants thus causing lung injury and cavitations. The MnSOD Ala16Val polymorphism alters its synthesis, transport and activity in the mitochondria and has been found to be associated with inflammatory disorders such as diabetes mellitus, obesity and cardiovascular disorders. This study aimed to evaluate serum levels of SOD2 along with Ala16Val (rs4880) polymorphism in the MnSOD gene to assess free radical injury in TB patients. **Methods:** 50 sputum positive, TB patients and 50 apparently healthy subjects were enrolled in the study.After obtaining due informed consent, 5mL venous blood was withdrawn in plain and EDTA vacutainers from all participants enrolled. Serum was separated and MnSOD levels were estimated using sandwich ELISA. Genomic DNA was extracted from whole blood and subjected to PCR-RFLP for studying the polymorphism in MnSOD gene. Statistical analysis was performed using SPSS. **Results:** The mean value of serum SOD2 was found to be nearly three-folds lower in TB patients as compared to healthy subjects (4.261 ± 3.03 vs 12.082 ± 10.58, p<0.001). The results showed that val/val genotype (mutant genotype) was higher in the patient group (44%) than the healthy subject group (26.6%). Further, majority of the healthy subjects (51.1%) were heterozygous for the MnSOD gene. In TB patients, the mean ± SD of serum SOD2 levels among different genotypes CC (homozygous wild-type), CT (heterozygous) and TT (homozygous mutant) were 4.78 ± 1.93, 3.8 ± 1.4 and 3.88 ± 1.28 respectively. The difference observed between values of serum SOD2 levels in the different genotypes were statistically non-significant (p= 0.391). **Conclusion:** The present study found that the serum levels of SOD2 were significantly lower in TB patients compared to healthy subjects. Lower antioxidant levels may be an important etiological factor in the pathogenesis of TB. Distribution of genotypes of Ala16Val polymorphism was such that in patient group, the TT (homozygous mutant) was the major genotype; whereas in healthy subjects, the major genotype was CT (heterozygous variant).

B-148

SARS-CoV-2 antibody profile of naturally infected and vaccinated individuals detected using qualitative, semi-quantitative and multiplex immunoassays

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Background: With more people vaccinated against SARS-CoV-2, the causative agent of COVID-19 disease, knowledge regarding the antibody profile of vaccinated versus naturally infected individuals is important in the laboratory evaluation of immunity and aiding diagnosis of infection and reinfection. This study examined antibodies against different antigen targets in healthcare workers (HCW) who have been fully vaccinated with mRNA vaccine, recovered from natural infection, and patients during active infection.

Methods: Samples were tested using four commercially available assays: Bioplex 2200 semi-quantitative SARS-CoV-2 IgG multiplex panel detecting and differenti-

ating antibodies against the spike protein (S) receptor-binding domain (RBD), S1, S2, and nucleocapsid protein (Bio-Rad, CA), Architect qualitative SARS-CoV-2 IgG against nucleocapsid (Abbott, IL), Atellica semi-quantitative SARS-CoV-2 IgG against RBD and Atellica qualitative SARS-CoV-2 total antibodies against RBD (Siemens, NY). The assay cutoff for positivity was 10 U/mL for Bioplex, 1.4 for Architect, and 1.0 for the Atellica assays.

Results: All 33 post-vaccination samples (collected ≥14 days after the second dose) were positive by the Bioplex anti-RBD, anti-S1, anti-S2, and the Atellica IgG and total RBD antibody assay. The total antibody assay showed an index value of >10 for all samples. After 5-fold onboard dilution, all samples fell into the reportable range of the Atellica IgG assay with a median index value of 179 and interquartile range (IQR) of 140-291. Among the different antigen targets within the Bioplex assay, the values of anti-RBD were highest (median >3200U/mL; IQR >3200 to >3200 U/mL; after 32-fold onboard dilution), followed by anti-S1 (median 2132U/mL; IQR 1649->3200 U/mL) and anti-S2 (median=42U/mL; IQR=24-76 U/mL). The recovered natural infection cohort included 52 seropositive HCW identified using the Architect nucleocapsid IgG assay and 10 PCR positive but seronegative (by the Architect assay) individuals. The seropositivity rate was 90% (median index >10; IQR 3.6->10) by Atellica total antibody, 73% by Atellica IgG (median index 3.3; IQR 0.9-9.4), 84% by Bioplex anti-RBD (median 62; IQR 12-269 U/mL), 77% by Bioplex anti-S1 (median 45; IQR 10-83 U/mL), 37% by Bioplex anti-S2 (median 7; IQR 1-12 U/mL), and 79% by Bioplex anti-nucleocapsid (median 69; IQR 20-185U/mL) respectively. The ratio of anti-S1 to anti-S2 was significantly lower in the infection recovery group (11.2±1.9) than in the vaccinated group (61.5± 7.1). The active infection cohort included 52 samples collected at various time points from 34 symptomatic patients. The sensitivity was 73%, 71%, and 64% for the Architect IgG, Atellica total, and IgG RBD assay respectively. The composite sensitivity of the Bioplex assay was 69% in this acute infection cohort with separate sensitivity of 60% and 58% targeting on RBD and nucleocapsid respectively.

Conclusion: Fully vaccinated individuals showed an average of 50-fold higher antibody levels than naturally infected unvaccinated individuals with immune reactivity strongly towards RBD/S1 and a very weak response to S2. RBD, S1, and nucleocapsid are good antigen targets for serology assays. Variation of assay sensitivity in detecting past versus active infection should be considered when selecting assays for surveillance or different clinical purposes. Standardization is need for semi-quantitative assays.

B-149

Performance of Immunoassays of Anti-SARS-CoV-2 in COVID-19 Infection and Vaccination

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Background: Several immunoassays of anti SARS-CoV-2 IgG antibodies that are FDA-approved under EUA are available for assessment of antibody response against SARS-CoV-2 to determine whether infected individuals are developing immunity against COVID-19. In this study, we compared the performance of three immunoassays of the IgG antibodies.Methods: The qualitative Abbott Architect assay detects antibodies against N-protein, while Beckman Dxl (qualitative and semi-quantitative) and Euroimmune assays detect antibodies against S-protein. Serum samples were obtained from individuals with or without COVID-19 infection (n=66). In addition, 15 serum samples are from previously COVID-19 infected and also vaccinated individuals (partially or fully with Pfizer vaccine).Results: In COVID-19 unvaccinated study, the concordance result between Abbott and Beckman was 91%. For the discrepant results, six samples were positive in Abbott assay but all were negative in Beckman assay. The concordance between Beckman and Euroimmun was 89% (Table 1). In COVID-19 vaccinated study, of the 15 previously COVID-19 infected patient specimens, 5 were positive and 10 were negative with the Abbott N-protein-based assay; however, all samples have high antibody levels when tested by Beckman or Euroimmun S-protein-based assay. The 10 negative results on the Abbott assay could be due to short half-life of infection-induced antibodies, particularly those individuals with prior infection 8-9 months ago. The average antibody level of the partial vaccination cases (n=2) is lower than the full vaccination (n=13) (Table 1). Conclusion: The N-protein IgG antibody assays are useful serological tests that can be utilized in diagnosis of COVID-19 infection, together with molecular testing. Further, our data suggest that the Abbott N-protein assay is more sensitive than the Beckman and Euroimmun S-protein assays, especially in early infection. On the other hand, the S-protein IgG antibody assays are useful diagnostic tests for monitoring and assessment of immunity following infection and/or vaccination.

Table 1. Testing Performance of Anti-SARS-CoV-2 IgG Antibody Immunoassays

		Abbott	Beckman*	Euroimmun
Unvaccinated Study	Number (66)	IgG Ab (+/-)	IgG Ab (+/-)	IgG Ab (+/-)
	33	+	+	+
	22	-	-	-
	4	+	-	-
	2	+	-	+
	2	+	+	-
	3	-	-	+
Vaccinated Study	Number (15)	IgG Ab + -	IgG Ab (AU/mL, Mean ± SD)	IgG Ab (Titer)
Partial Vaccination	2	1 1	147	>1:10,000
Full Vaccination	13	4 9	241 ± 150	>1:10,000

*Beckman qualitative and semi-quantitative methods are used for COVID-19 unvaccinated and vaccinated study, respectively.
-The positive cutoff values of Abbott, Beckman, and Euroimmun assays are index of 1.4, S/Co of 1.0, and absorbance ratio of 1.1 at the 1:100 dilution, respectively.

B-150

Diagnostic performance of seventeen rapid IgG/IgM antibody tests in COVID-19 patients.

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Background: Rapid tests (RTs), also termed point-of-care tests (POCTs), are excellent because they provide results in up to 30 minutes, in several situations and locations (they can be used in non-laboratory environments by capable personnel) and because they allow to broaden access to diagnosis in underserved communities. In the face of the health crisis caused by the COVID-19 pandemic, there are several efforts around the world in an attempt to reduce the spread of the virus, as well as for the accurate identification of infected patients. In this process, the rapid and accurate diagnosis of COVID-19 contributes to disease and outbreak management by enabling prompt and accurate public health surveillance, prevention and control measures. **Objective**:The aim of this work was to compare the analytical performance of a RT for diagnosis of evaluate seventeen COVID-19 infection RTs available in the Brazilian market regarding their analytical performance.

Methods: This was an observational, analytical, and concordance study, in which previously defined positive and negative samples, based on their RT-PCR (gold standard) results. We also evaluated the agreement between 17 kits (from manufactures: Abbott, Acro Biotech, Artron, Bioclin, Biocon, Eco Diagnóstica, eDiagnosis, Innovita, JD Biotech, Labtest, Leccurate, MedTest, Oltramed, Qingdao Hightop Biotech, Siemens, Vyttra and Wama) using Pearson's correlation and the quality of the reading pattern of the tests. Analytical performance and Pearson's correlation were calculated, considering a 95% CI and $p<0.05$.

Results: This study identified differences in the performances of the 17 tested kits. Seven out of 17 RTs showed good performance and can be used in the routine laboratory and health care units as screening tests. Among the sensitivity range for IgM antibody test, there was a variation from 5% to 100%, being that, among the seven kits that obtained good performance, this variation was of 62.8% to 90%. The sensitivity range for IgG antibody tests was a variation from 20% to 95%, which the seven better tests ranged from 58.6% to 95%. Regarding the specificity range for IgM antibody, there was a variation from 45.2% to 100%, being that, among the seven kits that obtained good performance, this variantion was 85.3% to 100%. The specificity range for IgG antibody tests was a variation from 89.8% to 100%. **Discussion:** RTs should be considered as screening tests, and it is more important that they have better specificity than sensitivity. The seven kits has better performed was Bioclin, Eco Diagnóstica, Innovita, Labtest, MedTest, Qingdao Hightop Biotech and Siemens. Considering that, the isolated IgM antibody test for COVID-19 is not clinically significant and the performance assessment of the isolated IgG antibody test, the Abbott kit was considered suitable for routine laboratory use.

Conclusion: In times of pandemic, in which a quick diagnosis makes all the difference in the evolution and treatment of the disease, the collaboration of RTs in the screening of patients is of great importance. However, one must take into account their accuracy, since there are several options, but not all have the same quality.

B-151

Analytical and clinical evaluation of three SARS-CoV-2 antibody assays in aiding diagnosis of COVD-19

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Background: Anti-SARS-CoV-2 antibody assays are useful in aiding the diagnosis of COVID-19 infection for patients who presented late in the disease course with a negative PCR result. Serology assays can also support the diagnosis of patients suspected of COVID complications, such as multisystem inflammatory syndrome. This study evaluated the analytical and clinical performance of one IgM/IgG lateral flow and two automated chemiluminescence immunoassays with different antigen targets. **Method:** A total of 103 blood samples collected from 48 PCR positive patients at various time points after onset of symptoms were used for assessing clinical sensitivity. Clinical specificity was evaluated using serum specimens collected before January 1, 2019. The assays we evaluated were: Healgen IgG/IgM rapid test against spike protein (Healgen Scientific LLC, TX), Architect SARS-CoV-2 IgG against nucleocapsid (Abbott, IL), and Atellica SARS-CoV-2 total antibodies against spike protein receptor-binding domain (RBD) (Siemens, NY). The cutoff for positivity was 1.4 and 1.0 for the Architect and Atellica assay respectively. **Results:** Assay characteristics are summarized in the Table. The rapid test showed lower specificity (95.6%) than the Atellica and Architect assays (both 100%), with sensitivity comparable to these automated assays except for samples collected within 7 days of symptom onset. One of the limitations of the rapid test is the subjective interpretation, especially of faint bands. By interpreting weak bands as indeterminate, specificity was improved to 100%. The agreement between the Atellica RBD and Architect nucleocapsid assay was 85%. When using both assays, sensitivity was increased to 65.9% and 75% for the samples collected 3-7 days and 8-11 days post-symptom onset respectively. **Conclusion:** All three assays are acceptable for clinical use in aiding the diagnosis of SARS-CoV-2 infection. Using antibody tests with different antigen targets may help resolve difficult cases. Adding an indeterminate interpretation category for the Healgen rapid test improved specificity.

Performance characteristics of three SARS-CoV-2 antibody assays

Precision	Architect IgG		Atellica total	
Samples	Intra-assay	Inter-assay	Intra-assay CV	Inter-assay CV
Negative control	1.9%	6.3%	SD=0.03	SD=0.02
Positive control	2.4%	3.1%	4.3%	3.9%

	Architect IgG	Atellica total	Healgen Rapid IgM / IgG	
Specificity	100% 33/33	100% 63/63	100% 45/45	95.6% 43/45
Sensitivity	n=103	n=103	n=44 M	G M+G
3-7 days post-symptom onset	48.8%	61.1%	21.4%	14.3% 21.4%
8-11 days	69.4%	69.4%	62.5%	75.0% 81.3%
12-14 days	92.3%	76.9%	92.9%	100% 100%
>14 days	100%	100%		

B-152

Gut Microbiome Evaluation in Congenital Generalized Lipodistrophy: Inferences About Causality Between Dysbiosis and Metabolic Diseases

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BACKGROUND: Studies have suggested the presence of intestinal dysbiosis is associated with metabolic diseases. However, the causal relationships between them are not fully elucidated. Microbiome evaluation of patients with congenital generalized lipodystrophy (CGL), a disease characterized by the absence of subcutaneous adipose tissue, insulin resistance, and diabetes since the first years of life, could provide insights into these relationships. METHODS: A cross-sectional study was carried out from October 2019 to March 2020. Patients with CGL, aged between 1 and 40 years (n=18), and healthy individuals, matched for age and sex (n=18), were included. The gut microbiome study was performed by sequencing the 16S rRNA gene through Next Generation Sequencing (Neoprospecta Microbiome Technologies, Brazil). RESULTS: The median age was 17.5 years (8.2; 30.5) and 66.7% (12) were female. There was no difference in pubertal stage, BMI, ethnicity, procedence (rural or urban area), delivery and birth conditions, previous breastfeeding, caloric intake, and macronutrient and fiber consumption. Lipodystrophic patients presented a lower alpha diversity - Richness index (53.5 versus 68.8; p = 0.002). The gut microbiome diversity in the groups is shown in figure 1. No differences were observed in the diversity parameters when analyzing the presence of diabetes, its complications or the CGL subtype. CONCLUSION: In this study, we demonstrated a reduction of gut microbiome diversity in individuals with insulin resistance and diabetes genetically determined. Dysbiosis was present despite dietary treatment and was observed in young patients with metabolic disease. Our findings allow us speculate that, in these patients, loss of intestinal microbiome diversity may be due to metabolic disorders presents since the first years of life, reinforcing the need for longitudinal studies to confirm the causal link between dysbiosis and diabetes in humans.

Figure 1 - Heat map of gut microbiome diversity in patients with congenital generalized lipodystrophy and healthy individuals.

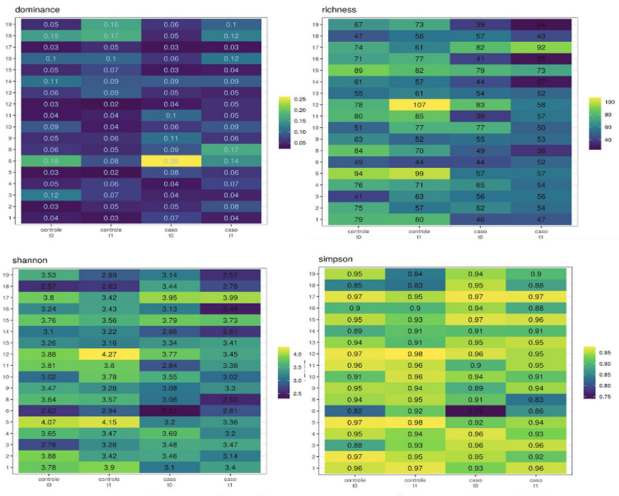

Figure 1 – Heat map of gut microbiome diversity in patients with congenital generalized lipodystrophy and healthy individuals.

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SARS-CoV2 Antibody Response in COVID-19 Positive Patients enrolled in the Canadian GENCOV Study

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Background: Testing for SARS-CoV-2 antibodies can identify patients who have had COVID-19 or received a vaccination. Serology assays target either the spike protein (sometimes targeting the receptor binding domain) or the nucleocapsid protein to detect total or isotype specific (IgG, IgM or IgA) SARS-CoV-2 antibodies. Patients show variable immune response to COVID-19 and the relationship between SARS-CoV-2 serological response and immunity is still under investigation.

Objectives: This study aims to assess serological response to COVID-19 in patients with mild and more severe clinical symptoms by assessing antibody isotype, titer and neutralization ability. To understand why patients have variable response to COVID-19, serological findings will be correlated with genetic variation, viral variation, biochemical/hematological results, T- and B-cell receptor dynamics, patient characteristics and clinical outcomes.

Methods: Recruitment will include up to 1500 patients with a positive COVID-19 nasopharyngeal, nasal or oral swab from six hospitals in Ontario, Canada. Blood samples will be collected at baseline (inpatient only), 1 month, 6 months, and 12 months after diagnosis or vaccination. Vaccinated cohort will be used to compare serological response. SARS-CoV-2 antibody analysis will be performed using two Roche immunoassays: 1) the Elecsys® Anti-SARS-CoV-2 qualitative assay targeting the nucleocapsid protein and 2) the Elecsys® Anti-SARS-CoV-2-S quantitative assay targeting the spike protein. Additional antibody characterization will be performed on research-developed ELISA to assess antibody isotype and neutralization ability. As the data is not normally distributed, the non-parametric test of Mood's Median is used to compare median antibody titer values across different age groups as well as sex.

Results: To date, 699 patients diagnosed with COVID-19 have been recruited and antibody analysis has been performed on a total of 434 inpatients and outpatients at 1 month post-infection. The median antibody titer was 73 U/mL (values range from 0.4 to 2500) and 11% of patients had titers greater than 500 U/mL. A significant difference was observed in the median antibody titers between young (19-35 years) and middle (36-55) age groups when compared to older age groups with median titers of 44, 71 and 200 U/mL, respectively. There was no significant difference observed in the median antibody titers between females (73 U/mL) and males (97 U/mL). Over 90% of patients had antibodies to both spike and nucleocapsid proteins, while 4% had only nucleocapsid antibodies or only spike antibodies, and 2% did not generate any SARS-CoV-2 antibodies.

Conclusion: Presence of SARS-CoV-2 antibodies does not necessarily indicate immunity, and additional analysis of antibody isotype, neutralizing antibodies and molecular profiling of T-and B-cell receptor dynamics will be performed to provide a more comprehensive examination of immune response. Assessment at later timepoints (6 and 12 months) will determine length of immunity. Preliminary data suggests antibody titer does not significantly change with sex but does increase with age. This data will help health care systems and public health to determine the clinical utility of serological testing for SARS-CoV-2 antibodies, aid in the development and implementation of appropriate serological assays, and prioritize populations for vaccinations if there is limited supply.

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Comparison of infection profile by COVID-19 between age groups in differents periods of Brazil's pandemic.

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Background: COVID-19 emerged in Wuhan, China with fast spread Worldwide. In Brazil, the first case of COVID-19 was confirmed in São Paulo, on 26 February 2020. Since then, Brazil became one of the centers of the COVID-19 pandemic, appearing in the second position in case number and deaths, behind only the USA. Nowadays, Brazil is traversing the worst moment of the COVID-19 pandemic, reinforcing the importance of comprehensive studies to understand the dynamics of dissemination of the disease. In this way, this study aimed to perform a descriptive analysis of COVID-19 in Brazil, evaluating age groups of the infected people.

Methods: We evaluated differences in the rates of COVID-19 positive tests between age groups, using data of RT-PCR for SARS-CoV-2 detection (database of Hermes Pardini Laboratory), between March to June 2020 (N=194,140) and December 2020 to March 2021 (N=1,040,812). The patients were grouped by age intervals (0 -10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90 and 91-100 years-old). The proportion of positive tests for each group were calculated as follows: (total of positive tests)/(total of tests) x 100%.

Results: At the beginning of the pandemic, people who were 21 to 50 years-old represented more than 66% of the tests performed, with an average positivity test of 32.2%. Already from December 2020 to March 2021, people who were 21 to 50 years-old also represented more than 66% of the tests performed, however, the average positivity test was 20,9%. There has been an increase in the rate of infection among group under 20 years-old (28%) and elderly aged 80 years or older (37%) in the period of December 2020 to March 2021, compared to the beginning of the pandemic (20% and 25% for group under 20 years-old and 80 years or older, respectively).

Conclusion: The increase of positive tests among young people and elderly may be associated with the circulation of new variants of SARS-CoV-2 in Brazil at the end of 2020, that have been described as more infectious. Besides that, these observations may indicate that people who have less mobility - since children and adolescents have been without presential access to school since the start of the pandemic, and the elderly are also being told to stay at home measures of social distance - may be being infected at home. Thus, these data reinforce the importance of genomic surveillance as a strategy to identify changes in the profile of the pandemic. On the social plane, the continuing need to guide the population on the correct use of NPIs (non-pharmaceutical interventions) such as wearing masks, hygiene and social distance, even at home, is a keystone for minimizing contagion among relatives.

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Evaluation of the SARS-CoV-2 Roche and Snibe spike antibody assays compared to prior nucleocapsid and spike antibody assays

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Introduction: We evaluated the Roche Elecsys Anti-SARS-CoV-2 S assay and the Snibe SARS-CoV-2 S-RBD IgG chemiluminescent immunoassays, comparing them to the prior Roche Elecsys/Abbott IgG nucleocapsid assays and the Abbott IgM spike assay.

Methods: We enrolled 184 SARS-CoV-2 RT-PCR positive samples and 215 controls (172 pre-pandemic samples and 43 current samples for cross-reactivity) to evaluate the Roche spike assay. For the Snibe evaluation, we included 119 RT-PCR positive samples and 249 controls (200 pre-pandemic samples and 49 current samples for cross-reactivity). 98 cases had been tested on all three spike assay platforms (Roche total antibody, Snibe IgG and Abbott IgM).

Results: The Roche spike assay had a CV of 0.5%/2.3% (at 0.82/8.72) and was linear from 1.16-240U/mL. The Snibe assay was linear from 6.43-77.7AU/mL and had a CV of 5.5%/8.8% (at 0.43/0.18AU/mL). The Roche spike assay was 100% specific (95% CI 98.3 to 100), but the Snibe spike assay had a specificity of 92.0% (95% CI 87.9 to 95.0) with cross-reactivity with 7 other antibody positive test samples (HBeAg 2/ Dengue 4/ANA 1). The Snibe spike assay was significantly more sensitive than the Abbott IgG assay at 0-6 days POS (difference 29.6%, 95% CI 17.5 to 41.8, $p<0.0001$), and was also slightly more sensitive than the Roche total antibody/Abbott IgM anti-SARS-CoV-2-S assays. We generated lower optimized LORs for all assays (average of ROC associated criterion + 99th percentile of negative controls) which significantly improved the sensitivity of the Roche spike assay (difference 8.7%, 95%CI 3.3-14.1, $p=0.004$), both nucleocapsid assays (Roche difference 20.2%, 95%CI 12.5-27.9, $p<0.0001$, Abbott difference 14.8%, 95%CI 5.3-24.3, $p=0.008$), and the Abbott IgM assay (difference 15.6%, 95%CI 5.0-26.2, $p=0.02$) in early disease (see Table 1).

Conclusion: Both spike assays show higher sensitivity than nucleocapsid assays in early disease, although optimized LORs provided the most significant improvements to assay sensitivity.

TABLE 1: Sensitivity of SARS-CoV-2-Ab assays by days post-first positive SARS-CoV-2 RT-PCR - recommended limits of reactivity versus optimized limits of reactivity

Days POS	Roche Nucleocapsid		Roche Spike		Abbott IgG (nucleocapsid)		Snibe IgG (spike)		Abbott IgM (spike)	
	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)	+ve/-ve	Sensitivity (95% CI)
Recommended limits of reactivity										
0 – 6	45/59	43.3 (33.6-53.3)	50/54	48.1 (38.2- 58.1)	2/52	3.6 (0.4 -12.3)	19/35	35.2 (22.7-49.4)	7/38	15.6 (6.5-29.5)
7 – 13	29/10	74.4 (57.9-87.0)	31/8	79.5 (63.5 -90.7)	23/8	74.2 (55.4-88.1)	25/6	80.6 (62.5-92.5)	17/7	70.8 (48.9-87.4)
≥ 14	39/2	95.1 (83.5-99.4)	39/2	95.1 (83.5-99.4)	33/1	97.1 (84.7-99.9)	34/0	100 (89.7-100)	23/6	79.3 (60.3-92.0)
Optimized limits of reactivity										
0 – 6	66/38	63.5 (53.4-72.7)	59/45	56.7 (46.7-66.4)	10/44	18.5 (9.3-31.4)	22/32	40.7 (27.6-55.0)	14/31	31.1 (18.2-46.6)
7 – 13	36/3	92.3 (79.1-98.4)	32/7	82.1 (66.5-92.5)	23/8	74.2 (55.4-88.1)	25/6	80.6 (62.5-92.5)	22/2	91.7 (73.0-99.0)
≥ 14	40/1	97.6 (87.1-99.9)	39/2	95.1 (83.5-99.4)	34/0	100 (89.7-100)	34/0	100 (89.7-100)	29/0	100 (88.1-100)

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Spike IgG (Abbott) and neutralizing antibody (Snibe) responses after complete Pfizer-BioNTech SARS-CoV-2 mRNA vaccine inoculation

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Introduction: There is a paucity of data on the spike (S-ab) and neutralizing antibody (N-ab) response to the Pfizer-BioNTech SARS-CoV-2 mRNA vaccine.

Methods: We assessed 36 subjects' SARS-CoV-2 nucleocapsid (Abbott), IgG S-ab (Abbott) and N-ab (Snibe) response pre-vaccination, 10 days after first, 20 and 40 days after full vaccination. The Abbott assay has a stated range of 21-40000AU/mL (≥50AU/mL positive), the Snibe immunochemiluminescent assay has a claimed range of 0.05-30ug/mL (≥0.3ug/mL reactive).

Results: Subjects were seronegative on all assays pre-vaccination. Expectedly, nucleocapsid antibodies were unaffected by vaccination. 10 days after dose 1, 20 subjects were S-ab reactive (55.5-802.9AU/mL) and 16 negative (6.3-49.1AU/mL); 2 subjects had N-ab (1.31 and 0.32ug/mL) with 34 negatives (0.0-0.28ug/mL). All cases were S-ab/N-ab positive 20 days post-vaccination (Abbott 5581-54673U/mL, Snibe 1.19-15.7ug/mL). S-ab/N-ab levels declined by day 40 to a median of 10892U/mL/2.69ug/mL; median day 20-40 decrease for S-ab 7888AU/mL (95%CI 6150-10502, p<0.0001) and for N-ab 1.01ug/mL (95%CI 0.64-1.61, p<0.0001) (see figure 1). S-ab/N-ab correlated well after complete vaccination (r=0.74). 4 samples (5.6%) exceeded the upper limit of the Abbott assay requiring dilution. No significant difference was found in the magnitude of rise of S-ab and N-ab post-vaccination from baseline between subjects ≥50 (n=11) and <50 years old (n=25).

Conclusion: More subjects were S-ab positive than N-ab positive 10 days after first vaccination. The measuring ranges of both assays are suitable to assess post-vaccination response. The Abbott S-ab correlates well with N-ab. Timing of sample collection is crucial in seronegative subjects after their first vaccination. Nucleocapsid antibodies do not rise in response to the vaccine and may be useful to assess vaccinated patients for infection. Both S-ab/N-ab decline by day 40 post-vaccination, reflecting the conversion of active T-cells to memory T-cells. There was no difference in the magnitude of increase of antibodies post-vaccination between age groups.

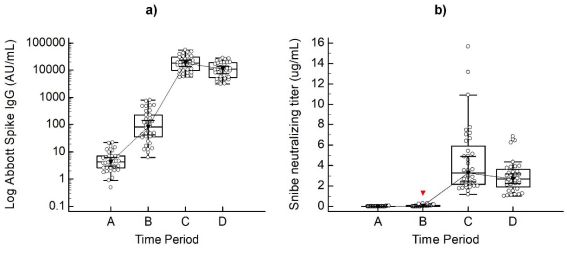

Figure 1: Progression of a) Abbott spike IgG and b) Snibe neutralizing antibodies at A: pre-vaccination, B: 10 days after the first vaccination, C: 20 days after the second vaccination and D: 40 days after the second vaccination.

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Comparing the spike (Roche) and neutralizing antibody (Snibe) responses after a completed course of Pfizer-BioNTech SARS-CoV-2 mRNA vaccine

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Introduction: There is a paucity of data on antibody response (spike S-Ab and neutralizing N-Ab) to the Pfizer-BioNTech SARS-CoV-2 vaccine.

Methods: We assessed SARS-CoV-2 nucleocapsid (Roche), spike (Roche) and neutralizing (Snibe) antibody response in 36 subjects pre-vaccination, 10 days after first, 20 and 40 days after full vaccination. The Roche assay has a stated range of 0.4-250U/mL (≥0.80U/mL positive), the Snibe immunochemiluminescent assay has a claimed range of 0.05-30ug/mL (≥0.3ug/mL reactive).

Results: Subjects were seronegative on all assays pre-vaccination. Expectedly, nucleocapsid antibodies remained undetectable after vaccination. Ten days after dose 1, twenty-three subjects were S-Ab reactive (0.82-178U/mL), 4 low (0.47-0.68U/mL) and 9 undetectable (<0.4U/mL); only 2 subjects had increased N-Ab (1.31 and 0.32ug/mL). All cases had elevated S-Ab and N-Ab 20 days post-vaccination (Roche 511-5853U/mL, Snibe 1.19-15.7ug/mL). S-Ab/N-Ab levels declined by day 40 to a median of 1520U/mL / 2.69ug/mL; the median day 20-40 decrease was: S-Ab 679U/mL (95%CI 456-951, p<0.0001) and N-Ab 1.01ug/mL (95%CI 0.64-1.61, p<0.0001) (see figure 1). There was good S-Ab/N-Ab correlation (r=0.77) after vaccination. All S-Ab samples exceeded the upper limit of the Roche assay post-vaccination. No significant difference was found in the magnitude of the S-Ab/N-Ab rise from baseline to day 20 between younger (50y, n=25) and older subjects (≥50, n=11).

Conclusion: More subjects were S-Ab positive than N-Ab 10 days after the first dose. The Roche assay requires a larger measuring range to assess post-vaccination titers. S-Ab correlate well with N-Ab. Timing of sample collection is crucial in initially seronegative subjects post first vaccination. Nucleocapsid antibodies are unaffected by vaccination and may be useful to assess vaccinated patients for re-infection. Both S-Ab and N-Ab decline by day 40 post-vaccination, reflecting the conversion of active T cells to memory T cells. There was no difference in the magnitude of increase of antibodies post-vaccination with age.

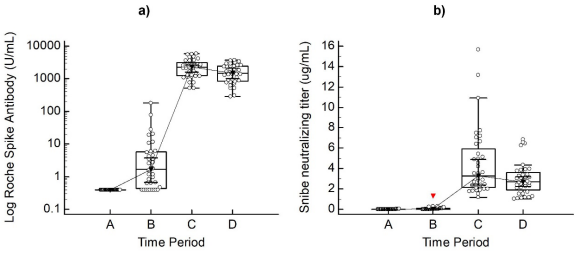

Figure 1: Progression of a) Roche spike antibodies and b) Snibe neutralizing antibodies at A: pre-vaccination, B: 10 days after the first vaccination, C: 20 days after the second vaccination and D: 40 days after the second vaccination.

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Consolidation of an Interferon Gamma Releasing Mycobacterium tuberculosis Assay to a High Throughput Random Analyzer

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Background: Interferon Gamma Releasing Assays (IGRA) are very useful as an aid in diagnosis of *Mycobacterium tuberculosis* (TB) in patients. The test is based on the release of gamma interferon from white blood cells *in vitro* when the cells are exposed to TB specific antigens post TB exposure or infection. QuantiFERON™ Gold has been approved for use in the investigations of TB and latent TB for many years. The test limitations for all IGRA assays involve proper specimen handling and testing within a short period of time to ensure white blood cell integrity. The original assay consists of the stimulation of white blood cells with TB antigen synthetic peptides ESAT-6, CFP-10 and TB7.7 and subsequent measurement of gamma interferon using ELISA. The United Arab Emirates (UAE) has a very large resident visa (expat) workforce. Many of the expats working in the UAE are from countries where TB is endemic. Testing and treatment of this population requires high volume and high throughput testing. The QuantiFERON Gold assay is a plate-based assay which has

some degree of manual handling. Traditionally, the test has also been performed in the microbiology departments of clinical laboratories. As staffing demands increase and the drive to reduce instrumentation and operational costs continues, applying this assay to an automated platform that handles many other assays is desirable. We have assessed the use of the Diasorin Liaison™ XL IGRA assay using QuantiFERON Gold. The use of the Diasorin system can reduce handling, consolidate tests to a common platform and reduce the dependency of microbiology technologists thereby improving laboratory efficiency. Method: Twenty nine specimens were tested using the original ELISA assay for QuantiFERON Gold and the Diasorin Liaison XL method. Results: The percent agreement was 94.4% with one indeterminant result (ELISA) reporting as negative on the Liaison XL system which is acceptable performance. As well the concordance between two Diasorin Liaison XL systems was 100 percent. Conclusions: The use of the Diasorin Liaison XL for QuantiFERON Gold testing can potentially reduce the number of testing platforms and therefore reduce maintenance and instrument costs. Laboratories that have the flexibility to support workflow and testing across disciplines can have increased capacity to expand menu offerings and potentially permit efficiencies in testing.

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GDH immunoassay as a screening test for *Clostridium difficile* diagnosis: a retrospective evaluation from a clinical laboratory in South Brazil.

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Background:*Clostridium difficile* infection (CDI) remains a major public health problem, with challenging aspects in laboratory diagnosis, patient safety and health costs. It occurs when the spores of a toxin-producing strain come in contact with someone who presents some alteration of the normal colonic microbiota, and causes clinical presentations ranging from asymptomatic colonization to severe diarrhea and colitis. Many countries are facing increased frequency and severity of CDI, and this has resulted in reevaluation of diagnostic test methods and development of new assays. The classical diagnosis method adopted by most laboratories, for a long time, was the enzyme immunoassays (EIAs), but despite its convenience, it lacked analytical sensitivity. The cell culture cytotoxicity neutralization assay (CCCNA) has been considered de gold standard to the laboratory diagnosis but is not feasible in routine due to the need of expertise in cell cultures and long turn-around time, besides being susceptible to preanalytical errors. Toxin immunoassays use antibodies to detect *C. difficile* toxins - there are different assays commercially available, including rapid immunochromatographic assays, with their sensitivities varying widely. Glutamate dehydrogenase (GDH) is a metabolic enzyme produced in all isolates of *C. difficile*, including both toxigenic and nontoxigenic strains, and represents a screening test for CDI. It is a low-cost test that allows a rapid turnaround time for negative results. The objective of this study was to evaluate the adequacy of GDH alone as a screening tool to rule out CDI, compared to the previously adopted strategy that was screening simultaneously for both GDH and toxin assays.

Methods:

We retrospectively evaluated all the *C. difficile* results performed in 2016, 2017 and 2018. All the samples were assayed by the TECHLAB® C. DIFF QUIK CHEK COMPLETE® immunochromatographic assay to detect simultaneously GDH, A toxin (tcdA) and B toxin (tcdB). The sensitivity and specificity given by the manufacturer was 90,5% and 93,1%, respectively. We searched for patients who presented GDH negative with tcdA/B positive.**Results:**We retrieved 3,518 results from this time course. Of this, 1,751 (49.77%) were from inpatients. GDH was negative in 2,968 (84.4%). Of these, all except 1 (0.03%) had the tcdA/B negative. Patient showing positive results for both GDH and toxin corresponded to 187 (5.31%).**Conclusion:**We found that, compared to toxin immunoassays, GDH testing is adequate as a screening test to CDI, validating the use of a sequential algorithm of GDH followed by tcdA/B assays, allowing appropriate turnaround time and costs.

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Evaluation of Two SARS-CoV-2 Antigen Assays as High Throughput Complimentary Assays to RT-PCR

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Background: Real time RT-PCR screening for SARS-CoV-2 is the recommended methodology for diagnosis of SARS CoV-2 virus. Early and rapid diagnosis of potentially contagious individuals permits disease containment through quarantine and tracking and tracing of contacts. Alternate methods that can potentially reduce costs, simplify analysis and improve turn around time and throughput in both symptomatic and asymptomatic individuals for mass screening purposed are desirable. Sensitive immunological assays to detect the presence of SARS-CoV2 antigen fit the criteria for improving mass screening. We have compared two antigen immunoassays directed against the nucleocapsid (N) protein to RT-PCR. The antigen assays use an aliquot of the viral transport medium from nasopharyngeal swabs transport medium to determine the relative sensitivity and create a proposed workflow. Method: Seventy RT-PCR (Ord1b and N gene) positive specimens (DiaplexQ, SolGent) with varying cycle time (ct) values were tested using both the Diasorin quantitative and Roche qualitative SARS-CoV-2 antigen assays according to manufacturers' instructions. Nasopharyngeal swab transport media was used as the specimen type. Results: The sensitivity of the Diasorin and Roche antigen assays were 61% and 66% respectively. The total agreement between Roche and Diasorin was 23 out of 70 specimens Diasorin did not detect 11 specimens which were detected by Roche and conversely 2 specimens were not detected by Roche which were detected by Diasorin. The highest ct detected by Roche was 36 and for Diasorin was 32. Conclusions: Antigen assays while being less sensitive than RT-PCR do provide a result in a shorter period with lower technical requirements. The Roche assay in this study demonstrated higher sensitivity to detection when compared to Diasorin especially at higher ct values. Mass screening is possible as the Diasorin assay has been approved by the FDA for both symptomatic and asymptomatic individuals. Antigen tests can potentially be a first line test with a reflex to PCR when the antigen test is negative. Alternatively, the assays may prove useful when time is critical, and the population studied is of low risk. For example, Airlines may find the antigen assay useful at check in prior to flying when passengers are RT-PCR negative within a 72- or 96-hour test window. This would additionally reduce the risk of boarding a SARS-CoV-2 contagious passenger.

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The landscape of the panorama of the coronavirus pandemic in Brazil: a look from the perspective of serological test

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Background: The WHO declared the COVID-19 outbreak as a world pandemic on March 2020. Scientists are still learning about the natural history of COVID-19; however, preliminary serological reports suggest that the SARS-CoV-2 antibody response follows the typical pattern, with a majority of patients seroconverting within 2-week following symptom onset. Antibody responses arise about a week following infection, IgA is associated with mucosal immunity and is considered to be an important player in respiratory infections, with the IgM, as the firsts antibodies arising, often concurrent with active infections. IgG antibodies appear towards the end of the active infection and can persist for months to years following infection. The development of an antibody response to infection can be host-dependent and take time. ELISA serological test technologies are characterized by high-throughput and low complexity and have helped us to use serological testing more accurately during both antibody development and monitoring the different phases of the disease. **Objective**: This study aims to evaluate the epidemiologic characteristics of the Brazilian population infected with SARS-CoV-2 based on ELISA serological tests. **Methods:** This is a retrospective and cross-sectional study conducted through our institute's results database from March 2020 to August 2020. The data was obtained using the Instituto Hermes Pardini (IHP) database, and descriptive statistics were performed. The kits Anti-SARS-CoV-2-IgA ELISA and Anti-SARS-CoV-2-IgG ELISA (EUROIMMUN, Germany) were used to detect IgA and IgG antibodies against COVID-19. Specimens with a value of ≥1.1 were considered positive for IgA or IgG COVID-19 antibodies. A value of ≥0.8 and < 1.1 was considered as an equivocal result (borderline) and a value <0.8 was determined to be negative. All samples with borderline results were tested twice. **Results:** From 271,643 samples analyzed, 142,379 (50.4%) were female and 129,082(45.2%) male. Among the total of patients, 66,605(25.6%) were positive (reactive result for one or both immunoglobulins), 185,354(71.3%) patients were negative (for both) and

8,016(3.08%) patients were undetermined (for both). Among the age group that had the highest number of patients was 30-39 years old, even though the average age was 41.39 years (41.33-41.45 years CI 95%). The North region presented the highest number of positive cases (34.6%), followed by the Northeast region (29.4%) and Mid-west region (27.7%). This study shows that have no statistically significant difference between gender (p-value = 0.01, CI = 95%) in infection by COVID-19, however, there is a statistically significant difference between the age groups(p-value = 0.71, CI = 95%) with the most positive group being the age group more than 70 years old (39.4%).

Conclusion: It was observed that the rate of positivity was higher in older people, it should be taken into account that this group only perform the test when they have symptoms, which increases the likelihood of positive results. In addition, the Brazilian population was characterized in terms of the immune response to the virus in the first moment of the pandemic, where a higher prevalence was observed in the North region. These data can be an important source of epidemiological information, even in asymptomatic patients.

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Serology testing after the first and the second dose of CoronaVac COVID-19 vaccine in Brazilian laboratory workers.

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Background: Currently, two vaccines against SARS-CoV-2 are in use in Brazil. Recent studies show the CoronaVac efficacy to prevent any symptomatic COVID-19 started in 50,7% and became more extensive as disease severity increase. CoronaVac is an inactivated vaccine containing inactivated SARS-CoV-2 and it is supposed that the vaccine triggers antibody response against all spike and nucleocapsid protein. Both spike and nucleocapsid are key structural proteins of SARS-CoV-2 with good immunogenicity and can serve as primary targets to check the immune response to vaccination. However, the duration of antibody responses and other potential measures of protective immunity need further investigation.

Methods: In this study, 180 laboratory workers who had previously enrolled to receive CoronaVac Vaccine according to availability in Brazil had the blood drawn 14 days after the first dose and 21 days after the second dose of vaccine. The interval between doses was 28 days. Serum was tested using Elecsys ® Anti SARS CoV 2 - Roche Diagnostics, an immunoassay electrochemiluminescence for the in vitro qualitative detection of total antibodies (including IgG) against nucleocapsid antigen. 36 laboratory workers have documented a history of SARS-CoV-2 infection.

Results: 14 days after the first dose of vaccine, 62 (34,4%) of 180 laboratory workers were antibody positive and 118 (65,6%) were antibody negative. Based on a documented history of infection, 32 of 36 participants tested positive and had higher antibody titer responses (cutoff index up to 190,5), but 4 remains negative. 21 days after the second dose of vaccine, 159 (88,3%) of 180 laboratory workers tested positive and 21 (11,7%) tested negative in serology testing. 34 of 36 who had documented infection tested positive and 2 remain negative.

Conclusion: The study showed that after a single dose, persons with past SARS-CoV-2 infection triggers more antibody response to SARS-CoV-2 (88,9%) than did those without a history of infection (20,8%). After the second dose of the vaccine, most participants developed detectable antibodies against nucleocapsid protein (88,3%), However, the duration of antibody responses and other potential measures of protective immunity need further investigation. Limitations of the study are the small sample size, lack of demonstration of vaccine efficacy, and lack of determination of antibody blocking of the receptor-binding domain to the human host receptor angiotensin-converting enzyme 2 (ACE2).

B-165

A Prospective Comparison of At-Home, Self-Collected Nasal and Saliva Specimens as an Alternative to Healthcare Worker-Collected Nasopharyngeal Swab Specimens for SARS CoV-2

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Background: The challenge of curtailing SARS-CoV-2 was initially limited by accessibility to adequate amounts of laboratory testing. Nasopharyngeal swab (NPS) specimens for RT-PCR testing are the standard for detection. However, recurrent shortages of swabs, personal protective equipment (PPE), and access to care prompted evaluation of sampling alternatives, including the use of patient self-collected samples with anterior nasal swab (ANS) and saliva.

Methods: From June through September 2020, 96 subjects were enrolled in a prospective, case-control clinical evaluation of at-home, patient self-collection versus standard of care (SOC) NPS collection. Twelve positive cases were identified through screening events and chart review for SARS-CoV-2 results from an FDA EUA test within 7-days of enrollment. The control cohort was identified by those who met CDC-defined criteria for testing and tested negative for SARS-CoV-2. Self-collected ANS and saliva samples were collected in an unsupervised, home environment and compared with contemporaneous healthcare worker (HCW)-collected NPS reference standard. All laboratory results for SARS-CoV-2 were obtained using high-sensitivity RT-PCR tests.

Results: Of enrolled subjects with HCW-collected samples, 51 and 59 subjects had paired ANS and saliva samples, respectively. High levels of overall agreement were observed between self-collected ANS and saliva specimens as compared with HCW-collected NPS reference (94.1%, 95% CI: 84.1%-98.0%; 96.6%, 95% CI: 88.5%-99.1%, respectively). One HCW-collected sample that was determined to be 'presumptive positive' was found to be negative for SARS-CoV-2 on both ANS and saliva samples.

Table 1. Performance of Self-Collected ANS or Saliva Samples Compared to HCW-Collected NPS Samples

		HCW-Collected NPS		
		Positive	**Negative**	**Total**
Self-Collected ANS	**Positive**	3	1	4
	Negative	2	45	47
	Total	5	46	51
		HCW-Collected NPS		
		Positive	**Negative**	**Total**
Self-Collected Saliva	**Positive**	5	0	5
	Negative	2	52	54
	Total	7	52	59

Conclusion: Early in the pandemic, data comparing self-collected specimens to the SOC for detecting SARS-CoV-2 were controversial. Questions surrounded specimen adequacy and assay performance, particularly the unknown influence of the sample matrix. This early evaluation demonstrated strong feasibility for detecting SARS-CoV-2 in self-collected ANS and saliva samples across multiple high-sensitivity assays.

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Microbiological profile of respiratory tract samples of the largest laboratory in Latin America, an observational study comparing findings before and during the COVID-19 pandemic

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Background: The Coronavirus Disease-2019 (COVID-19) pandemic has increased the number of critical care patients and the use of antimicrobials, consequently the risk of emergence of multidrug resistant organisms (MDRO). This study aims to compare microbiological findings from respiratory samples before and during COVID-19 pandemic.

Methods: This observational study assessed the microbiological findings in respiratory samples (tracheal aspirate and bronchoalveolar lavage) obtained from 37 different hospitals in São Paulo state, Brazil. Samples were obtained in two periods: September 1st to December 31st 2019 (group 1) and September 1st to December 31st 2020 (group 2). Variables analyzed were: number of samples, positivity rate, microorganism isolated and antimicrobial susceptibility profile. For Gram-negative organisms, Carbapenem-resistance was evaluated, whereas for Gram-positive organisms, Methicilin-resistance were evaluated.

Results: Group 1 consisted of 1459 respiratory samples, of which 827 (57%) were positive cultures. Group 2 comprised 2624 respiratory samples, of which 1634 (62%) were positive cultures. In Group 1, the most common isolates were Pseudomonas aeruginosa (18,2%), Staphylococcus aureus (8%) and Klebsiella pneumoniae (6,8%). In Group 2, the same bacteria were found more often, with 20%, 7,1% and 8,8% of samples, respectively. During pandemic, there was an increase in the prevalence of Carbapenems-resistant strains of Pseudomonas aeruginosa (from 34% to 42%) and Klebsiella pneumoniae (from 53% to 61%). There was no increase in Methicilin-resistant Staphylococcus aureus strains.**Conclusion:** This study highlights the increase

in the total of respiratory samples collected and the higher prevalence of MDRO (carbapenems-resistance) in the era of COVID-19. This might be justified by the rise of critical care patients, mechanical-ventilation and overuse of antibiotics.

Comparison between microbiological findings in respiratory tract samples

	Samples collected 2019 (%)	Carbapenem resistance (%)	Methicillin resistance (%)	Samples collected 2020 (%)	Carbapenem resistance (%)	Methicillin resistance (%)
Negative	632 (43,3)	-	-	990 (37,7)	-	-
Pseudomonas aeruginosa	265 (18,2)	34%	-	525 (20,0)	42%	-
Klebsiella pneumoniae	99 (6,8)	52%	-	231 (8,8)	61%	-
Staphylococcus aureus	116 (8,0)	-	33%	185 (7,1)	-	29%
Acinetobacter baumannii	42 (2,9)	78%	-	128 (4,9)	70%	-
Serratia marcescens	58 (4,0)	1%	-	100 (3,8)	4%	-
Others	247 (16,8)	-	-	465%	-	-
Total	1459			2624		

B-167

Evaluation of an Automated Assay for the Detection of SARS-CoV-2 IgG Antibodies to S1

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Background: SARS-CoV-2 has caused a global pandemic since early 2020. Bio-Rad Laboratories has designed the BioPlex 2200 SARS-CoV-2 IgG Assay, a highly specific, semi-quantitative, IgG serology assay for the detection of antibodies against the S1, including the RBD region of the COVID-19 virus. Here we evaluated the specificity and clinical sensitivity of the BioPlex 2200 SARS-CoV-2 IgG assay. We also correlated the semi-quantitative BioPlex 2200 SARS-CoV-2 IgG Assay results to the WHO International Standard (IS) for the binding activity of anti-SARS-CoV-2 IgG S1.

Method: The BioPlex 2200 SARS-CoV-2 IgG Assay employs fluoromagnetic dyed beads, each coated with S1 protein. The S1 protein-coated fluoromagnetic beads possess a unique fluorescent signature used to identify the presence of IgG antibodies to SARS-CoV-2 in a two-step assay format. To evaluate specificity, a total of 1078 samples from patients with potentially cross-reactant antibodies, as well as a total of 1557 samples from presumed healthy subjects and blood bank donors, were tested. All samples were collected prior to November 2019. To test for clinical sensitivity, a total of 305 samples from subjects who had been identified as positive for SARS-CoV-2 by PCR testing were evaluated with the BioPlex 2200 SARS-CoV-2 IgG Assay. To determine the correlation to the WHO IS, we serially diluted the IS provided by NBISC 1:2 in an immunodepleted negative serum pool to a level below the limit of quantitation (LoQ). Mean values at each serial dilution concentration were calculated for each level and a conversion factor was calculated to convert from the assay's U/mL to the international BAU/mL. To test the accuracy of the conversion factor, we also tested the BioPlex 2200 SARS-CoV-2 IgG Assay against the WHO International Reference Panel for anti-SARS-CoV-2 immunoglobulins.

Results: BioPlex 2200 SARS-CoV-2 IgG Assay exhibited an overall specificity of 99.9% (1556/1557). No significant cross-reactivity was observed. The CoV-2 IgG NPA, for all potentially cross-reactant samples, was 99.9% (1077/1078). The SARS-CoV-2 IgG PPA for all samples (N=305) was 80.6% (26/32) at 0-7 days, 96.3% (26/27) at 8-14 days, and 93.6% (231/246) at ≥15 days. Using the WHO IS,a factor of 2.2 was calculated to convert the S1 U/mL value to BAU/mL. for the WHO Reference Panel, the BioPlex 2200 SARS-CoV-2 IgG Assay results correctly classified positive and negative samples and the antibody titers for the S1 assay aligned with those reported by participants in the WHO international study.

Conclusion: The BioPlex 2200 SARS-CoV-2 IgG Assay demonstrated high specificity and excellent sensitivity and the semi-quantitative U/mL values can be easily converted to quantitative BAU/mL values with a simple conversion factor of 2.2. The BioPlex 2200 System offers practical advantages that allow for rapid, fully automated evaluation of IgG antibodies to the S1 protein of SARS-CoV-2.

B-169

Gold Nanoparticle-based Lateral Flow Immunoassay for the Quantitative Detection of Carcinoembryonic Antigen (CEA) in Human Pancreatic Cyst Fluid

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Background: This work describes a simple, rapid, inexpensive, and sensitive gold nanoparticle (AuNP)-based lateral flow immunoassay (LFI) for the detection of carcinoembryonic antigen (CEA) in pancreatic cyst fluid. CEA is a protein biomarker that has been linked with pancreatic cancer and some other gastrointestinal cancers. CEA levels in pancreatic cyst fluids can be used in consortium with other diagnostic information to distinguish pancreatic cancer and from other benign pancreatic conditions. A rapid test for the quantitative measurement of CEA levels in pancreatic cyst fluid will help healthcare providers make important patient care decisions in a timely manner. **Methods:** CEA was captured in a sandwich type immunoassay between an anti-CEA antibody pair; the capture antibody was immobilized on nitrocellulose membrane and the detection antibody was conjugated to AuNPs. The CEA dependent accumulation of AuNPs on the test zone on the nitrocellulose membrane resulted in a red colored band. The band was observed with the naked eye for qualitative data. For quantitative data, the test line intensities were read with a portable strip reader. Upon optimization of assay conditions, patient pancreatic cyst fluid was diluted 10-fold with assay buffer was tested for their CEA levels. **Results:** The developed LFI had a CEA detection limit of 2 ng mL^{-1}, based on a signal-to-noise ratio of 3. The linear dynamic range for the assay was 3 to 100ng mL^{-1}. The assay was tested against some common proteins found in bodily fluids and was found to be adequately selective. The developed assay was successfully applied for the screening of CEA levels in pancreatic cyst fluid and was able to distinguish mucinous from non-mucinous pancreatic cyst fluid based on a reference value of 192 ng mL^{-1}. **Conclusion:** From the results, the developed AuNP-based LFI shows good analytical performance. The developed biosensor presents a simple, rapid, and low-cost assay for the quantitative measurement of CEA levels in pancreatic cyst fluids. Upon further verification, the developed immunoassay has the potential to be applied for the point-of-care screening of CEA levels in pancreatic cyst fluids to aid patient care, particularly in limited resource settings.

B-171

Develop PCR-based genotyping assays to identify SARS-CoV-2 strains and study their prevalence and clinical presentations.

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Background: As a single-strain RNA virus, SARS-CoV-2 keeps changing at a rate of ~2 single-nucleotide polymorphism (SNP)/month. Next-generation sequencing (NGS), which can sequence viral RNA genome from thousands of samples in a massively parallel manner, has revealed SNP mutations and identified lineages/strains of SARS-COV-2. Genomic epidemiology investigation of emerging viruses can reveal the outbreak and track virus evolution. However, the clinical significance of SNP mutations are yet to be determined. To answer such questions with future clinical implementation in mind, more practical SNP genotyping methods are needed, ideally with less technical requirement, less cost, and shorter turn-around time. **Methods:** We develop PCR-based SNP assays for SARS-CoV-2 genotyping. Five SNPs of interest were selected, including three SNPs (28144T/C, 14408C/T, and 23403A/G) reported to distinguish Washington strain (WA1) strain from European (EU) strain, one characteristic SNP in CA strain (A23063T), and one common SNP (T22917G) in the UK/South Africa (SA) strains. We validated PCR-based SNP assays compared to NGS. After validation, we studied the prevalence and clinical presentations of SARS-CoV-2 strains from Mar 2020 to Mar 2021 in four states, including CA, NY, TX, and HI. **Results:** In Mar 2020, we observed a high prevalence of the WA1 strain at SF (76.7%) but a high prevalence of the EU strain at NY (83.9%). The EU strain became dominant (100%) in all locations by Jul-Sep 2020. Of interest, we noticed cases with EU strain infection tend to have a higher viral load than those with WA1

strain infection. Comparing the clinical presentations of these two strains, more gastrointestinal (GI) symptoms were reported in EU strain-infected cases but no obvious severity difference between these two strains. The prevalence and clinical significance of CA and UK/SA-related SNPs are still under investigation. **Conclusions:** We have developed and validated PCR-based SNP genotyping assays for SARS-COV-2. With these assays, we identified the strain types of COVID cases at different times and from different locations. Our study revealed the spread trend, consistent with the genomic epidemiology reports based on NGS, and minor clinical presentation differences of different strains. PCR-based SNP assays can identify SARS-COV-2 mutations and strains in an accurate and timely manner. It is a powerful and practical tool to investigate the clinical meaning of viral mutations to fill the gap between epidemiology to clinical practice.

B-172

External validation of the clinical validity and utility of an automated low cost cfDNA screening test for Down syndrome

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Background: Prenatal screening for the common aneuploidies is shifting from serum-based testing in over 200 laboratories in the US to cell-free (cf) DNA testing performed in fewer than 15 laboratories. This reduction is likely due to the complexity and costs of sequencing-based methods. In 2017 a more user-friendly and less costly platform based on a novel molecular probe technology was introduced and multiple validations were published. The VALUE study aimed at a more comprehensive and longer term study focusing the common trisomies and fetal sex. This report describes our results for trisomy 21, but results for trisomies 18 and 13 will al be provided.

Methods: The Vanadis® system (PerkinElmer, Waltham, MA) currently uses probes to capture fragments from chromosomes 21, 18, 13 and Y. Captured fragments are amplified via rolling circle replication and the resulting DNA products (balls) are fluorescently labelled and deposited onto a proprietary 96 well nano-filter plate. These objects are then imaged, counted and the results analyzed and interpreted for aneuploidy. We prospectively collected maternal plasma from pregnant women at 10 to 20 weeks gestation at 18 sites. The 'low risk' arm enrolled women with limited risk factors in the process of providing a sample for clinical cfDNA testing. The 'high risk' arm enrolled women after a positive clinical cfDNA test. Multiple Streck® tubes were collected and express shipped to Women & Infants Hospital, where plasma was separated and stored at −80°C until testing. Demographic information was collected along with results of the clinical testing. Discrepancies between clinical cfDNA results and ours were resolved by examining karyotypes, ultrasound findings and birth outcomes.

Results: Samples were tested in 39 batches of about 72 samples each. On 3 occasions the run failed to provide any results (7.7%). A second aliquot was tested for these and for single sample failures occurring in successful runs. The low risk arm included 2,218 women with 5 trisomy 21 and 2,213 unaffected pregnancies. Using this two-sample protocol, all cases were detected (DR 100%, 95% CI 51 - 100%) and 5 normal controls were screen positive (FPR 0.23%, 0.08 - 0.54%). The PPV was 5 of 10 (50%, 23 - 76%). The high risk arm included 81 additional trisomy 21 cases, all but one was positive; an overall DR of 98.8% (85 of 86, 93.1 - >99.9%). Two publications using the same methodology tested 208 additional trisomy 21 cases; all were detected and none of the 1,037 controls were positive. For all 294 cases the DR was 99.7% (97.9 - 99.9%) with a FPR of 0.15% (0.05 - 0.37%). One trained non-molecular laboratory technician processed and tested 430 samples over a two-week window; a rate over 10,000 samples per year.

Conclusion: The Vanadis system provides a high DR and low FPR for trisomy 21, the most common aneuploidy. Causes for the run failures can now be avoided. Turnaround time is improved by having a second sample available for testing in cases of failed runs or samples, but is not required.

B-173

Performance evaluation of Roche cobas Liat for the rapid detection of SARS-CoV-2 nucleic acid in a large academic hospital setting

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Backround: The SARS-CoV-2 global pandemic has highlighted the fundamental importance of the clinical laboratory in patient care. As the pandemic evolved, clinical laboratories responded aggressively to the ever-changing landscape of diagnostic testing needs. At our institution, we established multiple molecular platforms to test for SARS-CoV-2 and implemented testing algorithms to meet the varied clinical demands in our hospital. Trying to optimize these testing algorithms was challenging, especially considering ongoing reagent shortages. Additionally, our ability to provide rapid, highly sensitive testing was lacking. The Roche cobas Liat system consists of an RT-PCR-based instrument and assay appropriate for point-of-care testing in an acute care setting, as it provides results in approximately 20 minutes. The goal of this study was to verify the performance of this rapid system relative to an existing validated method, the Roche cobas 6800, to ensure the integrity of actionable results in an acute care setting. **Methods:** The analytical performance of the SARS-CoV-2 & Influenza A/B assay for the Roche cobas Liat was compared to the Roche cobas 6800 system for detection of SARS-CoV-2 on 248 nasopharyngeal swab specimens collected from patients with clinical suspicion for Covid-19 infection who presented to the Emergency Department. The samples were submitted for STAT testing and analyzed using the Liat: the remnant samples were used for parallel testing on the 6800. Samples with discordant results were further analyzed for SARS-CoV-2 using the Cepheid Xpert Xpress. **Results:** Results of this analysis revealed that the analytical sensitivity and specificity of the Liat system were comparable to that of the 6800 system. Specifically, the overall percent agreement for the detection of SARS-CoV-2 between the Liat and 6800 was 97.6% (242/248) with a positive predictive value of 92.1% (35/38) and negative predictive value of 98.6% (207/210). Samples with discordant results were further analyzed for SARS-CoV-2 using the Cepheid Xpert Xpress. Following discrepancy resolution, the overall percent agreement was 98.4% (244/248), with a positive predictive value of 97.4% (37/38) and a negative predictive value of 98.6% (207/210). **Conclusion:** This evaluation supports the excellent performance of the Liat for the rapid and reliable detection of SARS-CoV-2. This platform has provided our hospital additional testing capacity and has been utilized to support the management of patients presenting to acute care departments such as Labor and Delivery and the Emergency Department. As the pandemic continues to evolve, providing accurate and timely diagnostic testing is paramount and the Roche cobas Liat SARS-CoV-2 & Influenza A/B assay is a welcome addition the clinical laboratory's testing arsenal.

B-174

Advanced *SARS-CoV-2* Variants of Concern (VOC) whole genome materials for use as verification, external quality assessment (EQA) and prospective quality control samples

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Background: The *SARS-CoV-2* virus has evolved into several variants of concern (VOC), which pose a serious threat to public health. These variants, including B.1.1.7 (a.k.a. 501Y.V1), B.1.351(a.k.a. 501Y.V2), and P.1(a.k.a. 501Y.V3), are particularly dangerous due to their increased viral transmissibility, disease severity, and ability to reduce the efficacy of *SARS-CoV-2* vaccines and diagnostic tests. *SARS-CoV-2* nucleic acid amplification testing (NAAT) is a widely used diagnostic method for epidemiological surveillance, mass population screening, and clinical detection of the *SARS-CoV-2* virus. Given that several clinical laboratories have installed *SARS-CoV-2* molecular systems to detect *SARS-CoV-2* infections, it is good practice for operators to confirm that the assays in use can accurately detect infection with a VOC sample. Ultimately, accurate detection of *SARS-CoV-2* and VOC will be critical for determining the diagnostic outcome of the infected population and to establish appropriate epidemiological preventative measures. The use of VOC quality assessment products to verify and validate the performance of *SARS-CoV-2* and VOC diagnostic tests will help ensure successful identification and management of *SARS-CoV-2* infections.

Methods: Microbix formulated a novel swab-based positive sample based containing a whole genome cDNA of *SARS-CoV-2* B.1.1.7, B.1.351 and P.1 variant types. The material is desiccated on Copan FLOQSwab® in a proprietary matrix. The performance of the novel *SARS-CoV-2* swab-based VOC samples containing all the components found in the infected patient specimen (human cells, and viral nucleic acid) was evaluated in Original Equipment Manufacturer (OEM) and clinical laboratories. Major goals of the study were to show VOC sample usability in: 1. Verifying the performance of VOC-specific SARS-CoV-2 NAAT.2. Confirming the performance of the SARS-CoV-2 standard NAAT when challenged with VOC samples.3. Evaluating sample processing workflow, from swab elution in transport medium to final test results.

Results: All the mutations on *SARS-CoV-2* VOC were confirmed by NGS internally, further data from the participating OEM (Seegene and Elitech) showed excellent specificity towards E484K (S gene) HV69/70 deletion (S gene) N501Y (S gene) mutations. Additionally, EQA schemes with sample size n>1000 demonstrated the use of *SARS-CoV-2* VOC swab samples performance in multiple qPCR platforms by

evaluating the whole sample process workflow in the presence of the VOC specific mutations on a whole genome cDNA template by clinical labs using standard qPCR methods.

Conclusion: *SARS-CoV-2* VOC materials formulated using a widely accepted swab-based format, showed compatibility with the OEM platforms utilizing qPCR and mTOCE™ VOC methods of detection. The successful detection of various gene mutation targets demonstrates the achievement of constructing cross-platform compatible *SARS-CoV-2* VOC samples for use as prospective quality controls and verification panels. Furthermore, EQA schemes with VOC samples were able to successfully provide critical information to laboratories regarding their ability to detect general *SARS-CoV-2* infection when challenged with whole-process VOC samples.

B-175

Multiplex Fragment Analysis Identifies SARS-CoV-2 Variants of Concern

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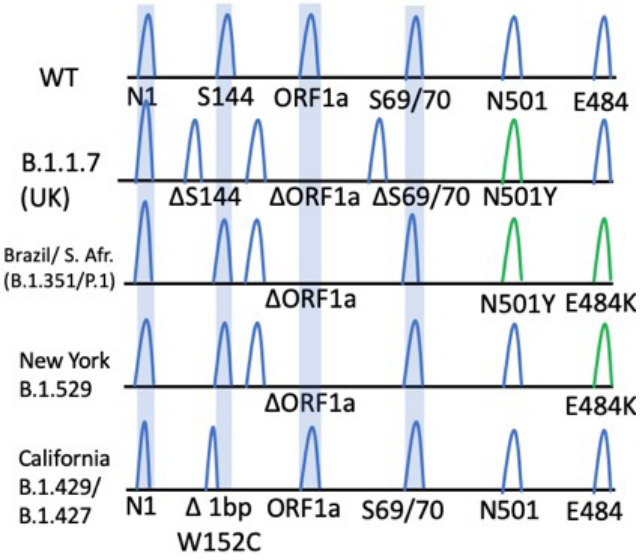
Background: The rapid spread of SARS-CoV-2 variants of concern (VOCs) necessitates systematic efforts for epidemiological surveillance. Whole genome sequencing (WGS) is the current method for identifying SARS-CoV-2 variants. Broad adoption of WGS is limited by instrument costs, bioinformatics support, technical expertise, and time required for implementation. Here we describe a scalable, multiplexed, non-sequencing-based capillary electrophoresis assay to rapidly and affordably identify and genotype SARS-CoV-2 VOCs.**Methods:** This RT-PCR fragment analysis method uses fluorescently-labeled allele-specific primers to detect characteristic mutations of SARS-CoV-2 VOCs. The assay targets deletions in the spike (del69_70, and del144) and ORF1A (del3675_3677) genes that are characteristic of the B.1.1.7 (UK) variant. As singular ORF1A deletions are also present in the New York, Brazil, and South African VOCs, allele-specific primers were added to detect the amino acid variants N501Y and E484K across different dye channels (blue (FAM) for WT, green (HEX) for mutant) for definitive identification. Lastly, the Spike del144 primers were modified to create a 1bp deletion when a characteristic California VOC spike protein mutation, W152C, is present.**Results:** 492 clinical specimens were assayed with concurrent WGS (March 1st-March 31st of 2021), and we detected 32 U.K. (B.1.1.7), 6 New York (B.1.526, B.1.525), 2 P.2, and 21 California (B.1.427, B.1.429) variants. The assay demonstrates high accuracy compared to WGS (sensitivity=99% and specificity=98%). The limit of detection varies for each target from 2,000 copies/ reaction (ORF1A del, N501Y) to <62.5 copies/ reaction for S:69_70del, S:144del, and E484K. The combination of mutations in the assay can classify VOC with 95% specificity, equivalent to WGS. Conclusion: This method can robustly and accurately identify COVID-19 VOCs utilizing a platform amenable to multiple targets (20-40 targets ranging from 100-500bp across four fluorescent channels) using equipment commonly found in routine molecular pathology laboratories. Future directions include validation and adding targets to detect new VOCs.



B-176

Comparing Sensitivity of Saliva versus Nasopharyngeal (NP) Swab for RT-PCR Detection of SARS-CoV-2

L. Contella, E. TerBeek, M. Snyder. *Luxor Scientific, Greenville, SC*

Background: The COVID-19 pandemic created the need for widespread screening for SARS-CoV-2 viral infection. The accepted standard for detecting SARS-CoV-2 is nasopharyngeal (NP) swab collection followed by reverse transcription polymerase chain reaction (RT-PCR) detection. Saliva has been promoted as an alternative and less invasive specimen source for SARS-CoV-2 detection. Use of saliva reduces need for limited collection supplies, as well as collector contact with a potentially contagious patient. A side-by-side study was conducted using paired saliva and NP samples to evaluate the sensitivity of saliva compared to NP swab for detecting SARS-CoV-2 infection. **Methods**: A total of 308 individuals were tested for SARS-CoV-2 infection by RT-PCR. Each individual provided a paired saliva and NP swab at the time of collection. Saliva (neat or in 1 mL saline for highly viscous samples) and NP swab (in 2 mL Viral Transport Media) were vortexed, and a 40 µL specimen aliquot was used for extraction. Samples were extracted with lysis buffer followed by a heat step at 56°C for 15 minutes and 98°C for 5 minutes. Extracted samples were run using Thermo Fisher TaqPath Combo Kit assay and master mix on an Applied Biosystems 96-fast block ViiA7. Samples with 2 or 3 genes crossing the determined threshold were considered positive; the presence of a single gene above the threshold was considered inconclusive (INC). The previously determined specificity of both specimen types was found to be 100%, so for the purposes of this study, all POS and INC results were considered to be evidence of active or resolving SARS-CoV-2 infection. **Results**: Of the 308 paired samples, 29 were positive and 5 INC for SARS-CoV-2 in their saliva and/or NP swab (11%; 34/308). Of the 34 total positives/INC samples, 29 (85.3%) were positive or INC by saliva and 94% (32/34) were positive or INC by NP swab; of the 5 that were negative in saliva, 2 were POS and 3 INC on the corresponding NP swab. Overall agreement between the two specimen types was 97.7% (301/308). **Conclusions**: Overall comparison of saliva versus NP swab for identifying SARS-CoV-2 infection was good (97.7%). While saliva detected fewer individuals (85.3% versus 94%) compared to NP swab, saliva detected 2 additional individuals who were negative by NP swab. These individuals reported general fatigue, GI symptoms, and body aches with no nasal symptoms. The use of saliva alleviates multiple bottlenecks when supplies are scarce, including nasal swabs and personal protective equipment (PPE). Additionally, patients can provide a sample themselves, painlessly and eliminating the need for direct interaction between health care workers and patients. Saliva testing also minimizes the likelihood of a false negative due to inadequate NP swab collection. This study demonstrates that saliva may be a valuable, non-invasive specimen source for detecting SARS-CoV-2 infection in instances in which the NP swab is not available or preferred.

B-177

Evaluation of a Simplified Extraction Protocol for RT-PCR Detection of SARS-CoV-2

L. Contella, E. TerBeek, M. Snyder. *Luxor Scientific, Greenville, SC*

Background: The recent high demand for COVID-19 testing placed a burden on laboratories to scale quickly with high-throughput RT-PCR testing options for detecting the SARS-CoV-2 virus. At the same time, reagent and consumable shortages were widespread, and many laboratories were forced to evaluate and validate multiple testing methods to provide supply chain flexibility. Our laboratory evaluated different extraction protocols to determine if a simple extraction process was comparable to an established extraction method for preparing NP swabs for detection by RT-PCR. We describe a laboratory-developed simple extraction protocol that can be performed manually in under an hour with minimal use of consumables and compare it to an established MagMax extraction method. **Methods:** A total of 460 NP swabs collected over the course of a week from individuals exposed to or infected with SARS-CoV-2 and were run via RT-PCR testing using a simple extraction protocol and compared to an established magnetic-bead based extraction protocol as the reference method. The simplified extraction was performed using 40 µL of Viral Transport Medium (VTM) from a NP swab and added to a 96 well plate along with MS2 internal control. Samples were lysed and extracted with Proteinase K followed by a heat step at 56°C for 15 minutes and 98°C for 5 minutes. MagMax Extraction was performed using 200µL of VTM and added to a 96-well plate and manually extracted using the MagMax Viral Pathogen Nucleic Acid Isolation Kit (Thermo Fisher). After incubation, the simple- and MagMax-extracted samples were cooled and added to a reverse transcription polymerase chain reaction (RT-PCR) using the TaqPath Combo Kit (Thermo Fisher) on an Applied Biosystems VIIA7. Samples with 2-3 genes cross-

ing the defined threshold were considered positive for SARS-CoV-2, while presence of a single gene was considered inconclusive (INC). Samples with internal control failures were considered indeterminate (IND). **Results:** Of the 460 individuals tested, 15.7% (72/460) samples were positive for SARS-CoV-2 by RT-PCR with MagMax extraction and another 1.7% (8) were INC. Simple extraction detected 60 (83.3%) of the 72, and 60 of 80 (75%) positive/INC samples with MagMax extraction. Simple extraction yielded an increased number of IND samples, 61 (13.3%), compared to 11 (2.4%) with MagMax extraction. Of the 12 MagMax-positive samples that were not positive following the simplified extraction, 8 were negative and 4 were IND with simple extraction. **Conclusion:** The simplified extraction protocol does not concentrate and purify the viral RNA to the same extent as MagMax extraction. Therefore, we observed lower sensitivity (83.3%) and increased rate of IND (13.3%) results compared to a full extraction protocol. In order to reduce the clinical impact of these limitations, laboratories could establish protocols to repeat all samples with 1-2 genes positive or evidence of amplification and IND results with another extraction protocol. The described simple extraction protocol is quick and efficient in terms of reagent and consumable usage.

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AccuPlex™ SARS-CoV-2 in Synthetic Oral Fluid: a Non-Infectious, Whole Process Reference Material for Detection of SARS-CoV-2 Virus in Oral Fluid

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Background: An outbreak of a novel coronavirus, SARS-CoV-2, occurred in Wuhan, Hubei Province, China in late 2019 followed by the worldwide coronavirus disease (COVID-19) pandemic. To date, more than 122,000,000 worldwide cases of the virus have been confirmed, with more than 2,700,000 confirmed deaths. The virus causes respiratory illness that can result in pneumonia, respiratory failure, and death. Mass testing combined with rapid and accurate SARS-CoV-2 diagnostic testing is essential for controlling the ongoing COVID-19 pandemic by allowing those infected to be properly quarantined and their contacts monitored. Studies show that saliva can serve as an alternative upper respiratory tract specimen type for SARS-CoV-2 detection. Testing saliva samples allows for self-collection and enables easy, frequent, non-invasive sample collection when compared to nasopharyngeal swabs, the gold standard for SARS-CoV-2 detection. SeraCare has developed AccuPlex SARS-CoV-2 in synthetic oral fluid, a non-infectious, stable, and reproducibly manufactured reference material, to aid diagnostic test developers design and validate molecular diagnostic assays.

Methods: AccuPlex™ SARS-CoV-2 in Synthetic Oral Fluid reference material has both positive and negative kit components. The positive components contain multiple recombinant Sindbis viruses bearing sequences from the 2019 coronavirus genome (SARS-CoV-2) at three titer levels: 24,000; 12,000; and 6,000 copies/mL. These titers were chosen to give Ct values of 30-35 using the SalivaDirect™ RNA extraction-free SARS-CoV-2 detection method, a U.S. Food and Drug Administration emergency use authorized duplex, reverse transcription qPCR method. The 12,000 and 6,000 copies/mL titers were selected because they are at the limit of detection (LOD) for the SalivaDirect assay. The recombinant viruses cover the entire SARS-CoV-2 genome (approximately 29.9 Kb), and there are ~200 bp overlaps between constructs, such that no matter where in the genome a PCR assay is targeted, the product will be compatible. The negative kit component contains recombinant Sindbis virus bearing human RNAseP sequences at 24,000 copies/mL. This product contains whole, heat-treated, replication deficient recombinant Sindbis viruses in synthetic oral fluid (saliva) so that the product is a whole process control.

Results: Viral RNA was extracted in triplicate from the reference material and TaqMan digital PCR assays based on primers and probes published by the US CDC for emergency use, CDC 2019 nCoV N1 and CDC 2019 nCoV RNaseP, were used to quantitate the recombinant viruses in synthetic oral fluid. The reference material was then tested in triplicate using the SalivaDirect RNA extraction-free SARS-CoV-2 method. All positive reference material tested positive (N1 Ct values 32.2 - 34.6 and RP Ct values 31.7-31.9) and negative reference material tested negative (N1 Ct values undetermined, RP Ct value 31.9) by this method.

Conclusion: SeraCare has developed stable, well-characterized reference material for performance monitoring and limit of detection studies for saliva-based SARS-CoV-2 (2019-nCoV Wuhan) molecular testing.

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

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Background:A little more than a year into the SARS-CoV-2 worldwide pandemic, new variant strains have arisen as the virus mutates. These variant strains are of great concern because it is unclear what effect the mutations have on disease transmission, disease severity, or vaccine efficacy. 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. In order to ensure the quality of this testing, whole process, reproducibly manufactured reference materials are needed. LGC SeraCare is developing AccuPlex SARS-CoV-2 Variant Reference Material Kit to fulfill this need.

Methods:LGC SeraCare developed the SARS-CoV-2 Variant Reference Material kit utilizing proprietary AccuPlex® recombinant virus technology. The reference material contains the entire genomic RNA from SARS-CoV-2 and includes four sample vials. Vial 1 contains virus with spike (S) gene sequences from 20I/501Y.V1 (B.1.1.7) strain, Vial 2 has sequences from 501Y.V2 (B.1.351) strain, and vial 3 has sequences from 501Y.V3 (P.1) strain. There is also a vial that contains virus with the reference/wild type Wuhan strain sequences. Recombinant viruses were heat treated and formulated in viral transport buffer at concentrations ranging from 40,000 copies/mL down to 5000 copies/mL and tested on LGC Biosearch SARS-CoV-2 Variant ValuPanels genotyping assays as well as on a variety of NGS panels

Results: Using PCR based SARS-CoV-2 genotyping assay, the mutations in the AccuPlex materials (including ΔH69/70V, ΔY144, K417N, K417T, E484K, N501Y, D614G, and P681H) were detected as expected. The AccuPlex materials behaved similarly to native pathogen on assay lower limits of detection studies, indicating that the reference material is comparable to patient samples.

Conclusion:LGC SeraCare has developed a non-infectious, multiplexed, 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. As labs prepare to add genomic surveillance to SARS-CoV-2 diagnostic workflows, this reference material will help them optimize and monitor assays to ensure detection of critical SARS-CoV-2 mutations.

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Comparison between molecular and culture methods when detecting Group A *Streptococcus* infection in clinical samples

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Background: Acute pharyngitis/tonsillitis, which is characterized by inflammation of the posterior pharynx and tonsils, is a common disease. Several viruses and bacteria can cause acute pharyngitis; however, *Streptococcus pyogenes* (also known as Lancefield group A β-hemolytic streptococci, GAS) is the only agent that requires an etiologic diagnosis and specific treatment. GAS is a common bacterial pathogen that has emerged as an increasingly important health concern in many parts of the world. Although GAS may appear harmless in healthy individuals, the ability of this bacterium to take advantage of a weakened or compromised host defence system is extraordinary. It is estimated that GAS accounts for 20% to 40% of cases of pharyngitis in children and 5% to 15% in adults. A new ready-to-use monoplex real-time PCR assay for the detection of *Streptococcus pyogenes* was developed and its clinical performance was compared with a CE-IVD marked molecular assay, the Streptococcus pyogenes Real-TM Quant kit (Sacace Biotechnologies). **Methods:** A total of 406 clinical samples already characterised as positive or negative to *S. pyogenes* by culture were analysed. Samples were human pharyngeal-swab (384 specimens) and ear-swab (22 specimens) clinical samples. Both collected in UTM (Vircell®). According to the initial diagnosis, 307 samples were negative for the studied pathogen and 99 were positive. After clinical diagnosis, using the leftover of the clinical samples, DNA extraction was carried out with the MagLEAD® 12gC nucleic acid extractor (PSS instruments) or Maxwell® RSC 48 Instrument (Promega). The acid nucleic were analysed in parallel with the VIASURE *Streptococcus pyogenes* Real Time PCR Detection kit and the reference molecular assay. **Results:** Compared with initial diagnosis, VIASURE results were concordant for 392 out of 406 specimens. Therefore, a total of 14 presumptive discordant samples were obtained, 13 presump-

tive false positive values and 1 presumptive false negative value. As mentioned before, all samples were analysed in parallel with the Streptococcus pyogenes Real-TM Quant kit molecular assay and from the 13 false positive, it was obtained that in 12 samples VIASURE result reported the true value. Regarding the false negative, it was obtained the same result as VIASURE assay indicating that this sample is a true negative sample. Compared to molecular reference assay, it was obtained an overall agreement of 0.99 (0.98-1). VIASURE assay presented a sensitivity of 1 (0.96-1) and a specificity of 0.99 (0.98-1). **Conclusion:**

With this evaluation, it was possible to observe that VIASURE *Streptococcus pyogenes* Real Time PCR Detection kit provided a good correlation with a reference molecular method, demonstrating to be a good tool for the identification of *Streptococcus pyogenes*. It is to note that both molecular assays were more sensible than a conventional methodology such as culture. A highlight feature of the evaluated product was that thanks to its lyophilized format, the manipulation was minimal, without intermediate mixtures, and was very useful for storage and transport at room temperature, instead of using refrigerated containers and occupying place in the freezers of the hospital.

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Molecular diagnosis of an emerging virus with increasing new variant mutations: when *in silico* analysis through bioinformatic assessment is the way to go

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Background: During the ongoing COVID-19 pandemic, RT-PCR-based diagnostic methods are considered the gold-standard. However, the virus recent emergence added to its vast and fast-paced spread potentially compromises detection based on nucleic acid sequence due to higher mutations rates. In addition, as laboratory analysis of all the newly appeared variants is not feasible on a geographic level, as it is usually associated to limited samples in a specific location. Therefore, bioinformatic analysis comparing the designed primers and probes with SARS-CoV-2 sequence data, known as *in silico* analysis, as become and essential tool to guarantee up to date sensitivity. **Methods:** The selected database was GISAID EpiCoV, as it provides complete, high quality and up-to-date information and is considered as the reference SARS-CoV-2 nucleotide sequence database. The sequences were filtered as complete and high coverage (<1% Ns and <0.05% unique amino acid mutations (not seen in other sequences in database) and no insertion/deletion unless verified by submitter). An in-house bioinformatic software based on Biopython analysis queries was used for the analysis, which is updated bimonthly to match GISAID database information. In case of presence of relevant mismatches in the alignments that could compromise the primers and probe set reactivity, further analysis of the alignment and sequence characteristics were performed. Additional analysis criteria were selected to determine relevance of detected mutations, such as dismissing ambiguous nucleotide codes as irrelevant and determining threshold per number of mismatched nucleotides, location and final calculated Tm for the alignment. **Results:**Using VIASURE SARS-CoV-2 test design, >99% of the analysed sequences would result in PCR amplification.57.71% of the sequences can be detected with 100% homology and 42.03% can be detected with 100% homology in one of the two target genes. 0.25% did not show 100% homology with either of the primers and probe sets. After individual analysis, 825 sequences were determined to correctly generate an amplification product with at least one of the targets and 98 (0.02%) sequences were determined not detectable using either ORF1ab or N targets primers and probes. **Conclusion:**

In an ongoing pandemic setting, bioinformatic analysis allows the prediction of detection sensitivity for upcoming variants, ensuring robustness of existing methods.

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The advantage of multiplex detection of gastrointestinal protists: Is single aim monoplex testing missing the big picture?

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Background: Gastrointestinal infections continue to be one of the main causes of morbidity globally. *Cryptosporidium*, *Entamoeba histolytica* and *Giardia duodenalis* are the three most common diarrhoea-causing protist species, despite being commonly misdiagnosed due to asymptomatic and chronic infections. Due to its high sensitivity and specificity molecular diagnosis techniques are considered the reference method for their diagnosis. Here a novel real-time PCR multiplex assay for the simultaneous detection and differentiation of these three protozoan parasites was evaluated. **Methods:** The VIASURE *Cryptosporidium, Giardia & E. histolytica* Real time PCR detection kit was assessed using genomic DNA from stool specimens of patients with signs and symptoms of gastrointestinal disease. Clinical samples were characterised at the National Centre for Microbiology (Majadahonda, Spain) between 2014 and 2019 using a real time PCR (*Giardia* and *Entamoeba*) and an in-house nested PCR (*Cryptosporidium*). Species and genotypes were identified by Sanger sequencing. **Results:** A panel of 326 genomic DNA samples were analysed: 96 *Cryptosporidium*, 115 *G. duodenalis, 25 E. histolytica* and 90 samples positive to other parasites. The evaluated assay reported 1 false positive result for *G. duodenalis* and 10 false negative results (5 *Cryptosporidium* spp., 4 *G. duodenalis* and 1 *E. histolytica*). Retesting with reference method revealed the 4 *G. duodenalis* samples (Ct values of 30.9, 37.1, 41.0 and 35.4) and the 5 *Cryptosporidium* spp. as still positive. Unnoticed coinfections involving *Giardia* and *Cryptosporidium* were detected in 5 *G. duodenalis* and 16 *Cryptosporidium* positive samples. These results were not considered false positives, as initial diagnosis focused only on the diagnosed pathogen of interest and no other parasitic species were tested. **Conclusion:**

With this evaluation, it was possible to observe that the tested product provided a very good correlation with the molecular-based routine analysis and therefore, demonstrated to be a good tool for the diagnostic of intestinal parasitaemia by diarrhoea-causing protists. The high frequency of coinfections detected revealed an apparently common underdiagnosis when performing single pathogen diagnostic testing as opposed to multiplexing. Therefore, testing for multiples pathogens would be advisable when caring patients with unspecific gastrointestinal signs and symptoms.

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Clinical performance and validation of a new real time PCR assay for the detection of human *Leishmania* spp infection in cutaneous and visceral samples

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Background: Leishmaniasis is a vector-borne disease caused by many *Leishmania* species, protozoans which can infect both humans and other mammals. Leishmaniasis is a complex disease, with heterogeneous clinical manifestations ranging from asymptomatic infections to lesions at cutaneous sites (CL), mucosal sites or in visceral organs (VL), depending on the species and host characteristics. A correct diagnosis is crucial to apply the appropriate treatment and the use of molecular techniques in diagnosis of leishmaniasis has become increasingly relevant due to their remarkable sensitivity, specificity and possible application to a variety of clinical samples. In this study a new real Time PCR monoplex assay developed for the detection of the main human pathogenic *Leishmania* species was validated. **Methods:** The evaluation was carried out with DNAs of clinical samples previously characterized as positive or negative to *Leishmania* using a validated Nested-PCR of the "Laboratorio de Referencia e Investigación en Parasitología del Centro Nacional de Microbiología; Instituto de Salud Carlos III, (Spain). DNAs were analysed with the VIASURE *Leishmania* Real Time PCR Detection kit using the CFX96 Touch™ Real-Time PCR Detection System following manufacturer's instructions. **Results:** A total of 168 specimens (61 biopsy, 53 blood and 54 bone-marrow aspirates samples) previously characterized as positive (88/168) or negative (80/168) to *Leishmania* were analysed. Compared with initial characterization (the routine Ln-PCR), VIASURE assay reported one incongruent result. One negative sample (total blood) resulted positive for VIASURE assay. Upon in-depth analysis of this sample, it turned out to be from a patient who had positive serology and the bone-marrow aspirate sample collected at the same time was also positive, therefore, it could be reported as a true positive for VIASURE assay. VIASURE assay obtained a sensitivity and a PPV of 1 (0.95-1) and a specificity and NPV of 1(0.95-1). All the included species in the study, *L. infantum*, *L. Viannia* subgenus, *L. tropica*, *L. major* and *L. mexicana* were correctly detected. **Conclusion:**

With this evaluation, it was possible to verify that the studied prototype provided a very good correlation with the reference molecular method in both, skin biopsy and visceral clinical specimens. It is noteworthy that the kit is presented in ready to use lyophilized format, reducing time-consuming steps in the lab, avoiding possible contaminations. Moreover, the stabilized format permits to transport and storage the kit at room temperature, which we considered an important advantage, as it does not take up space in an always cramped and space-limited laboratory refrigerator.

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Detection of *Neisseria gonorrhoeae* point mutation conferring ciprofloxacin resistance: evaluation of new real time PCR assay in comparison with reference methods

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Background: *Neisseria gonorrhoeae* is evolving into a superbug with resistance to the recommended antimicrobials for treatment of gonorrhea. Given the global nature of this disease, the high rate of usage of antimicrobials and treatment failures, it is likely that the global problem of gonococcal antimicrobial resistance will worsen in the future and that the complications of gonorrhea will emerge as a silent epidemic. Resistance to ciprofloxacin (CIP) in *N. gonorrhoeae* is due, in 99% of cases, to a point mutation located in the *gyrA* gene. By understanding its molecular mechanism, CIP resistance can be anticipated. We had the chance to validate a multiplex real-time PCR prototype designed for the detection and differentiation of *N. gonorrhoeae* sensitive or resistant to CIP in sexual clinical samples. **Methods:** The first analysis was carried out with 88 DNAs from *N. gonorrhoeae* isolates from clinical samples with different Minimum Inhibitory Concentration (MIC) of CIP. The initial characterization (gold-standard) was performed with MALDI-TOF identification and MIC values were determined by the gradient diffusion susceptibility testing using the EUCAST cut-off points of MIC <= 0.03 mg / L → sensitive and MIC > 0.06 mg / L → resistant. Once the correct functioning of the prototype was verified, a prospective-comparative analysis was performed with clinical samples. **Results:** Using *N. gonorrhoeae* isolates, VIASURE *Neisseria gonorrhoeae Ciprofloxacin resistance* Real-Time PCR Detection Kit results were 100% concordant with the reference method, identifying 58 *N. gonorrhoeae* CIP resistant and 30 *N. gonorrhoeae* CIP susceptible samples. Subsequently, twenty-two clinical samples were analysed on parallel and again it was obtained a 100% of concordance (13 CIP resistant and 9 CIP susceptible samples). **Conclusion:**

It was possible to observe that the developed prototype fulfils the stablished requirements of analytical sensitivity and specificity and it is adequate for the purpose and population for which it is intended. In addition to this, the assay was presented in a stabilized format and ready to use, reducing the number of time-consuming steps in the lab and avoiding possible contaminations. Finally, the stabilized format permitted the storage of the product at room temperature, which was an important advantage in our laboratory.

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Comparative study of the use of pooled samples for the detection of SARS-CoV-2 in infection surveillance in a controlled low-infection risk workplace setting

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Background: The ongoing COVID-19 pandemic is a substantial challenge for health-care systems and their infrastructure. RT-PCR-based diagnostic of individuals is crucial to contain viral spread because infection can be asymptomatic despite high viral loads. this situation is also being a challenge in terms of business management to guarantee safety in the work environment. Since the beginning of the pandemic, CerTest Biotec S.L.'s biosecurity committee established a protocol to ensure a SARS-CoV-2-free work environment. Among the newly appointed health safety measures, weekly SARS-CoV-2 Real-Time PCR testing was performed. **Methods:** Asymptomatic workers took the oropharyngeal sample with a sterile swab themselves and dipped it into 500 µL of UTM. The samples were analysed on the day of collection. To ease laboratory workload, validated pooled samples were prepared taking 40 µL of the 500 µL and adding them to a new empty tube, obtaining a 200 µL pool sample of 5 individual samples. The analysis was performed with *VIASURE SARS-CoV*-2 assay which detects the *orf1ab* and *N* genes of SARS-CoV-2 and with VIASURE *SARS-CoV-2 (N1 + N2)* assay which detects two different regions of *N* gene and the *RNaseP* as endogenous control which served as the control of correct sample collection and handling. **Results:** In ten months, 2365 pooled samples were analysed obtaining a positive signal in 14 of them. The specimens that made up these 14 pools were individually tested after positive pools result. In all 14 cases, the individual result was concordant with the result of the pooled sample, being the Ct values lower in individual samples due to the "dilution" effect of the pooling method. Ct value differences between pooled and individual positive samples were in the range of up to 6.89 in the case of *ORF1ab* and 4.45 in the case of *N* gene targets. Even if Ct values of single samples were up to 35, positive pools could still be confidently identified. **Conclusion:**

The implemented strategy was helpful to detect SARS-CoV-2 in asymptomatic workers, avoiding internal infections. Despite the potentially compromised sensitivity, this protocol allowed a fast epidemiological assessment of larger groups of people, avoiding prolonged occupational exposure risk.

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Comparative study of a real time PCR panel directed to seven sexual pathogens versus two validated reference molecular assays

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Background: Several of the STIs diseases still represent major epidemics worldwide. Microbiological diagnosis of syndromes caused by various of these pathogens may be challenging, and methods capable of simultaneously screening for multiple of these microorganisms in a clinical sample are essential. Molecular assays offer this characteristic in addition to presenting increased sensitivity and specificity compared to traditional methods. A new ready-to-use multiplex real-time PCR assay for the detection of 7 different pathogens implied in STIs diseases was developed and its clinical performance was studied compared with two CE-IVD marked molecular assays: GeneProof Chlamydia trachomatis and Allplex™ STI Essential Assay (Seegene). **Methods:** A total of 128 DNAs of male and female clinical samples were analyzed. Initial diagnosis was performed with a combination of culture methods, microscopy and the molecular assay Abbott RealTime CT/NG. Following initial diagnosis, 106 samples were identified as positive (29 *Candida*, 42 *C. trachomatis*, 10 *N. gonorrhoeae*, 7 *S. agalactiae*, 1 *S. cerevisiae*, 15 *T. vaginalis*, 1 *U. urealitycum*) and 23 as negative. **Results:** Molecular analysis results were: 43/128 *Chlamydia trachomatis* positive samples, 12/128 *Neisseria gonorrhoeae*, 3/128 *Mycoplasma genitalium*, 17/128 *Trichomonas vaginalis*, 18/128 *Ureaplasma urealyticum*, 58/128 *Ureaplasma parvum* and 31/128 *Mycoplasma hominis* positive samples. In comparison with the two molecular reference methods, the VIASURE *Sexual Health Panel I* Real Time PCR detection kit reported 3 false positive and 1 false negative value. The global panel sensitivity and specificity calculated for the analysed pathogens were 0.995 (0.970 - 1.000) and 0.996 (0.988-0.999), respectively. On the other hand, 50 samples were newly identified as co-infections, whilst initial diagnosis identified a single pathogen. Hinting by extension systematic underdiagnosis when using routine single pathogen analysis. **Conclusion:**

With this evaluation, it was possible to observe that VIASURE assay provided a good correlation with the two reference molecular methods, demonstrating to be a good tool for the identification of up to 7 sexual pathogens, including bacteria, parasites, and fungi, either in single infection or coinfection.

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Development of a rapid, high sensitivity, direct from blood assay for identification of biothreat organisms

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Overview: We discuss the development of an *in vitro* diagnostic assay for the identification of biothreat organisms direct from a patient blood sample. We have developed an assay that allows for the identification of six Centers for Disease Control and Prevention (CDC) Category A and Category B agents in less than 5 hours direct from blood. The broad scope and rapid time to result for the assay may allow for the initiation of effective therapy more rapidly than current diagnostic methods.**Context:** The use of biological agents to cause severe economic, agricultural and health consequences has occurred throughout history, most recently in the United States with 2001 Anthrax attacks that resulted in at least 22 infections and 5 deaths. The CDC has identified high priority biological agents that pose national security risk for their ability to cause human disease with high morbidity and mortality. These agents, however, have a low prevalence of infection in the United States making diagnosis difficult and delayed for physicians relying on culture-dependent molecular technologies and therefore delaying initiation of effective therapy. Due to this, there is a need for a rapid, high sensitivity and specificity, culture-independent *in vitro* diagnostic assay that provides species identification to improve diagnosis and patient care. Here we

discuss the development of a high sensitivity assay for the identification of six biothreat bacterial species direct from a patient blood sample without a positive blood culture. **Design:** A multiplex amplification reaction utilizing T2 Magnetic Resonance detection is under development for identification of *Bacillus anthracis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Yersinia pestis*, *Francisella tularensis*, and *Rickettsia prowazekii*. Functional testing was performed with both Biosafety Level 2 and 3 organisms in EDTA blood at clinically relevant concentrations. Culture methods were developed to provide accurate titer quantification of samples. Preliminary specificity was assessed via *in silico* analysis to identify potential cross-reacting species. Direct laboratory testing of near neighbors and similar genomic sequences was performed to demonstrate specificity. **Results**: Sensitive and specific detection of six bacterial species was achieved direct from blood samples in <5 hours on the T2Dx platform. The assay demonstrated a preliminary analytical sensitivity of ≤20 CFU/mL for all panel targets in samples that contained either a single or multiple species. The assay was able to distinguish between each species without cross-reactivity between channels. Closely related species were not detected at high concentrations of organisms, further demonstrating assay specificity. **Conclusions:** Low titer biothreat agents were detected direct from blood by T2MR using the T2Dx automated platform. Development of this assay may allow for rapid identification of biothreat organisms in patient samples independently of blood culture and allowing for more rapid initiation of effective therapy.

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Good from far but far from good, CSF IgG Index performance in Multiple Sclerosis and Central Nervous System Inflammation diagnosis.

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Background: Multiple sclerosis (MS) is a chronic inflammatory disease characterized by demyelination and axonal loss disabling the brain and central nervous system (CNS). Cerebrospinal fluid (CSF) analysis with oligoclonal (OLIG) banding and specifically immunoglobulin IgG index calculation (INDEX) have been used for decades as an aid in MS diagnosis. The INDEX provides objective quantitative measurement of intrathecal synthesis of immunoglobulins. We aimed to investigate the performance of INDEX as a diagnostic tool in a cohort of MS and CNS inflammation patients. **Methods:**A retrospective cohort of biobanked paired CSF and serum specimens (n=673) and a prospective collection of consecutively physician ordered OLIG CSF and serum samples (n=585) were analyzed combined, totaling 1258 specimens. OLIG was performed using isoelectric focusing electrophoresis. Albumin and IgG from serum and CSF were determined by immunonephelometry on a Siemens-BNII nephelometer. We interrogated the sensitivity and specificity of the INDEX with currently published cut-off of >0.85. ROC curve and Youden's index (J, 0-1) were used to evaluate improvements to the cut-offs. **Results:**There were 155 patients diagnosed with MS (155/1258) according to the 2010 McDonald criteria. Out of the non-MS patients, 326 were diagnosed with CNS inflammation (326/1103). The cohort was initially categorized by prevalence of MS (MS vs Non-MS cases). Using MS as true positives, an ROC curve for the 0.85 INDEX cut-off yielded a sensitivity of 36.1% and specificity of 95.6%, (J=0.32). A lower cut-off of >0.7 would improve sensitivity (49.7%) and specificity (92.1%), (J=0.42), correctly classifying 11 additional patients as MS who were classified as non-MS using 0.85. OLIG had a 73% sensitivity and 90% specificity (J=0.63). INDEX was >0.7 in 25% of positive OLIG-MS samples. INDEX was >0.7 in 3% of non-MS samples. INDEX is commonly ordered in other CNS conditions than MS. Therefore, we reviewed the non-MS subset into CNS inflammation and non-inflammatory cases. A >0.7 cut-off for the INDEX had 14.4% sensitivity and 86.4% specificity for non-MS/CNS inflammation. Diagnoses included chronic inflammatory demyelinating polyneuropathy, angiitis, limbic paraneoplastic encephalitis, neuromyelitis optica and non-traumatic myelopathy. OLIG had similar performance (15.3% sensitivity and 84% specificity) to the INDEX. However, in 9/326 (2.8%) samples in the CNS inflammation group, the INDEX >0.7 was the only CSF abnormality (negative OLIG). **Conclusion:**With this cohort, the value of INDEX in trending and prognosis of patients with CNS disorders was not possible to be reviewed. This study suggests that despite the broad use of INDEX, the current 0.85 cut-off could be lowered to 0.7 for an improved sensitivity and with a specificity of the test. Even with a cut-off improvement, the test is not as sensitive as OLIG for MS and did not identify any positive MS cases that OLIG had missed. For CNS inflammation adjunct testing, the assay shows similar performance to OLIG, but added value in rare cases. When CSF samples from heterogeneous conditions are reviewed in conjunction in the clinical lab, the value of the INDEX testing for diagnosis of MS is unremarkable and seems to be a prime candidate for future enhancement of test performance and utilization.

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Impact of blood culture-independent rapid diagnostics on early identification of sepsis causing pathogens

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Background: Early appropriate antimicrobial treatment is associated with reduced mortality in patients with sepsis and bloodstream infections (BSI). Blood cultures, the gold standard for diagnosing BSI, detect bacteremia in only about 50% of patients who are clinically suspected of having sepsis and that value may decrease after antimicrobial administration. Blood culture dependent assays have been associated with improvements in time to appropriate antimicrobial therapy by enhancing early identification of causative organisms. T2 Magnetic Resonance (T2MR) technology employs culture-independent testing, providing species identification directly from whole blood samples in 3 to 5 hours without the wait for a positive blood culture. T2Candida and T2Bacteria Panels are the only FDA cleared, commercially available assays for direct-from-blood detection of *Candida* and bacterial species most commonly implicated in BSI that can progress to sepsis.

Methods: A literature review was performed to evaluate the impact of T2MR culture-independent rapid diagnostics on accuracy and time to diagnosis of blood stream infections. The impact on antimicrobial stewardship and patient care outcomes were also described.

Results: The T2Candida Panel provided faster time to detection and speciation of *Candida* BSI (mean 4.4 hours) directly from whole blood compared to time to positivity (mean 43.6 hours) and species identification (mean 129.88 hours) of paired blood cultures. T2Candida has demonstrated a greater than 7-fold reduction in time to appropriate therapy of 5 hours versus 39 hours prior to implementation of T2Candida testing. The T2Bacteria Panel generated species ID results 2.9 days (69.7 hours) faster on average than blood culture providing opportunities for early antimicrobial escalation or targeting of therapy. Performance of both assays demonstrated high accuracy with overall sensitivity and specificity of T2Candida 91.1% and 99.4% and T2Bacteria 90% and 98%, respectively. Economic savings were described for both T2Candida and T2Bacteria Panels as demonstrated by reductions in intensive care and total hospital lengths of stay and improved antimicrobial utilization.

Conclusion:T2Candida and T2Bacteria Panels provide rapid and sensitive detection of bloodstream infections caused by the majority of concerning pathogens most commonly identified in patients with sepsis. These assays can be useful antimicrobial stewardship tools that have the potential to improve clinical and economic outcomes for patients.

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Comparison of Two SARS-COV-2 Pseudo Viral Control for COVID-19 Molecular Testing

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Background: Numerous molecular tests for COVID-19 have been launched worldwide by way of Emergency Use Authorization. Not all of these tests employed process controls that monitor the entire assay process including the viral extraction. With the increase of vaccination and the reduction of the total number of infections, competitions among assay providers will make those tests using better quality control more favorable. Pseudo Viruses carrying SARS-COV-2 RNA sequence provides a safe and effective way to mimic the SARS-COV-2 virus to be used as "full process" quality controls.

Methods: We compared two commercially available pseudo viral QC controls, from Anchor Molecular and company S, in their ability to perform similarly to the actual SARS-COV-2 virus. The heat-inactivated COVID-19 virus was used as reference in this study.

Results: Both pseudo viruses exhibited longer stability, measured by recovery of viral copies, than the COVID-19 virus when stored in 2 - 8 °C. Using one commercial viral RNA extraction kit, both pseudo viruses were comparable in linearity, dynamic range, free RNA content and PCR efficiency. However, one significant and important difference was observed. When comparing the extraction efficiency among two different extraction methods, one using bead-based kit and another using direct lysis method,

Anchor's control behaved the same as the COVID-19 virus in that the viruses exhibited about 1:3 ratio in the viral copy detected between the two extraction methods. The virus from company S showed a 1:1 ratio between the two extraction methods.

Conclusion: This showed that the pseudo virus can better represent the real COVID-19 virus in response to different extraction methods. It is a better control for COVID-19 test.

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A Novel Lung Cancer test: A Desirable Companion to LDCT for stage 0~I Lung Cancer Screening

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Background: Lung cancer is the most common cause of cancer-related deaths globally, accounting for about 18.4% of total cancer deaths. Most lung cancer cases are diagnosed at advanced stages because of the asymptomatic nature of lung neoplasms. The 5-year survival rate in lung cancer is meager. However, patients diagnosed at an early stage have a higher survival rate than the latter, localized, and metastasized stages (54.8% vs. 27.4% vs. 4.2%, respectively). Thus, there is great potential to reduce mortality by diagnosing lung cancer at stage 0~I through community screening. Low-dose computed tomography (LDCT) has emerged as a promising mass screening method for the early diagnosis of lung neoplasms. The advantage of the LDCT-based protocol is its simplicity and its high sensitivity. The LDCT has high screening efficacy in detecting stage I lung cancers. Thus, there is great potential to reduce mortality by diagnosing lung cancer at stage 0~I through community screening. LDCT is a promising method, but it has a high false-positive rate. Therefore, a biomarker test that can be used in combination with LDCT for lung cancer screening to reduce false-positive rates is highly awaited.**Method:** According to our recent report, the levels of CYFRA 21-1-Anti-CYFRA 21-1 autoantibody immune complex (CIC) are much higher than CYFRA 21-1 among cancer patients as compared to healthy individuals. Similarly, the cardiac troponin T (cTnT) levels are higher in treatment-naïve cancer patients than healthy people. N-terminal pro-brain natriuretic peptide (NT-proBNP) is also considered a valuable marker for the oncologic disease prognosis. Therefore, we used the 9G Test™ Cancer/Lung test that allows determining the lung cancer index (LC index = [(CIC/CYFRA 21-1) × (cTnT/NT-proBNP)]) by quantifying plasma levels of four biomarkers including CIC, CYFRA 21-1, cTnT, and NT-proBNP. Clinical samples from healthy individuals (n = 359) and lung cancer patients (n = 244) were collected and tested at various locations in South Korea. All healthy individuals and cancer patients were screened by LDCT, followed by the biopsy for individuals whose results were LDCT positive. The levels of CIC, CYFRA 21-1, cTnT, and NT-proBNP, were measured and used to determine the LC index.**Results:** The obtained results indicate that the 9G test™ Cancer/Lung can detect lung cancer with overall sensitivity and specificity of 75.0% (69.1~80.3) and 97.3% (95.0~98.8), respectively. The detection of stage I (n = 160), stage II (n = 32), stage III (n = 49), stage IV (n = 3) cancers with sensitivities of 77.5%, 78.1%, 67.4%, and 33.3%, respectively, at the specificity of 97.3% have

never been reported before. The receiver operating characteristic curve analysis allowed us to determine the population-weighted AUC of 0.93 (95% CI, 0.91-0.95). **Conclusion:** These results indicate that the 9G test™ Cancer/Lung can be used in conjunction with LDCT to screen lung cancer. Furthermore, obtained results indicate that the use of 9G test™ Cancer/Lung with LDCT for lung cancer screening can increase stage I cancer detection, which is crucial to improve the currently low 5-year survival rates.

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9G Cancer Screening Index: Stage 0 ~ I Cancers Screening with High Sensitivity and Specificity

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Background: There is an urgent need of a test that can screen stage 0 ~ I cancers because at these stages the recovery from cancer is 85-100% and only 1-10% at stage IV. A test that can detect stage 0 ~ I cancers in patients using blood-based biomarkers can screen the cancer patients from an apparently healthy population and allow the monitoring of treatment progress. This study aimed to investigate the applicability of 9G CS Index for screening stage 0 ~ I cancer patients.**Method:** This prospective study was conducted from May 22, 2017, through December 31, 2018. Five hundred sixty-seven participants comprised 213 individuals in the general population, and 354 cancer patients of eight types of stage 0 ~ IV cancers were enrolled in this study. The ratio of cTnT and NT-proBNP levels were used for the determination of the 9G Cancer Screening Index.**Results:** The 9G Cancer Screening Index value of 0·15 allows discrimination of healthy individuals and stage 0 ~ I lung, colon, stomach, rectal, thyroid, cervical, ovarian, and breast cancer patients. The sensitivities for the detection of stage I, stage II, stage III, and stage IV lung cancers were 92·0% (74·0-99·0), 70·0% (34·8-93·3), 76·9% (46·2-95·0), and 100·0% (15·8-100·0), respectively. The sensitivities for the detection of stage I, stage II, stage III, and stage IV colon cancers were 75·0% (19·4-99·4), 66·7% (43·0-85·4), 75·0% (47·6-92·7), and 75·0% (34·9-96·8), respectively. The sensitivities for the detection of stage I, stage II, stage III, and stage IV stomach cancers were 72·0% (50·6-87·9), 81·8% (48·2-97·7), 88·9% (51·8-99·7), and 88·9% (51·8-99·7), respectively. The sensitivities for the detection of stage 0, stage I, stage II, stage III, and stage IV rectal cancers were 100·0% (2·5-100·0), 85·7% (57·2-98·2), 100·0% (59·0-100·0), 83·3% (51·6-97·9), and 60·0% (14·7-94·7), respectively. The sensitivities for the detection of stage I, stage III, and stage IV thyroid cancers were 61·5% (40·6-79·8), 58·3% (27·7-84·8), and 33·3% (7·5-70·1), respectively. The sensitivities for the detection of stage 0, stage I, stage II, stage III, and stage IV cervical cancers were 100·0% (39·8-100·0), 76·5% (58·8-89·3), 71·4% (29·0-96·3), 33·3% (0·8-90·6), and 50·0% (1·3-98·7), respectively. The sensitivities for the detection of stage I, stage III, and stage IV ovarian cancers were 100·0% (39·8-100·0), 25·0% (0·6-80·6), and 80·0% (28·4-99·5), respectively. The sensitivities for the detection of stage I, stage II, stage III, and stage IV breast cancers were 65·2% (42·7-83·6), 76·2% (52·8-91·8), and 80·0% (28·4-99·5), respectively. **Conclusion:** These results indicate that the 9G CS Index can be effectively used in the cancer screening programs for the identification of stage 0 ~ I cancer patients from the general population.

The stage 0 rectal and cervical cancers were detected with 100% sensitivity, respectively. The 9G CS Index screens lung, colon, stomach, rectal, thyroid, cervical, ovarian, and breast cancers with 90·8% specificity and 79·8% accuracy. Therefore, the 9G CS Index can be applied for the identification of asymptomatic patients in a seemingly healthy population.

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BD COR™ System Usability: Improving User Experience and Increasing Efficiency

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Evolving healthcare paradigms have created pressure for molecular diagnostic laboratories to meet higher testing demand and lower the total cost of routine testing, while also providing clinicians rapid, actionable results. The BD COR™ MX analyzer module, part of the larger BD COR™ System that, when combined with the BD COR™ PX pre-analytical module, provides high throughput analysis and diagnosis of patient samples for an expandable menu of sexually transmitted infections and other pathogens. The inaugural assay for the BD COR™ MX is the BD COR™ CTGCTV2 assay, which detects *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC) and/or *Trichomonas vaginalis* (TV) infections which affect approximately 6 million Americans every year[1-3]. In order to evaluate system performance from a user experience perspective, the BD COR™ System development team conducted a usability and timing study during a recent clinical trial period at two external laboratories. The study

was designed with two parts: a usability survey specifically covering areas of the human-instrument interface (both software and hardware), and a timing study designed to better understand the amount of time our users spent doing common instrument tasks such as loading samples and reagents, daily cleaning and instrument preparation, and waste removal. Timing study results show that, between the four operators at the two external laboratories, average set up times for both the BD COR™ PX module and BD COR™ MX module were approximately 11 minutes each, with daily cleaning tasks totaling 6 minutes. Loading consumables needed to process 96 samples into the BD COR™ MX module, including pipette tips and all necessary reagent plates, took a total of 6 minutes. No user errors or consumable loading issues were recorded during the timing study, implying high ease-of-use and effective engineering controls preventing accidental improper loading of BD COR™ System consumables. When given a survey to evaluate the usability of certain interface features, users at the external laboratories expressed high levels of satisfaction with the BD COR™ System consumable loading and management scheme. Additionally, phrases used to describe the BD COR™ System throughout a recent clinical trial period included: efficient, impressive, useful, time-saving, and straight forward, indicating a high level of satisfaction with usability and accessibility design choices. [1] *Sexually Transmitted Disease Surveillance 2018 - Chlamydia*. Centers for Disease Control and Prevention, 30 Sep 2019 [2]*Sexually Transmitted Disease Surveillance 2018 - Gonorrhea.* Centers for Disease Control and Prevention, 30 Jul 2019

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Photothermal Mediated Rolling Circle Amplification for More Specific and Convenient Direct In Situ mRNA Detection

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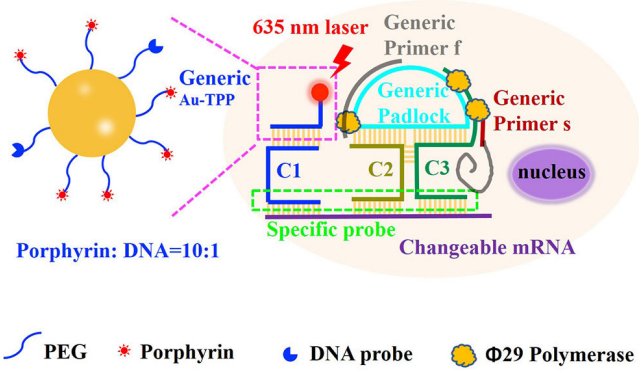
Background: Rolling Circle Amplification (RCA) has the advantage of in situ RNA biomarker detection at single molecule level, especially when good antibody is not available. However, RCA's wider clinical application is hindered by an inherent problem that its specificity is only controlled by the single interaction between the padlock template and the primer that binds to it. We aimed to develop a better in-situ direct mRNA detection technology by improving RCA specificity. **Methods**: We took temperature as another specificity controller by introducing photothermal activation via Au-TPP, gold nanoparticles modified with photothermal agent 4-(10, 15, 20-Triphenylporphyrin-5-yl)phenylamine (TPP) and DNA targeting oligo (Figure). Through photothermal effect, Au-TPP acted as a specific heat source upon irradiation of 635 nm laser on a confocal microscope. The photothermal mediated RCA would be initiated only when the padlock and Au-TPP bound adjacently to the same target mRNA. Furthermore, we introduced 'C' form target specific oligonucleotide linker probes to make generic padlock and Au-TPP for different mRNA targets, so that for a new mRNA target one does not have to redesign the padlock and the Au-TPP probe. **Results**: Using ACTB, GIPC2 and KCNJ5 genes as examples, and with 293T cells transfected with wildtype or mutant genes, as well as tissue sections of normal or gene-knockout rats, we demonstrated successful in-situ specific mRNA detection and in-situ specific point-mutation detection for both formalin-fixed paraffin-embedded (FFPE) and frozen tissue section, with either fluorescence or color detections. The whole process for a slide took about 2.5-3 hours. **Conclusion**: We have developed a novel photothermal-mediated RCA that can be readily integrated either in routine pathology diagnostics to quantify the abundance and to chart the spatial distribution of clinical biomarkers, or in the high-throughput screening of potential biomarkers in basic medical research.



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Exosome-Based Detection of KCNJ5 Mutations in Plasma from APA Patients

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Background: Primary aldosteronism (PA) is a major cause of secondary hypertension with higher rates of cardiovascular morbidity and mortality than patients with essential hypertension. About 50% of PA patients belong to the aldosterone-producing adenoma (APA) subtype which is curable through surgical operation. Adrenal venous sampling (AVS) is the gold standard laboratory procedure to diagnose APA. However, AVS is invasive, technical demanding and requires well-trained personnel, limiting its availability. As a result, a significantly large number of hypertensive patients who could have been cured by surgery were missed due to lack of access to proper diagnostics. Thus, noninvasive, simple and accurate diagnostic methods are urgently needed to identify APA. Potassium voltage-gated channel subfamily J member 5 (KCNJ5) mutations are the most common somatic variants in APA, especially in East Asian populations, with a prevalence of ~76.8% in Chinese APA patients, while very rarely detected in other PA subtypes. KCNJ5 mutations are therefore excellent diagnostic biomarkers for APA.**Methods:** We report the development of a novel exosome-based method for the noninvasive diagnosis of APA with KCNJ5 mutations. The method combines efficient capture of exosome from 1mL plasma with sensitive detection without nucleic acid extraction of three most common KCNJ5 mutations in a background of wildtype KCNJ5. To capture exosomes, a multi-layer high gradient magnetic separation (HGMS) technique was designed in which immunomagnetic beads (IMBs) monodispersed in the high gradient magnetic field were used to enrich and separate exosomes from flowing plasma, resulting in 4 times more blood exosomes captured than the gold-standard ultracentrifugation method. Exosomes were lysed and KCNJ5 nucleic acids captured onto 96-well plates to detect KCNJ5 p. G151R and p. L168R mutation status by a TaqMan-based qPCR with an allele-specific wild-type xenonucleic acids blocker (XNAs-blocker). **Results:** The exosome-based KCNJ5 mutation-detection system is capable of detecting mutant allele frequencies down to 0.05%. Preliminary application of exosome-based diagnosis on 22 APA patient plasma achieved a sensitivity of at least 69% sensitivity. **Conclusion:** The exosome-based liquid biopsy offers a promising alternative to the traditional, invasive AVS procedure in the diagnosis of APA.

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A real-time PCR approach for rapid high resolution of the HLA haplotypes associated to celiac disease

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Background: Celiac disease (CD) is an autoimmune enteropathy triggered by the ingestion of gluten in genetically susceptible individuals. Once thought to be a rare disease, CD diagnoses are increasing with current worldwide prevalence at 1%. Genetic susceptibility is associated with two sets of alleles, DQA1*05 - DQB1*02 and DQA1*03 - DQB1*03:02, which code for class II MHC DQ2 and DQ8 molecules, respectively. Approximately 90%-95% of celiac patients are HLA-DQ2 positive, and half of the remaining patients are HLA-DQ8 positive.Being able to quickly and efficiently determine the status of these DQA and DQB alleles in potential CD patients or risk population could dramatically aide in diagnostic work-ups. To improve the detection of these alleles, it was developed a simply and ready to use real-time PCR. **Methods:** A retrospective evaluation was performed using 94 specimens. From them, 76 were blood clinical samples, 4 were clinical sample negative controls, 7 were no-DNA controls, 4 samples were saliva clinical samples and 3 were synthetic samples. The routine diagnosis of these specimens was conducted with the CeliacStrip kit (Operon, Spain). This test consists in three steps: DNA extraction (not included in the kit), PCR amplification and DNA hybridization. The kit contains an endogenous control (ß-globin) which indicates that the PCR and hybridization processes have gone correctly. VIASURE *HLA Celiac* Real Time PCR Detection Kit consists of 2 tubes. The first one detects the alleles DQA1*05, DQB1*0302, DQB1*02 and the housekeeping ß-globin. Second tube detects the alleles DQA1*02, DQA1*03 and NO DQB1*02. **Results:** Except for one sample, both techniques yielded equal results. The discordance of this sample was that for DQ8, Operon test detect 3 alleles (DQA1*03, DQB1*0302 and DRB1*04) and VIASURE prototype detects 2 alleles (DQA1*03 and DQB1*0302). This sample contained the two alleles DQA1*03 and DQB1*0302 so, for operon was negative, as there was no DRB1*04 amplification, and for VIASURE was positive. Taking routine analysis as a reference values, the data for sensitivity (SE), specificity

(SP), negative predictive values (NPV) and positive predictive values (PPV) were calculated. VIASURE assay sensitivity was 1 (0.94-1) and specificity was 0.91 (0.59-0.99). **Conclusion:**

This new assay fulfils the criteria of accuracy, sensitivity and specificity. In addition, compared with the "reference" commercial assay, the obtained results were faster (2h vs 4h) and more reliable. A highlight feature of the evaluated product was that thanks to its lyophilized format, the manipulation was minimal, without intermediate mixtures, and was very useful for storage and transport at room temperature, instead of using refrigerated containers and occupying place in the freezers of the hospital.

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Real time PCR allows faster and more accurate characterization of carbapenemase-producing bacteria in clinical samples with suspicion of MDR infection

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Background: The overuse of wide-spectrum antibiotics has created a selective force that is leading to an increase of Multidrug resistant (MDR) bacteria, which are an emerging cause of health care-associated infections. A prompt genotypic characterization enables to implement the proper infection control measures to contain a MDR bacterial infection and to reduce cross-transmission. Molecular methods allow faster identification of carbapenemase-producing bacteria and precise differentiation of the carbapenemase type. The objective of the present study was to evaluate the clinical performance of VIASURE *carbapenemase-producing Enterobacteraeceae* Real Time PCR Detection with the routine laboratory reference method. **Methods:** The retrospective analysis was carried out with 97 previously characterized carbapenemase-carrying isolates and 50 rectal smears. To obtain bacterial isolates, clinical samples were streaked onto different media and bacterial identification and antibiotic susceptibility were performed by Maldi-TOF and micro-dilution using MicroScan® panels, respectively. Routine characterization was further confirmed by Real-Time PCR, using LightMix® modular carbapenemase assay (TIB Molbiol). Clinical samples were analyzed using the same methology. Specimens included OXA-48, OXA-405, VIM, NDM, KPC and IMP-carrying bacteria. **Results:** Regarding the enterobacterial isolates, β-lactamases corresponded to 17 ESBL and 80 carbapenemases [OXA-48 (n= 32), OXA-405 (n=1), VIM (n= 23), NDM (n= 19), KPC (n=2), IMP (n=7)]. The evaluation using bacterial isolates showed that all carbapenemase genes were detected correctly by both molecular methods. Regarding the rectal smears, β-lactamases corresponded to 16 ESBL and 39 carbapenemases [OXA-48 (n= 13), VIM (n= 11), NDM (n= 10), KPC (n=5)]. Detection of carbapenemase genes was fully coincident between VIASURE and LightMix® methods in all samples. Overall, there were 46 fully coincident between the molecular techniques and the conventional algorithm, with 5.12% increased detection of carbapenemases in comparison with the routine conventional algorithm. **Conclusion:**

Our results showed that VIASURE *carbapenemase-producing Enterobacteraeceae* Real Time PCR Detection kit offers a reliable method to detect carbapenemase genes both on MDR bacterial isolates and directly on clinical samples (rectal swabs). These two molecular techniques performed directly on rectal swabs increased the detection of carbapenemases when compared to the conventional algorithm through culture and bacterial isolation.

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An in-silico analysis of differentially expressed genes in HPV positive & Negative Head & Neck cancers

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Background Head and neck cancers (H&NC) are the sixth most common cancers worldwide. Consumption of alcohol and tobacco products along with environmental factors like human papillomavirus (HPV) are major etiological factors of H&NC. HPV subtypes 16 and 18 are majorly involved in causing infection with oral cavity and oropharynx being the predominant sites. Based upon HPV status H&NC are distinguished into HPV positive and HPV negative. The etiology of HPV positive H&NC is attributed to HPV infection while HPV negative H&NC are mostly related to tobacco and alcohol consumption. Despite several treatment modalities, the 5-year survival rates are low mostly because H&NC are diagnosed at advanced stage. It has been earlier established that HPV positive H&NC have a better prognosis compared to HPV negative. HPV positive patients show a better response to radiotherapy and chemotherapy. There is conflicting evidence with regard to distant metastasis in H&NC. Several studies have used microarray-based analysis to identify the differentially expressed genes (DEGs) in H&NC. Identification of potential candidate genes which can be used as predictors of prognosis in HPV positive/negative H&NCs are needed. **Methods**We initiated the study using publicly available microarray gene expression datasets from the Gene Expression Omnibus (GEO). Functional enrichment analysis of significant genes was done using the database for annotation, visualization and integrated discovery (DAVID). The protein-protein interaction (PPI) network was constructed in the Search Tool for the Retrieval of Interacting Genes and proteins (STRING) and visualized using Cytoscape. The CytoHubba plugin was utilized to identify the key genes in this complicated network. The top 10 genes with the highest scores were determined as hub genes and subjected to further analyses. The Molecular Complex Detection (MCODE) plugin was applied to screen out significant gene modules. Hub genes identified were analysed for overall survival (OS) in Gene Expression Profiling Interactive Analysis (GEPIA). Analysis of the miRNA and transcription factors targeting the hub gene with significance was done using miRNet. **Results**GEO datasets GSE39366, GSE40774, GSE117973 were used in this study. We identified 67 overlapping DEGs which were significant. Gene Ontology identified key terms as 'cellular component', 'biological process', and 'molecular function' respectively. Cytoscape and MCODE revealed two significant modules, first module consisted of five and the second module consisted of three genes. The common hub genes identified using cytohubb plugin were SPRR1B, CAV1, KRT16, KRT6B, KRT15, KRT19 & KRT75. Out of these DEGs, CAV1 has been reported to be significantly associated with overall survival in H&NC patients. 3 targeting miRNA (miR-7-5p, miR-145-5p and miR200b-3p) and 54 transcription factors were identified using miRNet. **Conclusion**In-silico survival and expression analyses revealed CAV1 as a candidate gene that may be used to predict the course of disease progression in HPV positive and HPV negative H&NC patients. Further invitro studies validating CAV1 and its three target miRNAs as predictors of prognosis in H&NC are highly warranted.

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Clinical evaluation of a new real time PCR assay designed for the detection of *Leishmania* infection in human samples compared to two reference molecular assays

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Background: According to WHO, leishmaniasis is one of the seven most relevant tropical diseases, present in 98 countries and with an increasing incidence in the last years. Currently, no gold-standard has been established for diagnosis, with traditional methods being of limited sensitivity. **Methods:** This study was designed for the clinical evaluation of the prototype VIASURE *Leishmania* Real time PCR Detection Kit (CerTest Biotec). The evaluation was carried out retrospectively with 49 DNA extracts from different clinical samples (44 cutaneous and 5 visceral) of patients with suspected *Leishmania* infection attended in Hospitals in Barcelona (Spain). This DNAs were used for the prototype analysis, alongside two commercial molecular assays: STAT-NAT *Leishmania* spp. (Sentinel) and *Leishmania* spp. Real-TM (Sacace). Samples were analyzed in duplicates using QuantStudio 6 (Applied Biosystems). **Results:** Results showed that studied prototype detected 28 *Leishmania* positive samples, STAT-NAT 17and Sacace 33. Of the 49 analyzed samples, only 15 positive samples and 15 negative samples showed concordant results with all three of the tested methods. Additionally, for 13 of the samples *Leishmania* identification at species level was available, obtaining one *L. braziliensis*, eight *L. infantum*, three *L. major* and one *L. panamensis*. Both the analyzed prototype and Sacace were able to detect *Leishmania* DNA for the 13 samples, whereas the other method showed some negative results, with no species-related sensitivity loss. The prototype under study showed Cohen's kappa coefficient values of 0.492 and 0.699 with STAT-NAT and Sacace, respectively. **Conclusion:**

The new prospective molecular assay fulfils the criteria of accuracy, efficiency, sensitivity, specificity, ease of handling and allows to detect the main species causing tegumentary leishmaniasis. In addition, compared with the analyzed reference commercial assays, similar results were obtained between tests with the same target (18S rRNA): Sacace and the tested prototype. On a side note, STAT-NAT was optimized for detection DNA parasite in blood samples. A highlight feature of the prototype was its lyophilized format, minimizing manipulation and allowing storage and transport at room temperature, with no need of refrigerated containers and taking no space in refrigerators and freezers of the laboratory.

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Evaluation of a new real time PCR assay directed to SARS-CoV-2 UK variant detection as compared with initial methods following B.1.1.7 variant emergence and characterization

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Background: In December 2020, the UK declared a new SARS-CoV-2 variant with a higher transmission capacity. Among the mutations of the B.1.1.7 variant, one of the most characteristic is the deletion 69-70 in the S gene. This variant is one of the greatest concerns of epidemiologists and those responsible for Public Health, therefore, the European Center for Infectious Diseases recommended close monitoring of the evolution of this variant. **Methods:** In Spain, following the instructions of the Ministry of Health, the epidemiological surveillance of B.1.1.7 is carried out mainly with two strategies with SARS-CoV-2 positive cases with Ct values lower than 30: 1) 1-2% of positive cases are sent to sequence, 2) All positive samples are analysed with the second molecular assay TaqPath COVID-19 test and if no amplification of the *S* gene is obtained (based on the deletion 69-70 in the S gene), the nucleic acid is sequenced. A group of 175 samples diagnosed with these algorithms were analysed with the prototype VIASURE SARS-CoV-2 UK variant (S_{uk}, *S* and *N* genes) Real Time PCR Detection kit which comprises the amplification of the B.1.1.7 *S* gene, two different targets of the non-UK variants and the *RNAse P* as endogenous control. **Results:** Following initial diagnosis, 20 of the 175 samples were SARS-CoV-2 negative, 70 were SARS-CoV-2 non-B.1.1.7 and 85 were SARS-CoV-2 B.1.1.7 variant. From the non-B.1.1.7 group, the prototype detected two samples as B.1.1.7 and from the B.1.1.7 group, two samples as non-B.1.1.7. The result of these four samples was confirmed by sequencing. Therefore, the TaqPath COVID-19 assay gave false negative due to amplification of the *S* gene and false positive due to lack of amplification on the *S* gene. **Conclusion:**

Although initially the TaqPath COVID-19 assay helped to monitor the B.1.1.7, the algorithms used left a large group of samples out of epidemiological follow-up (Ct>30). Furthermore, two PCRs and a subsequent sequencing must be performed, which implies time-consuming that, given the current situation, is not feasible. The developed prototype proved to be as sensitive and more specific than the reference assay, detecting SARS-CoV-2 and directly identifying the UK variant.

B-206

Diagnostic performance and validation of two point of care assays for detection of SARS CoV2

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Background: The detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by reverse transcription polymerase chain reaction (RT-PCR) is the primary method to diagnose coronavirus disease 2019 (COVID-19). Over the last few months, several point-of-care (POC) testing systems have gained health Canada approval. Some POC assays rely on qualitative and quantitative viral nucleic acid detection using RT-PCR or loop-mediated isothermal amplification (LAMP), while others rely on qualitative detection of viral antigens such as nucleocapsid antigen, using chromatographic immunoassay. It is unclear how these assays compare to each other. This work focuses on validating two POC assays and comparing their characteristics with our routine molecular diagnostic method.

Methods: Nasopharyngeal (NP) swabs in Yocon® VTM were collected from 80 patients presenting to community assessment centers. These samples were tested using a routine lab-developed and validated RT-PCR assay using ToughMix Ultraplex master mix (Quantabio®) and N/E/RNaseP primers (IDT). The bKIT Virus Finder COVID-19 kit used with the Hyris bCube miniaturized RT-PCR (Hyris^{LTD}) and SARS-CoV-2 rapid antigen assay (Roche) were evaluated as rapid POC assays in this study. Precision and sample stability were assessed using patient samples. A comparative study between two POC assays and the in-house routine molecular method was performed with 49 samples (25 positive and 24 negative samples by routine RT-PCR method). The level of agreement between assays was analyzed by the EP Evaluator® using the scoring method and Cohen's kappa coefficient.

Results: Precision was acceptable, and samples were stable for up to 5 days with both POC methods. When comparing the POC to routine PCR analysis, positive samples with a high viral load(Ct values <27 by RT-PCR) showed a 100% agreement on both POC assays. Positive samples with Ct ≥ 27 gave either negative or inconclusive results on POC assays. When comparing Hyris POC molecular assay to routine RT-PCR, the concordance between the two assays was 76.6 % (Cohen's k=60.7%, 95 % CI: 40.4-81.0%, n=47). The Roche rapid POC antigen assay had 87.5% agreement with routine RT-PCR assay (Cohen's k= 75%, 95% CI: 60.5-89.5%, n=80). When the same samples were tested on both POC assays, Hyris POC had a sensitivity of 90.48% (95% CI: 69.62%-98.83%, n= 39, 10 samples were excluded due to inconclusive results), whereas the Roche rapid antigen test had a lower sensitivity of 72% (95% CI: 50.61%-87.93%, n= 49). The specificity was high at 100% and 91.67% for Hyris and Roche POC assays, respectively. The Hyris POC samples with inconclusive results remained inconclusive despite repeated testing. For the Roche rapid POC antigen test, 2 out of 40 confirmed negative samples were falsely positive, which could be due to heterophile antibodies presence.

Conclusions: POC tests evaluated here serve as a tool to increase the speed and capacity of SARS-CoV-2 testing. Both POC assays showed an excellent correlation with routine RT-PCR tests for positive samples with high viral load. Although the Roche rapid antigen test provided a faster turnaround time than Hyris bKIT RT-PCR, both POC assays will add value by decreasing central laboratories' burden.

B-207

Validating Abbott ID NOW for COVID 19 During a Period of Low Disease Prevalence

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Background: Abbott's waived SARS-CoV 2 assay is performed on the ID NOW™ Instrument (IDNI) and is a rapid molecular in vitro diagnostic test using isothermal nucleic acid amplification technology. The Point of Care Department for Baystate Reference Laboratories was tasked with validating the IDNI test platform in the background of a low regional rate of COVID 19 infection. Hence, a source of purified intact viral particles was needed for positive samples. ZeptoMetrix (ZM) NATtrol™ SARS-CoV-2 External Run Control (Neat/Diluted) was used in lieu of fresh positive COVID 19 patient samples. **Methods:** Nasal swabs were used to procure the samples. To simulate positive patient samples (24), approximately 50 uL of freshly vortexed ZM positive control material (Neat/Diluted) was absorbed ("collected") by each nasal swab. Asymptomatic employee volunteers were used for negative samples (19), and underwent anterior nares sampling via nasal swab using the CDC recommended SARS-CoV 2 collection protocol. Samples were run in parallel on the IDNI and Cepheid Xpert Xpress® platforms. Two test scenarios were utilized to validate the IDNI: 1) Dry Nasal swab introduced to the IDNI *immediately* following "collection" and 2) Dry Nasal swab introduced to the IDNI following a *20 minute drying/waiting* period. For the Cepheid platform, the nasal swab samples were placed in 3.0 mL of VTM after sampling, either immediately or after 20 minutes of drying time. Then 200 uL (3333 viral copies) of the VTM/Sample mixture was introduced to the Cepheid platform for testing (per manufacturer). **Results:** The *Neat* ZM positive control material contained 25,000 viral copies/0.5 mL. Each swab absorbed approximately 50uL of freshly vortexed material or 2500 viral copies. Then, each swab was introduced to 2.5 mL of elution/lysis buffer in the Sample Receiver. The 2.5 mL of elution/lysis buffer containing the 2500 viral copies or 1000 viral copies/mL was then transferred to the Test Base, where 0.1 mL was used for amplification. Therefore, 100 viral copies were submitted per reaction. The *1:2 dilution* material contained 12,500 viral copies/0.5 mL. Each swab absorbed approximately 50 uL of freshly vortexed material or 1250 viral copies. Subsequently, each swab was introduced into 2.5 mL of elution/lysis buffer in the IDNI Sample Receiver. The 2.5 mL of elution/lysis buffer containing the 1250 viral copies or 500 viral copies/mL was then transferred to the Test Base where 0.1 mL was used for amplification. Therefore, 50 viral copies were submitted per reaction. Results from 24 positive samples and 19 negative samples demonstrated 100% sensitivity and 100% specificity when using Neat Positive Control and 1:2 diluted Positive Control compared to the Cepheid platform. **Conclusion:** Positive intact SARS-COV 2 control material may be used as a substitute for positive patient samples when validating IDNIs. This degree of validation provides assurance of the IDNI sensitivity when there is low prevalence of COVID 19 disease.

B-208

Detection of SARS-COV2 in saline solution gargle sample by RT-qPCR

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Background: The most often used and reliable clinical specimen for SARS-CoV-2 molecular detection is nasopharyngeal sample. However, acquiring a nasopharyngeal sample is not as easy as obtaining other types of specimens, such as gargle or mouthwash, which may result in suboptimal specimens, particularly if the specimens are

obtained by inexperienced personnel and the nasopharyngeal collec can cause coughing, increasing the risk of nosocomial spread of respiratory viruses during the collection procedure. Moreover, nasal swabs were in short supply during the SARS-CoV2 pandemics. We hypothesized if saline solution mouthwash or gargle could be a good candidate as an alternative primary sample in order to mitigate all these sample collection difficulties in the SARS-CoV2 detection process. The use of gargle samples for SARS-CoV2 detection would also allow for self-sampling. Thus, here, we investigated the diagnostic agreement between 0.9% saline solution gargle samples with nasopharyngeal samples for qualitative detection of SARS-CoV-2.**Methods:**Nasopharyngeal samples, in 0.45% saline as transport medium, and paired 0.9% saline solution gargle samples were collected from 80 volunteers referred to our laboratory for SARS-COV2 molecular detection. All samples were submitted to the fully automated Roche Cobas 6800 SARS-CoV-2 test (Roche, Pleasanton, CA, USA). The applied test is a single-well dual target assay targeting ORF1, a nonstructural region that is unique to SARS-CoV-2, and a conserved region in the structural protein envelope E gene for pan-sarbecovirus detection. The total, positive and negative agreement between both specimens were computed along with their respective 95% confidence interval using the online Westgard QC 2x2 Contingency calculator (http://tools.westgard.com/two-by-two-contingency.shtml). RT-PCR quantitative cycle (Cq) difference (gargle sample Cq value minus nasopharyngeal sample Cq value) was calculated for all samples that tested positive in both specimens. The median, minimum and maximum Cq differences were presented and compared.**Results:**Valid results were obtained for both nasopharyngeal and gargle samples from all tested volunteers. All specimens that tested negative for SARS-COV2 in the nasopharyngeal sample also tested negative for the saline solution gargle sample. Twelve out of fourteen positive nasopharyngeal samples also tested positive in the gargle sample. The total, positive and negative agreement between both tested specimens were 97.5% (95%CI: 91.3 - 99.3%), 85.7% (95%CI: 64.0 - 96.5%) and 100% (95%CI: 94.3 - 100%), respectively. Considering the twelve samples that tested positive in both specimens the median, minimum and maximum Cq differences were 9.21 (1.24-15.71) for the target ORF1 and were 10.25 (0.95-17.14) for the target E. Nasopharyngeal samples always presented lower Cq values.**Conclusion:**Saline solution gargle samples presented a highly acceptable total agreement with nasopharyngeal samples for the SARS-COV2 detection by RT-qPCR (97.5%). Negative agreement was complete and positive agreement was substantial (85.7%). Saline gargle samples Cq values were lower than nasopharyngeal samples in all instances indicating decreased viral load in this specimen which could explain the lower positive agreement between them. However, the use of gargle samples allows for self-sampling dismissing the complexities associated with nasopharyngeal sampling being a suitable alternative specimen for SARS-COV2 detection in remote areas or at home avoiding the need to go to a sample collection unit.

B-209

Ultrasensitive Determination and Tracing of Cellular MicroRNA In-Situ

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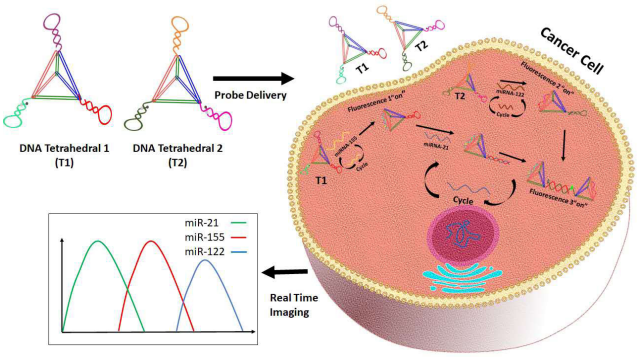
Background: Breast cancer is considered as highest cause of death in women globally and microRNAs are promising biomarkers in prognosis and diagnosis of breast cancer. But tracking multiple intracellular microRNAs *in-situ* at real time is particular challenging owing to its short length and low abundance. **Methods:** We deliberately designed a pair of DNA tetrahedrons with rigid geometry for higher cellular membrane penetration efficiency. With fluorescently labelled catalytic hairpins (CHA) pairs in each vertex for *in-situ* visualization of microRNAs, the proposed system was validated by *in-situ* molecular imaging of three representative microRNAs: miR-21, miR-155, and miR-122. We evaluated the feasibility of the proposed approach by comparing the analytical results from clinical samples with RT-qPCR results.**Results**: We designed two DNA tetrahedrons with fluorescently labelled three pairs of CHA at the terminal of each vertex. The presence of specific microRNAs bridges the two tetrahedron scaffold to become a dimer while the hairpin loop would be opened and fluorescence "on" could be readout (Figure1). Additionally, a target concentration dependent FI increased linearly in the range from 10pM to 50nM and a detection limit of 1-10 fM was observed. The cell membrane penetration of DNA tetrahedron was validated by delivering the mere CHA probes or designed origami into MCF-7 cell. We utilized the nanoprobe to test the artificially modulated miRNAs and the results indicated that identical results were observed for both the proposed method and RT-qPCR. **Conclusion**: We described a sensitive, specific, and non-destructive dual tetrahedron CHA for multiplexed imaging of microRNAs *in-situ*. By utilizing a DNA origami, a CHA probes can expediently penetrate the cells, enabling the detection of various microRNAs profiles simultaneously. This method provides a novel strategy to investigate microRNAs abundance in real time and explores subsequent clinical applications.



B-210

Validation of chromosomal microarray analysis in a clinical setting

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Introduction:

Chromosomal microarray analysis (CMA) is a wide-genome screening method that allows the identification of submicroscopic chromosomal imbalances and has been considered as a first-tier test to detect copy number variations (CNVs) in multiple congenital anomalies and intellectual disabilities. Clinical laboratories are incorporating the method into its test portfolio. However, careful validation of the assay and adherence to international standards and guidelines is essential in order to provide a reliable CMA. Thus, the aim of this work was to validate the CMA in our clinical laboratory setting.

Methods:

A total of 18 blood samples with previously known results were used in this validation. Fourteen samples which CMA was outsourced to a reference laboratory (4 samples with 1 microdeletion and 1 microduplication, 5 samples with 1 duplication, 2 samples with 2 microdeletions, 1 sample with 1 deletion, and 2 samples with no alterations, accounting for 18 micro alterations on total) and 4 with altered G-band karyotype (2 deletions, 1 duplication, and 1 balanced rearrangement (to demonstrate the known limitation of the CMA method), accounting for 3 alterations on total. Blood was submitted to Pharm Lyse solution (Becton Dickson) in order to separate the leukocytes from red blood cells and DNA was extracted using the kit QIAamp DNA Mini Kit (QIAGEN) according to manufacturer instructions. Applied input DNA concentration ranged from 45-60 ng/µ;L and, when necessary, DNA samples were concentrated using KAPA Pure Beads (Roche). All samples were submitted to CytoScan 750K Suite (Thermo Fisher Scientific) performed according to manufacturer instructions. CytoScan 750K is composed of 750,000 probes (200,000 for single nucleotide polymorphisms and 550,000 for non-polymorphic sites), allowing CNV and loss of heterozygosity detection. Analysis and interpretation of the data were performed on Chromosome Analysis Suite following international standards and guidelines. The sensitivity and the positive predictive value (PPV) of the proposed method were computed at CNV and final report level considering the previously known results as the true (total of 21 CNV and 18 patients).

Results:

The proposed method was able to detect 19 out of 21 (90.5%) expected alterations and classify correctly 17 out of 18 patients (94.4%). A discrepant result was in one sample with 2 microdeletions (90 and 86 kb, respectively) that tested negative by the tested method. The assay sensitivity and predictive positive values at CNV level were 90.5% and 100%, respectively, and at final result level the sensitivity was 93% and PPV was 100%. The case of balanced rearrangement returned negative results demonstrating the limitation of the CMA. The two negative results were concordant in both methods.

Conclusion:

Sensitivity and PPV values at CNV level and at final report level observed in this CMA validation indicate that the assay is acceptable to be offered to our patients. The origin of the unique discrepant result in this validation can be the QC metrics and filter parameters used in the CNV calling process of each laboratory. This sample will be submitted to a third laboratory to resolve the dispute.

B-211

Upgrading a previous validated RT-qPCR assay for SARS-CoV-2 detection

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Background:In February 2020, our laboratory started to offer an RT-qPCR assay for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The validated method was a single SARS-CoV-2 target assay based on N1 primers/probes described by the Centers for Disease Control and Prevention (US-CDC) multiplexed with RNAse P assay as internal control. N1 assay presented superior analytical sensitivity and specificity compared to other targets in our validation. However, SARS-CoV-2 can mutate during its adaptation to new hosts and environmental conditions and these mutations can potentially disrupt RT-qPCR primers binding sites leading to false positives results. Thus, the aim of this validation was to add a second SARS-CoV-2 RT-PCR target into our method upgrading it to a single-well multiplex dual target assay to preventively avoid false-positives results. **Methods**:Left-over nasopharyngeal swabs samples (n=375, 100 positives and 275 negatives) referred to our laboratory for SARS-CoV-2 RNA detection were used in assay upgrading. The reference method was N1 assay from US-CDC protocol. The candidates for second assay were N2 (from US-CDC protocol) and UTR assay (this study). The reactions were performed with LightCycler® Multiplex RNA Virus Master on LightCycler 480 II according to the manufacturer instructions (Roche). Tested assays combinations were: 1) N1 *versus* N2 and N1 *versus* N1 + N2 using 188 samples (66 positives and 122 negatives) and 2) N1 *versus* UTR, and N1 *versus* N1 + UTR using 186 samples (33 positives and 153 negatives). N1 was labeled with FAM, N2 and UTR with CY5 and RNAseP assay labeled with HEX was used as internal control in all instances. The total, positive and negative agreement between each comparison were computed and presented considering N1 as a reference method. **Results**The N1 *versus* N2 assays comparison returned total, positive and negative agreements of 187/188 (99.5% - 95%CI 97-99.9%), 65/66 (98.5% - 95%CI 91.9-99.7%), 122/122 (100% - 95%CI 96.9-100%), respectively. The N1 *versus* N1 + N2 assays comparison returned total, positive and negative agreements of 186/188 (98.9% - 95%CI 96.2-99.7%), 65/66 (98.5% - 95%CI 91.9-99.7%), and 121/122 (99.2% - 95%CI 95.5-99.9%), respectively. The N1 versus UTR assays comparison returned total, positive and negative agreement of 184/187 (98.4% - 95%CI 95.4-99.5%), 31/34 (91.2% - 95%CI 77-97%), 153/153 (100% - 95%CI 97.6-100%), respectively. The N1 *versus* N1 + UTR assays comparison returned total, positive and negative agreement of 184/187 (98.4% - 95%CI 95.4-99.5%), 31/33 (93.9% - 95%CI 80.9-98.3%), 153/154 (99.4% - 95%CI 96.4-99.9%), respectively. **Conclusion:**Both N2 and UTR assay presented have very good agreements with N1 assay. A slightly higher agreement with N1 in positive samples was observed for N2 (98.5%) compared to UTR (91.2%). In the experiments evaluating the introduction of a second assay to the N1 reaction configuring a multiplex reaction this trend was maintained, both multiplex (N1+N2 and N1+UTR) showed an overall very good total agreement, but N1+N2 (98.5%) presented a higher agreement in positive samples for compared to N1+UTR (93.9%). In conclusion, US-CDC N2 assay was chosen as the second assay in our RT-qPCR for SARS-CoV-2 detection.

Preanalytical and Postanalytical

B-212

Distributions of potassium (K^+) and hemolysis index (HI) among reported and cancelled K^+measurements: comparison between Emergency Department and inpatients

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Background: At our institution, plasma K^+ measurements (Roche Cobas) are cancelled for samples with hemolysis index (HI) > 100. For the Emergency Department (ED), cancellation variably affects between 4-6% of all K^+ samples submitted, compared to 1-2% for all other inpatients (IP). Recently, ED services requested the laboratory to report all K^+ measurements. Distributions of K^+ and HI among cancelled K^+ measurements were previously unassessed. We documented distributions for all measured K^+ and HI among all ED samples, and compared these to distributions for measured K^+ and HI among all IP specimens. **Methods**: Data for K^+ and HI were obtained for a one-month interval (Dec 2020). Records for reported K^+, and for reported and cancelled HI, were available from hospital information system reports. K^+ measurements cancelled due to hemolysis were retrieved manually from Roche instrument records. Statistical analyses were performed by programming in Excel, Python, or R. **Results**: ED data comprised 4132 measurements, with 230 (5.6%) cancellations. IP data comprised 11862 measurements, with 165 (1.4 %) cancellations. For ED, median age was 57 years, with 47% female; for IP, median age was 62, with 41% female. For distributions of reported K^+, average values (X) were nearly identical for ED (X=4.06 mmol/L) and IP (X=4.08 mmol/L) ($p>0.05$). For distributions of cancelled K^+, X was also nearly identical for ED (X=5.22 mmol/L) vs. IP (X=5.29 mmol/L) ($p>0.05$). For distributions of HI among reported specimens, the average value (Y) for ED (Y=15.3) was greater than that for IP (Y=8.7) ($p<0.05$). For distributions of HI among cancelled specimens, Y for ED (Y=201) was instead less than that for IP (Y=235) ($p<0.05$). For ED, 43% of cancelled K^+ were below the upper limit of the reference range (ULRR) for plasma K^+ (4.8 mmol/L). For IP, 40% of cancelled K^+ were of this classification. Note that such results would generally rule out hyperkalemia despite elevated HI. Empirically, separation of K^+ distributions between reported and cancelled K^+ measurements for ED was consistent with an average effect of hemolysis to increase K^+ by a factor F=0.6 mmol/L K^+ per 100 HI. For IP, data were consistent with F=0.53 mmol/L K^+ per 100 HI. These values were both high in comparison to the range of values for F reported in the literature (0.3-0.5 mmol/L K^+ per 100 HI). **Conclusions**: Correspondence of both reported and cancelled K^+ distributions between ED and IP suggest that they were derived from the same underlying basis distribution for non-hemolysed plasma K^+ in both settings. For cancelled K^+, HI data show a greater average driving force of hemolysis to elevate K^+ for IP samples compared to ED samples, but applied to a lesser fraction of samples submitted. For both ED and IP, approximately 40% of cancelled K^+ were below the ULRR for plasma K^+, warranting consideration of unrestricted reporting for this class of results. For both ED and IP datasets, values deduced for the apparent average effect of hemolysis on K^+ (F = delta K^+ per delta HI) were high compared to literature values.

B-213

Automated Sample Interference Indices on the Alinity c System

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Background: The Alinity c system Sample Interference Indices (HIL) provides a more accurate and consistent method for interpretation of interferents than time consuming visual interpretation. The Alinity c system uses specific wavelength pairs and an algorithm to provide a Sample Interference Index that can correlate with sample interference due to hemolysis, bilirubin and turbidity present in serum/plasma samples. From a single aspiration from a sample, the individual or all 3 indices can be selected to be reported. These values in combination with assay specific endogenous interference can be used to determine the potential for HIL interference in the Alinity c clinical chemistry assays.

Methods: The HIL methodology will be reviewed and discussed. The study followed Clinical and Laboratory Standards Institute (CLSI) protocol EP7-A2. Interferences were studied up to concentrations of 1000 mg/dL for hemoglobin, 30 mg/dL for Bilirubin (unconjugated) and 1000 mg/dL for triglycerides in serum. Serial dilutions of the sample pools were analyzed in replicates of 4 on the Alinity c system. A cumulative summary was compiled of the Hemolysis, Icterus and Lipemia indices for 80 Clinical Chemistry assays.

Results: Using known concentrations of hemoglobin, bilirubin and Intralipid, the Abbott Semi-Quantitative Index (concentration range in mg/dL) and Qualitative Index (Blank, 1+, 2+, 3+ 4+) were confirmed on the Alinity c system. Correlation studies show a linear relationship ($r = 1.0$) of the indices with increasing concentration of analyte. An easy to use guide was created that combines the HIL Qualitative and Quantitative Index scores with the assay specific interference results to provide a guide to potential interferents for the Alinity c clinical chemistry assays. This guide provides background information on the causes of HIL interferences, conversion factors from Conventional to SI units and denotes the specific concentrations of interferents which could lead to an over or under estimation in the presence of hemoglobin, bilirubin and/or Lipemia. In addition, the effects of the influence of two or more HIL interferents on assay reported results is discussed. The majority of the 80 clinical chemistry assays were not compromised using samples with elevated H, I or L index values.

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

B-214

Compatibility of BD Vacutainer Urine Preservative Tubes with the CLINITEK Novus Automated Urine Chemistry Analyzer

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Background:Urinalysis is time-sensitive: it is recommended that urine samples be preserved through refrigeration, freezing (not suitable for all analytes) or chemical preservatives if not analyzed within two hours of collection. Commercially available preservative tubes are a convenient way to preserve samples, which adds flexibility to the busy clinical lab. However, preservatives are potential interferents for urinalysis, so the compatibility of a particular preservative and urinalysis method needs to be assessed.BD Vacutainer Urinalysis Preservative Plus Conical Urine Tubes were tested for compatibility with the CLINITEK Novus Automated Urine Chemistry Analyzer in this study, using contrived clinical urine specimens. Methods:Urine pools were prepared at positive and negative levels for all analytes measured by CLINITEK Novus 10 Reagent Cassettes. Twelve (12) replicates of each pool were tested on the CLINITEK Novus Analyzer in BD Vacutainer Urinalysis Preservative Plus Conical Urine Tubes and standard urine tubes without preservative. Performance (in terms of semi-quantitative or qualitative "block" outputs) for each analyte level was compared between BD Vacutainer tubes and tubes without preservatives. Results:BD Vacutainer Urinalysis Preservative Plus Conical Urine Tubes caused a slight increase in pH (within one color block) for one tested sample. No changes in clinical block output were observed for other analytes.Conclusion:Results indicate that BD Vacutainer Urinalysis Preservative Plus Conical Urine Tubes are suitable for use on the CLINITEK Novus Automated Urine Chemistry Analyzer.

B-215

Storage of FiT samples at fridge temperature for 5-6 days may impact the clinical interpretation based on analytical precision and chosen cut-point.

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Background: Faecal immunochemical testing (FiT) for colorectal cancer screening is now used in most developed countries. It has been demonstrated that storing the collection devices for OC-Sensor Diana (Eiken Chemical Co Ltd, Tokyo Japan) at 2-8 °C, maintains haemoglobin (Hb) stability for approximately 37 days. For laboratories with lower volumes of testing, the ability to store samples and analyze in batches is an important consideration. In our high volume facility, we had the opportunity to perform repeat analysis on a large number of samples, approximately one week after initial analysis.Methods: Samples were collected by residents in the province of Ontario, who were eligible for the colon cancer screening program. Collection kits with detailed instructions are mailed out and returned via Canada Post, or dropped off at one of 219 sites across the province. Samples are received and analyzed on the same day in the laboratory. Analysis is performed on one of three, OC-Sensor Diana instruments. As part of troubleshooting a technical issue on one of the instruments, we stress tested the instrument with a batch of approximately 496 samples on 6 different days using randomly selected samples that had been previously reported. Results: 2976 paired samples were available for analysis. The samples had been stored at fridge temperature for 5-6 days after analysis in the laboratory. The original results ranged from 0 ng/mL to 939 ng/mL, the paired analysis demonstrated significant differences in mean 1.6 (1.4-1.9) ($p < 0.0001$) and median -1.0 (-2.0 - 1.0) and IQR -4.0 to -1.0 ($p < 0.0001$). Using the recovery targets of 80%, 70% and 50% as suggested by Catomeris *etal*, we found median recovery of 92% (91.5-93.2) and IQR 78%-109% with the 0.1 quantile at 64% and 0.3 quantile at 81%, demonstrating that at least 70% of samples meet the 80% recovery target and at least 90% the 50% target. Using the dataset to probe the clinical cut points of 50, 75, 100 and 150 ng/mL there are significant differences for the 50 and 75 ng/mL ($p < 0.001$) cut-points and not at the 100 and 150 ng/mL cut points ($p = 0.32$ and 0.06 respectively). If we allow for "optimal" analytical variation of 10% in the repeat sample, then the lower cut-points have no significant differences while the higher cut-points become significantly different (≥100 ng/mL $p = 0.003$ and ≥150 ng/mL $p = 0.002$).

Conclusion: The storage of samples at fridge temperature has been deemed an acceptable practice using percent recovery from original spiked concentration of Hb. In this evaluation of in-laboratory storage of patient samples, the degradation of Hb at fridge temperature over 5-6 days suggests that the storage of samples in a laboratory prior to analysis, may impact the interpretation of the result and this will be dependent on the chosen cut-point and the imprecision of the method.

B-216

Evaluation of the Tasso-SST and TAP-II Commercial Automated Devices to Collect Capillary Blood For Detection of Tetanus Toxoid and SARS-CoV-2 Antibodies

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Background: Capillary blood is a suitable specimen for detecting and measuring a variety of analytes, including antibodies to infectious agents. New commercial devices such as the Tasso-SST (Tasso, Inc.) and TAP®-II (Seventh Sense Biosystems, Inc.) allow for collection of up to 500µL of liquid capillary blood from the upper arm, potentially providing sufficient volume for use with automated test systems. These devices are relatively simple to use and are suitable for self-collection. We evaluated the use of capillary blood collected using the Tasso-SST and TAP-II devices for detection of antibodies to SARS-CoV-2 and Tetanus Toxoid and compared the results to those obtained by routine venous blood testing. We also evaluated the ease-of-use of the devices.

Methods: Trained phlebotomists obtained three consecutive blood specimens during a single visit from healthy donors: TAP II (Lithium heparin tube), Tasso-SST (serum separator tube), and a venous blood draw (serum separator tube). Specimens were transported to the laboratory at ambient temperature, spun within 20 hours of collection and stored refrigerate for up to 5 days prior to testing by all assays. Plasma (TAP-II) or serum was tested by the Euroimmun anti-SARS-CoV-2 and anti-Tetanus Toxoid IgG enzyme-linked immunosorbent assays (ELISAs; Euroimmun Inc., Lubeck, Germany) using the Dynex Agility (Chantilly, VA) automated ELISA processors. The volume collected by each device was recorded, along with any technical difficulties experienced in collecting or accessing the capillary blood.

Results: At least 20 µL of serum or plasma was successfully collected from 45 (80.4%) and 36 (62.3%) of the 56 donors by the Tasso-SST and TAP-II, respectively. All venipunctures acquired at least 750 µL of serum, meeting the minimum 300 µL requirement for testing on the Agility platform. In contrast, none of the TAP-II collected samples and only one sample collected by the Tasso-SST had sufficient volume for automated testing on the Agility platform. Mean and median volumes of serum or plasma obtained were 80.7 and 100 µL using the Tasso-SST, and 74 and 50 µL using the TAP-II, respectively. Laboratory technologists reported that following processing of the microtubes, sample was easier to obtain from the Tasso-SST than the TAP-II. All SARS-CoV-2 antibody tests were negative, while all tetanus antibody tests were positive.

Conclusion: Capillary blood arm collection devices show promise for semi-automated, at-home collection, but suffer from high failure rates and do not provide adequate volume for use on most automated systems.

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Evidence-Based Harmonization of Adult Reference Intervals Across Canada using Big Data Analytics: A Report of the CSCC Working Group on Reference Interval Harmonization (hRI)

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Background: Marked variation in reported reference intervals for many well-standardized laboratory tests continues to exist across clinical laboratories, increasing the risk of inaccurate and inconsistent test result interpretation. The Canadian Society of Clinical Chemists (CSCC) Working Group (WG) on Reference Interval Harmonization (hRI) aims to establish harmonized reference intervals for key laboratory tests and support their implementation across Canada. Harnessing the power of a novel big data analytics approach, both direct (healthy adult population data) and indirect (outpatient lab data) were examined from across Canada. Common reference ranges were

found to be feasible and were established for 17 routine biochemical markers. **Methods**: Retrospective laboratory data was extracted from four community reference laboratories across Canada over a two year period (Jan 2018-Dec 2019), including: 1) DynaLIFE Medical Labs (Alberta, Siemens Advia), 2) LifeLabs (British Columbia, Roche Cobas & Abbott Architect), 3) LifeLabs (Ontario, Roche Cobas & Abbott Architect), and 4) Dynacare (Ontario, Roche Cobas/Modular). Data was extracted for the following 17 assays: alanine aminotransferase, albumin, alkaline phosphatase, lactate dehydrogenase, total bilirubin, total protein, phosphate, calcium, creatinine, free T3, free T4, thyroid stimulating hormone, sodium, potassium, magnesium, total CO2, and chloride (serum). Age-, sex-, and center-specific differences for each laboratory test were assessed using the Harris and Boyd method, as recommended by CLSI guidelines. Age and sex partitions were established accordingly and outliers were subsequently removed. Reference intervals were then established based on the indirect truncated maximum likelihood (TML) method. Results were compared to direct data from healthy Canadian volunteers and common reference intervals were proposed taking health-associated data and clinical expertise into consideration. **Results**: Initial datasets from community reference laboratories were comprised of up to 14 million test results in total per assay. Big data analysis revealed few statistical differences between laboratory centers/provinces using different analytical platforms. The minor statistical differences observed between provinces or analytical platforms were found not to be clinically significant based on analyte-specific reference change values (RCVs), supporting the feasibility of RI harmonization. Considering both direct and indirect data sources, common sex-specific hRIs were established for the following tests: alanine aminotransferase, alkaline phosphatase, creatinine, and total bilirubin. Age-specific hRIs were recommended only for alkaline phosphatase. Common reference ranges for other assays required no age- or sex-specific partitioning. **Conclusions**: The current report describes a novel approach to RI harmonization developed by the CSCC hRI WG, including: (1) extraction of data from community reference laboratories across Canada, (2) assessment of outliers, (3) statistical evaluation of age, sex, and center-specific differences, (4) derivation of preliminary hRIs using the TML method, and (5) comparison of established hRIs to direct data in the healthy Canadian population. RI harmonization in clinical laboratories will directly contribute to more accurate test result interpretation and improved clinical decision making and patient outcomes. Future work is also planned to expand this approach to other analytes of interest.

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Emergence of New Pre-Analytical Errors in the Total Testing Process: Identify, track, and solve transient errors

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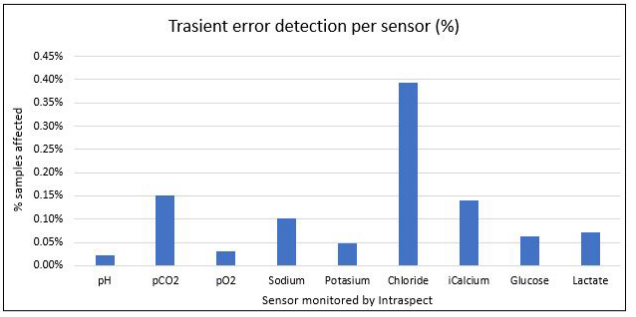
Background Since the seminal presentation by Plebani and Carraro [1] called attention to the crisis of pre-analytical errors, great strides have been taken in the improvement of the total testing process. IntraSpect™ is a patient-based real time quality control (PBRTQC) technique, based on proprietary technology, that takes 15 measurements of each patient sample, characterize the sensor signal and flag any result that does not meet the expected behavior. These flags identify transient errors that impact only single patient specimens and are virtually impossible to catch with traditional statistical quality control (QC) or even by traditional electronic checks. **Methods** The QC solution of the GEM Premier 5000 blood gas analyzer (intelligent quality management 2 [iQM2®]), which includes IntraSpect, has been used to monitor and flag transient errors caused by potential pre-analytical factors from numerous institutions worldwide. Data on 1,013,391 patient samples collected from 4,985 cartridges out of 2,765 instruments were used in this analysis. **Results** iQM2 detected errors derived from transient factors in 8,240 samples (0.81%). A wide range of sensors were affected by transient errors, which included those using different measuring principles (e.g., potentiometric, amperometry, diffusion-controlled). Error detection ranges varied from 0.02% for the pH sensor to 0.39% for the chloride sensor. Results are summarized in Table 1. **Conclusion** Transient errors caused by pre-analytical factors are present in the regular clinical environment. iQM2 can detect transient errors, where traditional QC cannot, nor can it be modified to detect these errors. The only way to detect transient errors is to build an individual patient specimen check into the testing process. Now that it is possible to detect these "new" errors, laboratories can no longer ignore them. Even if undetectable, these errors are occurring. New efforts must be martialed to monitor, manage, and minimize transient errors.

Table 1. Summary data for iQM2 detecetd errors caused by a transiant factors (samples n = 1,013,391)



	Sensors monitored by Intraspect								
	pH	pCO2	pO2	Sodium	Potassium	Chloride	iCalcium	Glucose	Lactate
Number of detected errors	225	1526	303	1019	490	3987	1418	633	718
% results affected	0.02%	0.15%	0.03%	0.10%	0.05%	0.39%	0.14%	0.06%	0.07%

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Emergency Department Hemolysis Incidence Affected by Changes in Sample Handling and Quality Cutoffs

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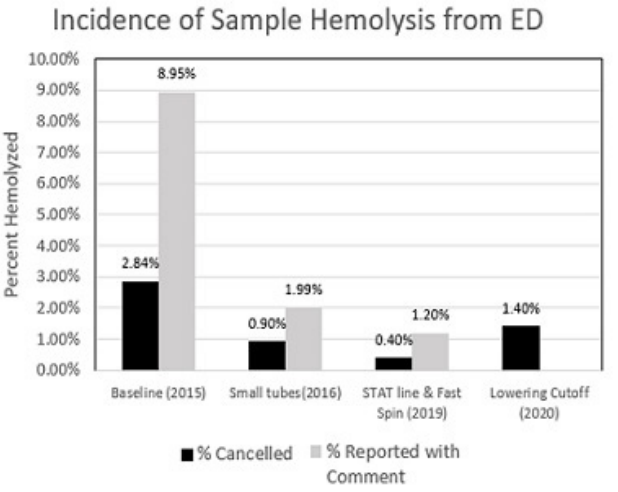
Background: Lab sample hemolysis plays a significant role in patient care and flow, especially in emergency departments, which often submit the largest percentage of hemolyzed samples to the hospital lab. We tracked the incidence of hemolyzed blood samples arriving from a large, tertiary care emergency department to the automated chemistry lab over several years, while various quality and process improvement initiatives were implemented. Methods: Data was obtained from the LIS and business intelligence software. The baseline process consisted of sample collection in 6 mL lithium heparin plasma vacutainer tubes, analyzed on Roche c702 chemistry analyzers. Hemolysis index (HI) was measured on any potassium order. Samples were centrifuged for 6 minutes at 1,959 g. Potassium results in samples with HI<90 were reported without quality flags. For samples with 90≤HI<300, potassium results were reported with a cautionary comment that hemolysis likely elevated the potassium result. When HI≥300, the potassium results were cancelled. Plasma tubes were changed to 2mL tubes in 2016. In 2019, process improvement projects to reduce TAT resulted in faster centrifugation times (4,000 g for 3 min) and addition of a dedicated STAT line for samples from the ED. In 2020, potassium results were cancelled when HI≥120, and the cautionary comment for 90≤HI<300 was eliminated. Results:



Conclusions: Switching to smaller tubes resulted in an immediate reduction in hemolyzed samples. The process improvements in 2019 may have contributed to the further reduction in hemolysis incidence, but these causes are inferred (hypothesized) due to the duration of implementation and possible split workflows at times. Of note, the more vigorous centrifugation did not increase hemolysis. As expected, lowering the

cutoff threshold increased the incidence of cancelled potassium results. The increased cancellation rate had other effects, including a significant increase in notification phone calls from the lab to the ED.

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Comparison of the 25-Gauge BD Vacutainer® UltraTouch™ Push Button Blood Collection Set with PentaPoint™ 5-Bevel Needle to a Standard 23-Gauge Butterfly Needle in an Adult Cancer Population

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Background: Phlebotomy is one of the most commonly invasive procedures performed in healthcare and can be a significant source of patient discomfort. Generally, higher gauge needles are less painful to patients, however, their smaller inner bore size can lead to greater instances of in-vitro hemolysis and other specimen integrity issues. 23-gauge needles are typically the highest gauge acceptable for routine phlebotomy. The BD Vacutainer® UltraTouch™ Blood Collection Set with PentaPoint™ 5-bevel needle and Right-Gauge™ technology is unique in that it is a 25-gauge needle with a decreased cannula wall thickness, affording an inner bore similar to that of a standard 23-gauge needle. The collection system includes a button-activated in-vein retraction mechanism to prevent accidental needle sticks. In this study, we compared the 25-gauge UltraTouch needle to the 23-gauge BD Vacutainer® Safety-Lok™ needle, assessing patient pain felt during phlebotomy, patient satisfaction, number of needle sticks required for a successful draw, and the resulting specimen integrity.

Methods: 200 male and 180 female oncology patients ages 18 and over were voluntarily recruited for the study and divided into two cohorts of 190 patients each. Each cohort had phlebotomy performed using the 25-gauge UltraTouch needle or a standard 23-gauge needle. After phlebotomy, each participant was asked to fill out a survey ranking the pain they experienced during the procedure, how many needle sticks were required for a successful draw, and their overall satisfaction. Pain was assessed from 0 - 10, where 0 is no pain and 10 is the worst pain imaginable. Patient satisfaction was also assessed from 0 - 10 where 0 is very dissatisfied and 10 is completely satisfied. In addition, sample integrity was assessed between the two collection systems by determining the presence of hemolysis in the sample by measuring the hemolysis index on the Abbott Architect c8000.

Results: No statistically significant difference in median pain scores was observed between the two cohorts, P-value: 0.068. However, as pain scores were strongly right-skewed in both cohorts, we split the ratings into 4 categories to afford a better analysis. Patients in the 23-gauge cohort more frequently reported 3+ pain than in the 25-gauge cohort, 14/190 compared to 5/190. Pain scoring 1 and 2 was reported more frequently in the 25-gauge cohort than the 23-gauge cohort, 84/190, compared to 54/190. Patients in the 23-gauge cohort more frequently reported a pain score of 0 than the 25-gauge cohort, 122/190 compared to 101/190. Stratification of the pain scores was statistically significant, P-Value: 0.003. No statistically significant difference in patient satisfaction or in number of needle sticks required was observed between the two cohorts, P-Value: 0.6 and 0.4. The 25-gauge needle did not show any increase in the rate of hemolysis, P-Value: 0.5.

Conclusion: Sample integrity was identical between both blood collection systems. Furthermore, the 25-gauge needle resulted in less 3+ pain during venipuncture. Paradoxically pain scoring 1 and 2 was reported more frequently in patient's in the 25-gauge cohort than the 23-gauge cohort. These findings are pertinent in cancer patients who require frequent blood draws.

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Reporting Potassium Results from Hemolyzed Specimens, Clinical Response and Patient Outcome

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Background: Preanalytical issues causing in-vitro hemolysis of specimens can falsely elevate potassium levels (pseudohyperkalemia). Our laboratory reports potassium results with an interpretative comment if specimen has mild/moderate hemolysis or cancels the potassium result if severe/gross hemolysis. Clinician interpretation and response to potassium values in a specimen with hemolysis can be a source of post-analytical error. We investigated the post-analytical interpretation of critical potassium values in both hemolyzed and non-hemolyzed potassium specimens. **Methods**: Institutional databases were searched and specimens were identified for each category as follows: mild/moderate hemolysis with critical hyperkalemia (>6 mmol/L), no hemolysis with critical hyperkalemia, and mild/moderate hemolysis with potassium within normal limits (3.7-5.1 mmol/L). Hemolysis level was determined by an automated hemolysis index (H-Index) where <90 was no hemolysis present, 90-300 was mild/moderate hemolysis present, and >300 was gross/severe hemolysis. Pertinent clinical information, time interval of redraw, and clinical outcomes were recorded. **Results**: As summarized in Table 1, data included 133 patients/specimens in group 1 (mild/moderate hemolysis with critical hyperkalemia), 146 specimens from 129 unique patients in group 2 (no hemolysis with critical hyperkalemia), and 150 patients/specimens in group 3 (mild/moderate hemolysis with normal potassium). Table 1 summarizes data on clinical outcome, draw location, redraw rate, and time interval of redraw. **Conclusion**: Specimens with critical hyperkalemia values (groups 1 and 2) are more often redrawn, and are more often redrawn within 24 hours, compared to specimens with hemolysis and normal potassium value (group 3). The similar redraw rates between groups 1 and 2 suggest that clinicians acted on critical values, regardless of specimen hemolysis. No morbidity or mortality was related to lack of redraw. In conclusion, data support that specimens with critical hyperkalemia were more often redrawn within 24 hours, regardless of specimen hemolysis.

Table 1. Summary of Clinical Characteristics of Patient Populations, Clinical Response, and Patient Outcome

	Group 1: Mild/ Moderate Hemolysis (H90-300) with Critical Hyperkalemia (>6 mmol/L)	Group 2: No Hemolysis (H<90) with Critical Hyperkalemia (>6 mmol/L)	Group 3: Mild/ Moderate Hemolysis (H90-300) with Normal Potassium (3.7 – 5.1 mmol/L)
Unique Patients	n = 133	n = 129	n = 150
Known History of Hyperkalemia, n (%)	Yes: 101 (76)No: 32 (24)	Yes: 88 (68)No: 41 (32)	Yes: 47 (31)No: 103 (69)
Unique Specimens	n = 133	n = 146	n = 150
Draw Location: Inpatient, n (%)	107 (80)	91 (62)	149 (99)
Draw Location: Outpatient or Emergency Department, n (%)	26 (20)	55 (38)	1 (1)
Total Redraws (%)	123 (92)	128 (88)	118 (79)
Redraw within 24 hours (%)	103 (77)	115 (79)	82 (55)

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Canada-Wide Harmonization of Adult Lipid Reporting in Clinical Laboratories: *Evidence-Based Recommendations of the CSCC Working Group on Reference Interval Harmonization (hRI)*

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Background: Through a national survey, the CSCC hRI Working Group highlighted extensive variability in lipid reporting between laboratories, including cut-offs and interpretive comments, despite Canadian guidelines on cardiovascular risk management. This inconsistency is likely due to the lack of laboratory implementation guidance provided alongside clinical guidelines. This study developed a recommended harmonized adult lipid report to support common lipid reporting across Canada. **Methods:** Using the 2021 Canadian Cardiovascular Society (CCS) guideline as the primary resource, a uniform reporting model for adult lipid parameters for all Canadian laboratories was developed. The recommended report outlines cut-offs to use to flag abnormal results, interpretive comments to include for each lipid parameter, and how to incorporate non-fasting cut-offs for triglycerides. The CSCC hRI Working Group, consisting of clinical and medical biochemists, consulted with physicians (e.g., family physicians, cardiologists, lipidologists) to finalize the harmonized lipid report. **Results:** The recommended harmonized adult lipid report is shown in the **table** below. Interpretive comments and flagging cut-offs for all lipid parameters are recommended to ensure rapid and consistent result interpretation. LDL-C, non-HDL-C, and apoB cut-offs are based on the treatment initiation for low and intermediate risk

patients from the 2021 CCS guideline. The total cholesterol, HDL-C, triglyceride, and Lp(a) cut-offs are based on a combination of the 2021 CCS guideline, the Framingham study, NCEP ATP III and the 2016 EAS/EFLM guideline. Fasting hours should be reported on the lipid report, with specific triglycerides cut-offs based on fasting status. **Conclusion:** A harmonized lipid report, consistent with up-to-date Canadian guidelines, will improve consistency and continuity of lipid test interpretation across Canada. The CSCC hRI Working Group plans to support implementation of the harmonized lipid report in Canadian laboratories in a collaborative manner with other medical specialities. Lastly, we plan to perform a follow-up survey to assess uptake.

Analyte	Decision Limit	Interpretive Comments for Individual Analyte Reporting(Always include a comment)
Total Cholesterol	<5.2 mmol/L	Use in Framingham Risk Score estimation, Cardiovascular Life Expectancy Model, and to calculate non-HDL-C.
HDL-C	>1.0 mmol/L	Use in Framingham Risk Score estimation, Cardiovascular Life Expectancy Model, and to calculate non-HDL-C.
LDL-C	<3.5 mmol/L	If triglycerides ≥1.5 mmol/L, use non-HDL-C or apoB instead of LDL-C for initial lipid screening and as a treatment target.
Triglycerides	<1.7 mmol/L (fasting) <2.0 mmol/L (non-fasting)	If triglycerides >4.5 mmol/L, measurement of fasting lipids recommended. If triglycerides >11 mmol/L, patient at increased risk for pancreatitis.
Non-HDL-C	<4.2 mmol/L	Refer to 2021 Canadian Cardiovascular Society guideline for information on treatment initiation recommendations and treatment target values.
ApoB	<1.05 g/L	Refer to 2021 Canadian Cardiovascular Society guideline for information on treatment initiation recommendations and treatment target values.
Lp(a)	<100 nmol/L	Earlier and more intensive health care management recommended when >100 nmol/L
Fasting (hours)		Fasting and non-fasting testing acceptable
Lipid Panel (TC, LDL-C, HDL-C, triglycerides, non-HDL-C) Interpretive Comment: Total cholesterol and HDL-C are used in the Framingham Risk Score estimation, Cardiovascular Life Expectancy Model, and to calculate non-HDL-C. If triglycerides ≥1.5 mmol/L, recommended to use non-HDL-C or apoB instead of LDL-C for initial lipid screening and treatment target.If triglycerides >4.5 mmol/L, measurement of fasting lipids recommended. If triglycerides >11 mmol/L, patient at increased risk for pancreatitis.See the 2021 Canadian Cardiovascular Society guideline for information on treatment initiation and treatment target values.		

B-223

Evaluation of Bio-Rad's Improved Liquichek Serum Indices, Hemolysis

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Background: Hemolysis is one of the most frequently monitored assay interferents on an automated chemistry platform because it affects key chemistry analytes. Liquichek Serum Indices, Hemolysis is intended for use as part of laboratory interference testing to monitor instrument's ability to detect hemolyzed samples. Bio-Rad had made formulation improvements to ensure reliable and consistent performance throughout shelf life for this product. In this study, we performed stability testing to support the product shelf life and in-use (open-vial and closed-vial) claims with the current and improved formulation.

Methods In the shelf-life study, Bio-Rad utilized a predictive modeling based on real-time stability data to determine the shelf life of the product. In the in-use stability study, Bio-Rad utilized real-time stability data for two product claims: 1) Open Vial Stability: the product was opened daily and tested at T_0 and T_f. 2) Closed Vial Stability: The product was thawed on T_0 and tested on T_f. T_0 represents data from a separate vial of the same lot.

Results In the shelf-life study, it was determined that the current formulation has 6 months of product claim with a loss of 10% versus the improved formulation has 24 months of product claim with a loss of approximately 5%. Based on this study, the new formulation is 18 months more stable than the current formulation.

In the in-use stability study, it was determined that the original formulation met stability claim of 5 days open-vial stability and 7 days of closed-vial stability with a loss of <5% versus the improved formulation would achieve 14 days for both open-vial and closed-vial stability claim with a loss of less than 5%.

Stability Study	Formulation	T_0	T_f	% of T_0	Claim
Shelf Life (Predictive Modeling)	Original Formulation	212	191	90%	6 Month
Shelf Life (Predictive Modeling)	Improved Formulation	215	205	95%	24 Month
Open Vial Stability (Opened Daily)	Original Formulation	192	195	102%	5 days
Open Vial Stability (Opened Daily)	Improved Formulation	219	214	98%	14 Days
Closed Vial Stability (Thawed on T_0, Tested on T_f)	Original Formulation	192	197	103%	7 days
Closed Vial Stability (Thawed on T_0, Tested on T_f)	Improved Formulation	219	211	96%	14 Days
Note: T_f is based on claim timepoint					

Conclusion The improved formulation will have 24 months of shelf life, 14 days of open vial stability claim, and 14 days of closed vial stability claim.

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Reducing Preanalytical Variation with the Becton Dickinson Barricor™ blood collection tube

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Background: The BD Barricor™ blood collection tube uses a mechanical separator during centrifugation to separate plasma from the cellular elements of blood. Compared to the use of the standard plasma gel separator tubes (PST™), Barricor™ plasma contains significantly fewer platelets and is associated with fewer episodes of pseudohyperkalemia. **Methods:** To determine if Barricor™ results in a reduction in pre-analytical error compared to PST™, we used a model previously published (Clin Biochem 2017;50:936-941) that utilizes serial differences between intra-patient consecutive measurements transformed into a Taylor series of variation vs time with the y-intercept (yo) equal to short term analytic variation (sa), preanalytic (pa) variation and biologic variation(b): $yo = (sa^2 + pa^2 + sb^2)^{1/2}$. We evaluated the intra-patient variation of sodium, chloride and potassium obtained from the Emergency Department of a large tertiary care center sampled with either the PST™ tube (May 2016-April 2018, n=27,400 specimens) or Barricor™ (May 2018-May 2020, n=28,500 specimens). All specimens were analyzed on the Roche Cobas® 8000. For each analyte, we tabulated the pairs of intra-patient results separated by either 2h or 3h intervals. The average between-pair variations were then regressed against time. We also regressed potassium against platelet count and compared rates of high potassium outliers for each tube. **Results:** The Table summarizes the differences in the y-intercepts for the electrolytes. Sodium and potassium exhibited reduced variation with Barricor™(-0.8 and -4.1% respectively, p<0.05). Furthermore, there were fewer elevated potassium results with the Barricor™ tube in specimens with elevated platelet counts (≅1000 X 10^9/L). Similarly, the high potassium (>6.0 mmol/L) outlier rate was lower for Barricor™ (41/27444 vs 60/26062, p=0.03). **Conclusion:** We demonstrate that Barricor™ reduces sodium and potassium variation, is associated with fewer potassium outliers and reduces potassium elevations with extreme thrombocytosis. .

	Barricor			PST				
Analyte	yo	Std.Error	Mean	yo	Std.Error	Mean	Level of Sig.	Error Reduction (%)
Na, mmol/L	1.43	0.06	139.2	1.78	0.13	139	0.042	0.8
Cl, mmol/L	1.76	0.55	100.9	2.23	0.83	100.7	0.301	1.4
K, mmol/L	0.213	0.006	4.04	0.27	0.024	4.04	0.048	4.1

B-225

Mitigation of Hospital Pneumatic Tube Transport Effects on pH, pCO_2 and pO_2 Measurements

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Background: Specimen transport is an important pre-analytical factor that can influence the accuracy of clinical laboratory measurements. Blood gas analytes can be affected by pneumatic tube transport, since gas exchange can occur between the specimen and air bubbles introduced during specimen collection and/or tube trans-

port. There is wide variation in the literature as to whether pneumatic tube transport is acceptable for blood gas specimens, which may reflect variability between institutions in pneumatic tube system design and parameters such as pressure and transport time. We sought to determine the acceptability of specimens transported via the pneumatic tube for pH, pCO_2 and pO_2 measurements compared to measurements made at the point-of-care.

Methods: Blood gas measurements were performed on Siemens RAPIDPoint 500 analyzers. Arterial blood gas specimens were analyzed at the point-of-care then sent through the pneumatic tube system for repeat measurement. CLIA or CAP total allowable error limits were used to establish acceptable limits for pH (0.04), pCO_2 (greater of ± 5mmHg or ± 8%) and pO_2 (± 3 times the average usual standard deviation).

Results: A total of 60 specimens were analyzed pre- and post-tube transport. 96.7% (58/60) of pH results, 88.3% (53/60) of pCO_2 results and 50.0% (30/60) of pO_2 results were within the acceptable limits. The average biases between pre- and post-pneumatic tube specimens were -0.0048 for pH, 1.25 mmHg for pCO_2 and 0.99 mmHg for pO_2. The largest differences were observed in specimens with high pO_2 values. Given the large number of pO_2 specimens that fell outside of the acceptable limits for pO_2 we implemented two mitigation strategies: 1.) syringe caps were replaced with rubber stoppers before transport and 2.) specimens were padded so that they were held more tightly in place within the transporter using an insert from the pneumatic tube system manufacturer. An additional 54 specimens were measured pre- and post- transport with the interventions, 74.1% (40/54) of which were within acceptable limits for pO_2, with an average bias of -0.59 mmHg. 92.6% (50/54) of pCO_2 measurements and 100% (54/54) of pH measurements were within acceptable limits. To determine the clinical significance of the pO_2 differences, we further examined data from specimens with pO_2 values outside of the reference interval (70-100 mmHg) that may have resulted in changes in patient management. Ten specimens were < 70.0 mmHg pre-transport, all of which had higher post-transport pO_2, with an average increase of 7.4 mmHg. Importantly, 100% of these specimens remained at < 70.0 mmHg pO_2 post-transport. 35 specimens were > 100 mmHg pre-transport, with one specimen measuring within the reference interval post-transport and an average bias of 5.0 mmHg. None of the specimens that were in the reference interval pre-transport measured outside of the of the reference interval post-transport.

Conclusion: Immobilization of specimens and use of rubber stoppers on blood gas syringes may mitigate the effects of pneumatic tube transport on blood gas measurements. Utilizing these mitigation strategies, this study concluded that the pneumatic tube system was an acceptable means to transport specimens for arterial blood gas measurements.

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Verification of Manufacturer-defined Hemolysis Thresholds for Multiple Chemistry Analytes on the Beckman AU680

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Background: Hemolysis is a well-known interference in the measurement of several analytes in the clinical laboratory. While most manufacturers provide hemolysis thresholds for each analyte, multiple studies have demonstrated that these thresholds may vary between laboratories, even when measurements are performed on the same analyzer. This study sought to verify the hemolysis thresholds for 13 different chemistry analytes on the Beckman AU680 analyzer, with the goal of setting autoverification rules that would allow specimens with hemolysis levels that were within in-house established acceptable error limits to be reported. **Methods:** Hemolysate was purchased from Sun Diagnostics and spiked into pooled serum to achieve concentrations ranging from 25 mg/dL to 800 mg/dL of hemoglobin. These concentrations spanned all five levels of hemolysis as described by the manufacturer. ALB, ALP, ALT, AMY, AST, CHOL, CK, Fe, GGT,K^+,LDH, MG and PHOS were measured in spiked specimens on the Beckman AU680 analyzer. Acceptable limits were defined as the half of the CLIA or CAP allowable error at the medical decision level, with sufficient replicates measured to achieve 90% power. **Results:** Table 1 summarizes the study results. Eleven analytes: ALP, ALT, AMY, AST, CK, Fe, GGT, K^+, MG and PHOS were within the acceptable error limits at hemolysis levels that exceeded those defined by the manufacturer as acceptable. CHOL exceeded the acceptable limits at 250 mg/dL, which is considered a hemolysis level of +++, and is below the manufacturer's hemolysis threshold of 500 mg/dL (++++). ALB and LDH exceeded the acceptable limits at the manufacturer's threshold. **Conclusions:** Manufacturer hemolysis thresholds should be independently verified by clinical laboratories. The results of these studies can guide reporting of test results for hemolyzed specimens that are difficult to recollect or required within a rapid turnaround time.Table 1. Summary of Measured and Manufacturer-defined Hemolysis Thresholds for Thirteen Chemistry Analytes

Analyte	Acceptable Change Limits	Measured Hemolysis Threshold	Manufacturer Hemolysis Threshold
ALB	± 0.18 g/dL	++++	++++
ALP	± 22.50 U/L	Not exceeded	++++
ALT	± 4 U/L	Not exceeded	++++
AMY	± 15 U/L	Not exceeded	+++
AST	± 15 U/L	+++++	Avoid hemolysis
CHOL	± 10 mg/dL	++++	+++
CK	± 30 U/L	Not exceeded	++++
Fe	± 15 mg/dL	+++++	Avoid hemolysis
GGT	± 7.50 U/L	+++++	++++
K^+	± 0.25 mmol/L	++	Avoid hemolysis
LDH	± 25 U/L	Avoid hemolysis	Avoid hemolysis
MG	± 0.5 mg/dL	+++++	Avoid hemolysis
PHOS	± 1.50 mg/dL	Not exceeded	++++

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Evaluating Serum Quality and Shipping Stability Using the Torq ZDrive MR and BD Microtainer for Decentralized Capillary Blood Sample Collection

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BackgroundThe growing trend toward decentralized capillary blood collection for diagnostics faces challenges in ensuring proper plasma and serum sample preparation. Capillary blood samples are simple to collect in remote and decentralized settings, but separation requires laboratory equipment and is typically performed by trained personnel. Delays in plasma or serum separation can increase hemolysis and decrease serum or plasma yield, negatively impacting test accuracy and accessibility. The Torq™ ZDrive™ with Microvolume Rotor (ZDrive MR) is a lightweight, hand-portable centrifuge powered by two AA batteries for rapid separation of capillary blood collection tubes such as BD Microtainers®. The ZDrive MR is self-balanced and automated to separate blood in a single Microtainer in less than five minutes at the point of collection prior to immediate analysis or shipment to a centralized laboratory. Here we present results of verification studies evaluating the sample quality and shipping stability of capillary and venous serum samples processed using BD Microtainers and the ZDrive MR. **Methods**Samples were collected from n=11 subjects. Each subject had capillary blood collected directly via finger stick into one (1) Microtainer for serum separation, and venous blood was drawn into uncoated blood collection tubes, which was immediately transferred into three (3) additional Microtainers for serum separation at each of three (3) different loading volume conditions (minimum volume, maximum volume, and the same volume of capillary blood collected into the Microtainer from that subject). All Microtainers were processed using ZDrive MRs. Red blood cell (RBC) concentration and serum yield were immediately measured in serum. Serum was then reloaded into Microtainers, packaged, and subjected to ISTA-3A standardized simulated shipping treatment including drop testing on specified edges of the package and vibration at 600, 1200, and 1500 rpm with and without a one (1) kg weight secured to the package. Samples were then stored overnight, and these steps were repeated the following day. Following shipping simulation, quantitative hemolysis in each serum sample was measured using the Fairbanks spectrophotometric method. **Results**The mean RBC concentration for the minimum loading, maximum loading, volume equivalent to capillary blood, and capillary blood conditions were found to be 0.11 M/mL, 0.13 M/mL, 0.21 M/mL, and 0.37 M/mL respectively. Mean percent serum yields w.r.t. the original blood volume were found to be 44.04%, 41.61%, 41.58%, and 43.17% respectively. After shipping simulation, the mean hemoglobin concentrations were found to be 116.7 mg/dL, 76.6 mg/dL, 62.2 mg/dL, and 126.2 mg/dL respectively. All results passed pre-stated acceptance criteria developed using literature and industry standards for testable serum quality: <6 M/mL RBC, >30 % serum yield, and <200 mg/dL hemoglobin.**Conclusion**The ZDrive MR system was shown to successfully separate capillary and venous blood into testable serum. These results indicate that the ZDrive MR system is a viable option for providing

point-of-care serum or plasma that can be used to test onsite or shipped to a centralized laboratory. This ability to collect and generate high quality serum or plasma at the point-of-care can improve access to diagnostics, particularly for tests sensitive to sample degradation.

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Evaluating the Torq MiniDrive2 System for Decentralized Capillary Blood Collection and Plasma Separation

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Background: Today's methods for at-home, self-collection of blood samples are limited to fingerstick lancets paired with dried blood spot stabilization or whole blood shipment in a microvolume collection tube. Both methods suffer from the persistent inability to separate capillary whole blood into plasma samples and blood cells at the point of collection, leading to sample degradation including hemolysis which accounts for an estimated 60% of rejected samples. To address this deficiency, we have developed the Torq™ MiniDrive2™ System for self-collection and transport of pre-separated liquid plasma samples. The System contains the MiniDisc™, a disc-shaped, anticoagulated capillary blood collection device, and the MiniDrive2, a 2.5-inch diameter, lightweight, disposable centrifuge powered by a single AAA battery separates blood contained in the MiniDisc in 4 minutes. With its low-cost design and compact footprint, the MiniDrive2 is easy to deploy via the mail, does not require balancing, and can be used with only written instructions. Following collection and processing, liquid plasma samples can be shipped to a centralized laboratory for testing without the hemolysis caused by shipping whole blood. Here we present study results evaluating plasma yield and quality from whole blood samples processed with the Torq MiniDrive2 System.**Methods:**Venous blood samples were collected from n=5 subjects and immediately transferred to replicates of EDTA MiniDiscs for anticoagulation and blood separation. For each subject, venous blood was also transferred into a control BD Microtainer®. A range of blood input volumes (210 µL - 290 µL) were used to replicate the range of blood volume collected via finger stick. Each MiniDisc was then spun on a MiniDrive2 and plasma was extracted and transferred to a plasma collection tube. Plasma yield (volume) was then measured and hemoglobin concentration was measured in all samples using the Fairbank's spectrophotometric method. **Results:**The mean plasma yield for Subjects A, B, C, D, and E were 84.04 µL, 93.07 µL, 56.00, 62.68 µL, and 75.12 µL respectively. The mean hemoglobin concentrations for Subjects A, B, C, D, and E in MiniDiscs were 57.17 mg/dL, 30.68 mg/dL, 23.86 mg/dL, 35.29 mg/dL, and 23.48 mg/dL respectively. Microtainer controls had a hemoglobin concentration of 19.92 mg/dL, 12.83 mg/dL, 13.21 mg/dL, 22.89 mg/dL, and 8.44 mg/dL respectively. All MiniDisc and Microtainer control results passed pre-stated acceptance criteria developed using literature and industry standards for testable plasma quality >50 µL of plasma and <100 mg/dL hemoglobin concentration. **Conclusion:**The MiniDrive2 System was shown to successfully collect and separate whole blood from multiple subjects. The system met and exceeded pre-stated acceptance criteria for both plasma yield and hemolysis with multiple donors and blood input volumes. These findings suggest the MiniDrive2 is a valid solution for at-home, self-collection of capillary liquid plasma and may thus expand at-home testing options by eliminating the sample dilution and degradation disadvantages of today's at-home collection tools.

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Reference Intervals for Some Minerals of the Adult Population in Mongolia

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Background: Minerals are important for the proper body functioning. They also play a role in preventing and fighting diseases. Reference values for minerals help physicians evaluate the mineral status of patients and make clinical decisions. The aim of this study was to determine the reference intervals for some minerals of the Mongolian adult population.

Methods: Three hundred healthy adults (150 males and 150 females) of 19 to 70 years of age were selected for the study based on CLSI C28-P3 criteria Defining, establishing & Verifying reference interval in the clinical laboratory; Proposed Guidelines, 2008. The study was approved by the ethical committee of the Ministry of Health of Mongolia. Informed consents were taken from the selected individuals. Morning blood samples of the participants were collected under aseptic conditions. Levels of iron (Fe), zinc (Zn) and copper (Cu) were measured by graphite furnace atomic absorption spectrometry. The lower and upper reference limits were defined as the 2.5th and 97.5th percentiles, respectively. The data were analyzed using Excel program.

Results: The mean level of blood iron was 30.50 µmol/L (95% CI 29.71-31.29) for men and 30.91 µmol/L (95% CI 30.03-31.79) for women. The calculated reference interval for males was 21.39-37.72 µmol/L and 19.87-39.67 µmol/L for females. The mean concentration of zinc in the blood of males was 11.00 µmol/L (95% CI 10.69-11.31) and that of females was 11.79 µmol/L (95% CI 11.39-12.19). The reference interval for blood zinc was 8.20-14.92 µmol/L in men and 8.52-16.67 µmol/L in women. The mean level of blood copper was 15.28 µmol/L (95% CI 14.64-15.89) for men and 18.08 µmol/L (95% CI 17.30-18.86) for women. The calculated reference interval for copper in males was 9.72-22.34 µmol/L and that in females was 11.18-27.27 µmol/L.

Conclusion: The reference values for zinc, copper and iron of Mongolian adults do not differ significantly from those observed in European and Asian countries. The calculated reference intervals can be used for evaluation of the mineral status of the Mongolian adult population.

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Automated separation and washing of red blood cells from whole blood samples

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Background: We developed an automated method for the separation of red blood cells (RBCs) from whole blood (WB) and washing of the RBCs. Performance of the method was evaluated by comparison of the concentrations of three biomarkers measured in RBC samples, with RBC separation and washing performed either manually or by the automated method. Our data confirmed the effectiveness and utility of the automated method for RBC separation and washing. **Methods:** 1 mL aliquots of WB samples were transferred in tubes and placed in the rotor slots of an Ultra CW® II (Helmer Scientific, IN) cell washer. RBCs were separated from plasma by centrifugal packing; plasma was then decanted by centrifugation, while the tube holders in the rotor were repositioned. The decanting step was followed by two cycles of RBC washes, consisting of addition of saline to the tubes, agitation of the tubes, pelleting the RBCs and decanting the supernatants. The resulting volume of washed RBC suspension was ~0.3 mL. The method's performance was assessed by comparison of concentration of magnesium in RBC samples, with RBC separation and washes performed using automated or manual methods; the magnesium testing was performed using an in-house validated ICP-MS method. Assessment of the potential for sample cross-contamination during the RBC separation and washing was performed by simultaneous processing sets of samples positive and negative for 6-Thioguanine (6-TG) and 6-Methylmercaptopurine (6-MMP); tubes with the positive and negative samples were placed in the alternating positions in the rotor of the washer. Measurement of 6-TG and 6-MMP was performed using LC-MS/MS.**Results:** Magnesium concentrations in RBC samples (n=24) processed using the automated and the manual methods were in good agreement; slope of the regression line, y-intercept and R^2 were 0.967, 0.0424 and 0.943, respectively. No carryover was detected from 6-TG and 6-MMP positive samples in the proximally located negative samples, confirming the absence of cross-contamination during the automated RBC separation and washing. Among the steps of RBC separation and washing, the decantation step and centrifugation time were the most critical parameters. The maximum recovery of RBCs with the least volume of residual supernatant left in the tubes was achieved at the decanting force of 13 RCF +/-1. Centrifugation time of 240 sec +/-30 sec was found to provide maximum recovery of RBCs.

Conclusions: We developed an automated method for RBC separation from WB samples and washing of the separated RBCs. The method is simple and automates sample preparation for use in analytical methods where large initial WB sample volume is required. The manufacturer suggested maximum WB volume for the processing is ~0.1 mL, whereas our data indicate that up to 1 mL of WB may be processed. Hands-on time for processing 24 WB samples using this automated method is ~15 minutes whereas manual processing of an equivalent batch size of WB samples takes ~2.5 hours.

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The Impact of Therapeutic Concentrations and Dose-Dependent Effects of Hydroxocobalamin Interference on D-Dimer and Anti-Xa Assays

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Background: Hydroxocobalamin (OHCob), marketed as Cyanokit®, is an FDA-approved antidote for the treatment of cyanide poisoning and has been used off-label for treating vasoplegic syndrome. Usually, a 5g intravenous (IV) dose is recommended; however, depending on case severity, a total of 10g may be administered. Therapeutic concentrations of OHCob cause a pink discoloration of urine, serum, and mucous

membranes. This discoloration evades the hemolysis-index flag due to spectral differences in the absorbance of hemoglobin and OHCob. Considering the rise in the use of interventional OHCob, it is imperative to characterize its impact on common coagulation assays that utilize spectrophotometric readout to prevent reporting spurious results that could compromise patient management. The objectives of this study were to investigate the impacts of estimated therapeutic concentrations of OHCob on the D-dimer and anti-Xa assays and to characterize its dose-dependent effects. **Methods:** A stock solution of OHCob was prepared by reconstituting pharmaceutical-grade OHCob powder with 0.9% sodium chloride. Retrospective patient specimens were used to create two base pools of normal and abnormal concentrations of D-dimer (0.34 ng/dL and 2.59 ng/dL, respectively) or anti-Xa (0.64 U/mL and 1.05 U/mL, respectively). Final OHCob concentrations of 0.1 mg/mL and 1.5 mg/mL were used, and they were selected based on the reported therapeutic levels (0.4-1.3 mg/mL) after administration of a 5g IV dose of OHCob. To investigate dose-dependent effects, additional concentrations of 0.2, 0.4, 0.8, 1.2, 2.6, and 3.9 mg/mL were tested. An equal volume of saline, instead of OHCob, was added to separate paired control sample pools to adjust for dilution effects. The experimental and control samples were tested in triplicate with the D-dimer and anti-Xa assays on the Stago STA Compact® instrument (Asnières-Sur-Seine, France). Our defined acceptable error limits for D-dimer and anti-Xa were 30% and 20%, respectively. Data analyses were performed using Prism software (LaJolla, CA). **Results:** At normal D-dimer concentrations, 5.4% and 237.5% positive biases were observed at OHCob concentrations of 0.1 mg/mL and 1.5 mg/mL, respectively. However, at abnormal concentrations, D-dimer showed biases of 3.6% and 21.5% at drug concentrations of 0.1 and 1.5 mg/dL, respectively. Interestingly, no significant biases were observed in the anti-Xa assay at normal or abnormal concentrations. Drug concentrations above 1.5 mg/dL significantly affected the D-dimer and anti-Xa assays. Lastly, we observed a dose-dependent positive bias in the D-dimer results (up to 1193%) and a negative bias in the anti-Xa results (down to -79.5%) in response to increasing concentrations of OHCob. **Conclusion:** Our data suggest that therapeutic levels of OHCob at a 5g dose cause significantly spurious elevations with the D-dimer assay at normal concentrations and tolerable increases at abnormal concentrations. Alternatively, no significant interference was seen with the anti-Xa assay at normal or abnormal concentrations. OHCob concentrations above 1.5 mg/dL interfered with both D-dimer and anti-Xa assays. We acknowledge that therapeutic concentrations of these analytes are variable and influenced by metabolism; however, our data would help clinical laboratories, in conjunction with clinicians, establish a protocol for accepting or rejecting specimens from patients exposed to OHCob.

B-233

Evaluating extraction reproducibility of BÜHLMANN Calex® Cap stool extraction device on fecal calprotectin measurement

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Background: Fecal calprotectin (FC) is a protein released by the neutrophils during intestinal inflammation. The non-invasive FC test has high sensitivity for differentiating between pathological inflammatory bowel disease (IBD) from functional disease such as irritable bowel syndrome, thus reduces the number of patients requiring specialist referral for invasive endoscopy and biopsy testing. Current FC test requires a stool extraction step prior to analysis. This sample preparation has resulted in great measurement variability between laboratories. Although it is still the gold standard, the manual weighing method is time and labor consuming, prone to errors and limits assay throughput. Here, we evaluated a commercial stool extraction device - BÜHLMANN Calex® Cap for preparing different stool samples for FC measurement. The device contains a pin with grooves able to carry an approximate amount of stool, the sample is then diluted in corresponding buffer volume. We aimed at assessing the reproducibility of stool extraction among various stool types and operators across multiple consecutive days' stool extraction.

Methods: Nine fresh stool samples including solid (n=3), semi-solid (n=3) and liquid (n=3) were included in the study using a 3 (stool type) x 3 (sample number) x 3 (operator) x 3 (day) study design. Each of the nine stool samples were extracted using the BÜHLMANN Calex® Cap extraction device on day 1 by each of the three operators. The same extraction process was repeated on day 2 and then day 3. For liquid stools, 10ul of stool sample was pipetted out and directly added to the extraction device buffer according to the IFU. The nine stool samples were stored refrigerated during the whole experiment, which is within the IFU stated specimen stability. Then, the extracted sample was analyzed immediately using the BÜHLMANN fCAL® turbo reagent on a Beckman Coulter UniCel® DxC 800 platform. In addition, both within and between run CV were performed using two levels of QC and three levels of patient stool samples.

Results: Total analytical imprecision was calculated from within and between run CV, ranged from 0.99% to 4.25%. Using the BÜHLMANN Calex® Cap extraction device, the overall CV of FC measurement was determined to be 15.04% across all stool types, operators and different days of extraction. Among the three stool types: solid, semi-solid and liquid had CV of 11.72%, 16.85% and 16.54% respectively. For the three operators, the achieved CV is 9.57%, 12.47% and 14.20%. Moreover, the determined CV of the three days of extraction is 15.95%, 11.04% and 14.67%.

Conclusion: Previous literature has shown both under- and over-recovery of FC when using commercial extraction device compared to the stool weighting method; however, those studies did not take stool and preparation consistency into consideration. In the current study, we observed a larger CV when extracting semi-solid and liquid stools compared to the solid stools. This is most likely due to the non-homogenous nature of the stool sample, the sampling location and also the accuracy of pipetting liquid stool. In addition, there is a minor CV variation between different operators and days of extraction.

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Validation of Anton Paar Ball Mill BM500 for Automation of Sample Preparation in the Analysis of Kidney Stones

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Background: Kidney stone formation is multifactorial and its incidence is increasing worldwide. Stone analysis is essential to identify chemical composition and etiologies of stones, guide therapy and prevent recurrence. Grinding of stones to fine powder is a pre-analytical step that is essential for reproducible and accurate spectral analysis. Mortar and pestle (M&P) remains the most commonly used tool for stone grinding in the majority of medical laboratories. However, this approach is ergonomically challenging, especially in high-volume laboratories, where it can lead to repetitive use injuries such as shoulder strain. Ball mills are used as automated tools to pulverize and homogenize a broad range of organic and inorganic materials, which could also make them suitable for the automated grinding of kidney stones. Despite this, ball mills are, to the best of our knowledge, not commonly used for automation of stone grinding in medical laboratories. **Methods:** Here we assessed the quality of grinding, robustness, ease of use, and length of processing time using Anton Paar Ball Mill BM500 (BM500) compared to manual grinding using M&P. A split sample comparison was also performed to explore any possible impact of BM500 grinding on the accuracy of testing using Fourier-transformed infrared spectroscopy (FTIR). QC and patient samples were selected to cover all common kidney stone types such as Calcium Oxalate (mono- and di-hydrate), Calcium Phosphate, Magnesium Ammonium Phosphate, Uric Acid, Ammonium Urate, Calcium Carbonate and Cystine. The spectra were compared to the in-house spectral library to define top % match for each sample. **Results:** BM500 was robust throughout the study, and similar to manual grinding, consistently produced fine powder which was suitable for FTIR spectral analysis. FTIR background signal was similar for powder generated by BM500 and M&P. FTIR analysis showed no significant difference in the quality of the IR spectra for powder generated by BM500 or M&P. The average top % match (±SD) for QCs representing 7 single chemical compositions was 88.0 (±7.4) vs. 91.0 (±4.4) (p=0.38) for BM500 vs. M&P, respectively. For patient samples representing 8 different single or double compositions, the average top % match was 84.8 (±4.4) vs 82.6 (±6.6) (p=0.38), respectively. Total processing time per specimen was longer for BM500 (33.5 seconds) compared to M&P (12.5 seconds), caused by longer loading/unloading and grinding times. Furthermore, repeated manual capping/uncapping of the BM500 grinding chamber caused significant arms and wrist strain. **Conclusion:** BM500 provides a suitable alternative for M&P for kidney stone pre-analytical processing in medical laboratories. However, in high-volume settings, longer processing times and ergonomic challenges may diminish its overall benefits.

Special Patient Populations

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Vanadate-based Total Bilirubin Assays: Overestimating Risk when Using Diazo-based Bhutani Nomograms

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Objective: Monitoring total serum bilirubin (TSB) in newborns helps prevent clinically significant hyperbilirubinemia and potential kernicterus from occurring. New-

born patient's TSB concentrations above critical concentrations may trigger aggressive medical intervention to lower the TSB concentration to safer levels. The use of the Bhutani nomogram for the designation of risk is a widespread practice to estimate the risk of extreme hyperbilirubinemia in newborns and is recommended by the American Academy of Pediatrics. This nomogram was constructed by measuring TSB in 13,003 newborns using a diazo-based TSB methodology. In recent years, a newer methodology using vanadate oxidation has replaced the diazo method in many of the newer automated analyzers. This method change is based on the claim that hemolysis interferes less with bilirubin measurements in vanadate-based methods when compared to diazo-based methods. There has not been a new nomogram constructed using the vanadate method. Upon implementation of a new vanadate-based method in our laboratories hospital system, we learned from our neonatologists about an increase readmission of newborn patients due to hyperbilirubinemia, causing potentially unnecessary interventions to occur. This prompted the laboratory to compare the vanadate and an open-channel diazo method to investigate a possible TSB concentration bias, and the clinical implications of assessing risk when using vanadate-based TSB assays in the Bhutani diazo-based nomogram. **Methods**: Correlation was performed by comparing TSB concentrations measured by a diazo based third-party method (Sekisui) with a vanadate oxidation method (Siemens Diagnostics Inc) in the Siemens Atellica Solutions automated analyzer. 41 patients' specimens with TSB results between (0.1 and 24.9 mg/dL) were included in the study (7 samples had TSB concentrations above 10 mg/dL). Additionally, 5 serum specimens were spiked to TSB concentrations of 14, 16, 18, 20 and 22 mg/dL and used in the correlation analysis. Precision (within run and between run), and linear range for the Sekisui assay in the Siemens Atellica analyzer were evaluated by using QC, and linearity materials. Hemolysis interference in the Sekisui assay was calculated by adding increasing amounts of hemoglobin (red cell lysate) in a serum containing a single bilirubin concentration originally reported as 5.43 mg/dL. Data analysis was performed using EP evaluator and Microsoft Excel. **Results**: The vanadate-based assay had a consistent bias across the TSB concentration tested. The bias increased at higher concentrations and ranged from 1 to 3 mg/dL for TSB concentrations above 10 mg/dL. **Conclusions**: The vanadate assay has an unacceptable analytical performance in the Siemens Atellica Solution analyzer at critical diagnostic TSB concentrations when interpreted using the Bhutani diazo-based nomogram. The Sekisui diazo TSB assay was found to provide more reliable results when using the Bhutani nomogram and was placed into production to replace the original vanadate assay. Although vanadate-based methods may be superior in terms of avoiding some analytical interference, vanadate-based nomograms for hyperbilirubinemia will need to be created to identify the clinical risk associated with the increase TSB in newborns.

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Mining the gap for trimester-specific reference intervals for key hematology markers: A novel approach using a large healthy Canadian pregnancy cohort

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Background: During pregnancy maternal physiology adapts to support fetal growth and development. Increases in plasma volume, renal blood flow, cardiac output, and erythropoietin synthesis occur throughout gestation which can impact hematology test results. Despite routine needs for hematological assessment in pregnancy, most clinical laboratories provide non-pregnant adult reference intervals for test interpretation due to limited availability of pregnancy-specific studies. The current study aims to establish trimester-specific reference intervals (RIs) for three hematology parameters in a large pregnancy cohort by applying indirect and direct statistical techniques. **Methods**: A 5-year pregnancy cohort was defined by extracting all live births in British Columbia, recorded in the British Columbia Perinatal Data Registry (BCPDR) between Jan 1, 2010 and Dec 31, 2014. Clinical information in the BCPDR was used to exclude high-risk pregnancies, those with complications, preterm delivery, multiples or with documented maternal comorbidities, to define a cohort of healthy mothers with healthy pregnancies for RI inclusion. Retrospective laboratory data, including complete blood count, were extracted from BC Children's & Women's Hospital and LifeLabs BC laboratory information systems and linked to demographic and clinical data in the BCPDR. Only the first test result per mother per trimester was retained. RIs were calculated using both direct (i.e. nonparametric method) as well as indirect (i.e. truncated maximum likelihood) approaches. Associations of maternal variables, including age, body mass index and ethnicity, with laboratory values were assessed using linear regression. Trimester-specific RIs were determined for white blood cell (WBC) count, red blood cell (RBC) count, and platelet (PLT) count. **Results**: We included 142,817 pregnancies representing 121,209 mothers with ~150,000 CBC results available for inclusion in RIs. Maternal age varied from 13 to 52 years old (mean: 30.7, SD: 5.2). Average gestational age of pregnancies was 39.1 weeks (SD: 1.2); 45% were primiparous; 47% had normal pre-pregnancy BMI; and 5.9% were smokers. Ethnicity data was available for 28% of pregnancies, of which 60% were Caucasian by maternal self-report, 22% east Asian, 10% south Asian, and 7% all other ethnicities combined. WBC count RI limits increased during pregnancy (**T1**: 4.8-12.4, **T2**: 6.0-13.9, **T3**: 6.0-14.2 $x10^9$/L). In contrast, RBC count (**T1**: 3.75-4.95, **T2**: 3.33-4.52, **T3**: 3.36-4.61 $x10^{12}$/L) and PLT count (**T1**: 164-362, **T2**: 147-340, **T3**: 131-336 $x10^9$/L) decreased slightly throughout gestation. Similar lower and upper reference limits were obtained using direct and indirect calculation methods (direct reported here). Within our healthy cohort, statistically significant associations were observed between CBC parameters and the following variables: age, body mass index, smoking status, assisted reproduction, and ethnicity, although the degree of impact on lab values may not be clinically significant. **Conclusions**: The current study underlines the importance of establishing and implementing trimester-specific hematology RIs. It also demonstrates the feasibility of using retrospective data to derive pregnancy RIs when population-based perinatal data are available to define a healthy pregnancy cohort. Future work will use this approach to derive RI for additional analytes commonly assessed in pregnancy, including thyroid markers, liver enzymes, electrolytes, and renal markers.

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Pediatric Reference Interval Verification for 28 Common Biochemical Assays on the Abbott Alinity System

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Background: The quality of clinical laboratory service depends on quality laboratory operations and accurate test result interpretation based on reference intervals (RIs). As new analytical systems continue to be developed and improved, previously established RIs must be verified. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has established comprehensive RIs for many biomarkers on several analytical systems. Here, published CALIPER RIs for 28 chemistry assays on the Abbott ARCHITECT were assessed for verification on the newer Alinity system. **Methods**: An analytical validation was first completed to assess assay performance. CALIPER serum samples (100) were analyzed for 28 chemistry assays on the Alinity system. The percentage of results falling within published pediatric ARCHITECT reference and confidence limits was determined for each analyte. Based on Clinical and Laboratory Standards Institute guidelines (CLSI) guidelines, if ≥ 90% of test results fell within confidence limits of ARCHITECT assay RIs, they were considered verified. **Results**: Of the 28 assays assessed, 26 met the criteria for verification. Reference values for calcium and magnesium did not meet the criteria for verification with 87% and 35% falling within previously established ARCHITECT confidence limits, respectively. However, both assays could be verified using pediatric RIs provided in the Abbott Alinity package insert. **Conclusion**: In this study, CALIPER ARCHITECT RIs were verified on the Alinity system for several chemistry assays. These data demonstrate excellent concordance for most assays between the Abbott ARCHITECT and Alinity systems and will assist in the implementation of the Alinity system in pediatric healthcare institutions.

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Pediatric Reference Interval Verification for Endocrine and Fertility Hormone Assays on the Abbott Alinity 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. In this study, pediatric RIs were verified for key immunoassays on the Abbott Alinity system based on the analysis of healthy children samples and comparison to comprehensive RIs previously established for Abbott ARCHITECT assays. **Methods**: Analytical performance of Alinity immunoassays was first assessed. Subsequently, 100 serum samples from healthy children recruited with informed consent were analyzed for 16 Alinity immunoassays. The percentage of test results falling within published CALIPER ARCHITECT reference and confidence limits was determined. If ≥ 90% of test results fell within the confidence limits, they were considered verified based on CLSI guidelines. If <90% of test results fell within the confidence limits, additional samples were analyzed and new Alinity RIs were established. **Results**: Of the 16 immunoassays assessed, 13 met the criteria for verification with test results from ≥ 90% of healthy serum samples falling within the published ARCHITECT confidence limits. New CALIPER RIs were established for free thyroxine and prolactin on the Alinity system. Estradiol required special considerations in early life. **Conclusion**: Our data demonstrate excellent concordance between ARCHITECT and Alinity immunoassays, as well as the robustness of previously established CALIPER RIs for most immunoassays, eliminating the need for *de novo* RI studies for most parameters. Availability of pediatric RIs for immunoassays on the Alinity system will assist clinical laboratories using this new platform and contribute to improved clinical decision-making.

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Pediatric Reference Interval Establishment for Common Cardiac Markers on the Alinity ci System

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Background: The clinical use of cardiac biomarkers, such as brain natriuretic peptides and cardiac troponins, is routine in adult assessment of heart failure and myocardial infarction, respectively. Recent evidence suggests clinical utility in pediatric settings, including in the evaluation of myocarditis, heart failure, and cardiac-involvement in immune conditions. Despite emerging clinical utility, there is a paucity of evidence-based reference intervals (RIs) for cardiac biomarkers in healthy children and adolescents, limiting implementation. The current study establishes RIs for high sensitivity cardiac troponin I (hs-cTnI) and N-terminal fragment prohormone B-type natriuretic peptide (NT-proBNP) in the Canadian Laboratory Initiative on Pediatric RIs (CALIPER) cohort on the Abbott Alinity ci system. **Methods**: An analytical validation was first completed, including precision, linearity, and method comparison with an Abbott Architect system according to Clinical and Laboratory Institute guidelines. Healthy children and adolescents were then recruited from the community in the Greater Toronto Area (0 to 18 years, n=200). Serum samples were collected upon informed consent via venipuncture. NT-proBNP and hs-cTnI were then measured on the Abbott Alinity ci system at the Hospital for Sick Children and RIs were established. **Results**: NT-proBNP and hs-cTnI assays demonstrated acceptable analytical performance, demonstrating precision estimates below 5% and high concordance to Architect assays. Pediatric reference value distributions for both hs-cTnI and NT-proBNP demonstrated relatively elevated and variable concentrations in the first month of life followed by consistently low concentrations throughout the remainder of the pediatric age range, requiring age partitioning only in the neonatal period. Only 4% of pediatric samples had detectable hs-cTnI concentrations (LoD=10 ng/L). **Conclusion**: The current study reports evidence-based RIs for hs-cTnI and NT-proBNP on the new Alinity system in a healthy pediatric cohort for the first time. Study findings will support clinical decision-making in laboratories utilizing the Alinity NT-proBNP and hs-cTnI assays in the diagnosis and prognosis of cardiac conditions in children and adolescents. Future studies are planned for other cardiac biomarkers including BNP, creatine kinase, and creatine kinase-MB.

B-240

Multianalyte Reference Intervals in Adult Black Population: A Systematic Review and Meta-analysis

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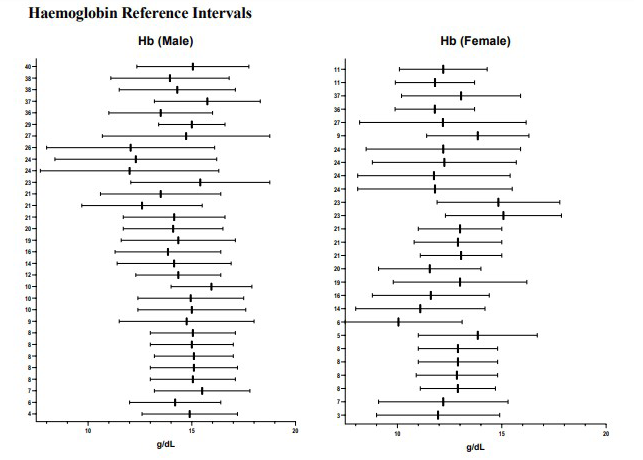
Background: Reference intervals (RIs) are dependent on several factors including, age, sex and ethnicity. Although the majority of the population in Africa is black, most studies conducting research to establish RIs in the black population in African countries have often reported variability in the RIs for commonly measured analytes. We conducted a systematic review and meta-analysis of the available literature on the similarity and variation of RIs of multiple analytes established for the adult black population.

Method: An electronic database search of PubMed, Google Scholar and Scopus was performed from inception to February 2021 based on defined inclusion and exclusion criteria. A combination of the following keywords was used as search items: biochemical, hematological, reference, normal, intervals, ranges, values, African, black, adult. The data was extracted and organized for quality assessment by independent investigators in the research team. Data included details of RIs for 21 hematological analytes, 32 biochemical analytes, and 14 electrolytes in the black adult population. Statistical analysis was conducted using R and GraphPad Prism. Outcome measures such as lower and upper reference limits were assessed for heterogeneity.

Results: A total of 173 articles met the inclusion criteria on the basis of title and abstract. Imposition of stricter exclusion criteria (including non-human subjects, non-black population, unavailability of publications) resulted in 44 articles. Significant variations were observed for analytes among the studies performed for black population in addition to comparison with other RIs sources with predominantly Caucasian population. For hemoglobin in males, the mean lower and upper RIs were 11.7 g/dL (SD 1.6) and 17.0 g/dL (0.8) respectively.

Conclusion: Major gaps exist for reference intervals related to the black population. We report for the first time, a comprehensive systematic review and meta-analysis of literature that has established reference intervals for biochemical and hematological analytes in the black population.



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Are amphetamines the new opiates? Evolving trends of drug positivity rates and concentrations in a mega cohort of neonatal meconium specimens

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Background: Maternal drug use during pregnancy can predispose the newborn to neonatal abstinence syndrome (NAS) but also to long-term effects including hearing difficulty, vision problems, and learning disability. According to a 2017 estimate, 7.3 infants per 1000 births suffer from NAS, which is commonly associated with opioid use. The Substance Abuse and Mental Health Services Administration (SAMHSA) annually publishes self-reported rates of drug use during pregnancy; however, com-

prehensive long-term laboratory data are lacking. Furthermore, the impact of COVID-19 pandemic on maternal drug use is unknown. The objective of this study was to investigate drug positivity rates, concentrations, and patterns in a large cohort of patient specimens to decipher trends in maternal drug use over the past six years. **Methods:** A large number (>150,000) of meconium specimens from 46 US states were analyzed between the years 2015 and 2020. We retrospectively investigated drug positivity rates for 28 analytes from 5 drug classes (i.e. cannabis, opioids, stimulants, benzodiazepines, and barbiturates). An immunoassay screen reflexed to quantitative drug detection was conducted by LC-MS assays. Data were analyzed using R-programming, Microsoft Excel, and GraphPad Prism packages. **Results:** The total meconium drug positivity rate was lowest in 2015 (47.25%) and steadily increased over six years, reaching a peak in 2020 (54.44%). 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC) was the most frequently detected analyte across all six years. The second most frequently detected analyte during 2017-2020 was amphetamines in contrast to morphine for the years 2015-2016. THC positivity rate rose to 38.21% (2020) from 29.71% (2015). Conversely, the opiates positivity rate consistently declined over time in the range of 1.71-2.25%. The positivity rates for stimulants increased over time in the range of 0.41-2.85%. We also investigated multidrug positivity combinations. For the years 2015-2016, the most common two-drug combination was THC-opiates (2.44%), which was replaced by THC-amphetamines (2.60%) in 2017-2020. The most common three-drug combination was THC-opiates-amphetamines through all six years. Increasing median concentrations of THC continued to be detected after 2015, declining in 2019 followed by a 1.37-fold increase in 2020. In the opiates drug class, decreasing concentrations of morphine were detected up until 2019 (1.26-fold reduction), followed by an upward trend in 2020. An increasing concentration trend was also observed for methadone, reaching a 1.41-fold increase in 2020 compared to 2015. In the stimulant drug class, an increasing concentration trend was seen for both amphetamine and cocaine, reaching a peak in 2019 with 1.14- and 1.60-fold increases respectively relative to 2015. **Conclusion:** Positivity rates for commonly abused compounds including opiates and benzodiazepines declined over six years, while those for THC, amphetamines, and cocaine grew. As well, the median drug concentrations for THC, amphetamines, and cocaine increased over time, while opiate concentrations declined. Lastly, the most common two-drug combination shifted from THC-opiates in 2016 to THC-amphetamines by 2020. Thus, our study suggests that maternal drug use rose in 2020 compared to 2019 with amphetamines replacing opiates. The impact of this change on neonatal health will be realized with future reports on NAS incidence.

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Pediatric Reference Intervals for Essential Trace Elements in the CALIPER Cohort of Healthy Children and Adolescents

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Background: Essential trace elements play critical roles in cellular metabolism and neurocognitive function. Deficiencies in essential trace elements have serious medical implications, particularly in pediatrics wherein the risk of developing elemental deficiencies is higher. Pediatric reference intervals (RIs) for essential trace elements are lacking on modern analytical systems, increasing the risk of inaccurate test result interpretation. The current study establishes comprehensive pediatric RIs for essential trace elements in whole blood and plasma samples collected from the Canadian Laboratory Initiative on Pediatric RIs (CALIPER) cohort of healthy children and adolescents. **Methods**: Approximately 175 community children and adolescents (5 to 18 years) were recruited as part of CALIPER with informed consent. Blood was collected through venipuncture into Royal Blue Top trace metal analysis tubes. Whole blood and plasma were collected from each participant when possible. Trace metal analysis was performed on two Agilent 8800 triple quadrupole ICP-MS/MS instruments connected to autosamplers. Analytical methods utilized helium and/or oxygen as collision gases when operating in kinetic energy discrimination mode. Oxygen was also used as a reactive gas for select elements. Measurements were obtained for the following essential trace elements chromium, cobalt, copper, manganese, molybdenum, nickel, and selenium in plasma and whole blood. Iodine and zinc were also evaluated in plasma only. RIs were then established according to Clinical and Laboratory Institute Guidelines. **Results**: Of the nine essential trace elements assessed, only copper and selenium demonstrated significant age-specific changes throughout growth and development, requiring partitioning. Copper concentrations decreased in early adolescence (12 to 18 years), whereas selenium concentrations increased. Remaining essential trace elements demonstrated constant reference value distributions from 5 to 18 years. No statistically significant sex-specific differences were observed. **Conclusion**: This is the first study to establish comprehensive RIs for essential trace elements via ICP-MS/MS in a healthy Canadian pediatric cohort. These data greatly contribute to our understanding of normative pediatric values for essential trace elements towards more accurate clinical-decision making and will be of value to pediatric clinical institutions offering trace element panels.

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Pediatric Reference Intervals for Heavy Metals in the CALIPER Cohort of Healthy Children and Adolescents

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Background: Biomonitoring is an important tool in assessing human exposure to toxic metals, informing risk assessment and clinical management at the patient and population level. In children, exposure to toxic metals can cause significant neurodevelopmental delays that may go undetected without proper laboratory investigation and test interpretation. Despite clinical importance, few studies have established reference intervals (RIs) to define toxicity in the pediatric population using modern analytical methods. The current study establishes comprehensive pediatric RIs for key heavy metals in whole blood and plasma samples collected from the Canadian Laboratory Initiative on Pediatric RIs (CALIPER) cohort of healthy children and adolescents. **Methods**: Healthy children and adolescents (5 to 18 years, n=175) were recruited via community initiatives with informed consent as part of CALIPER. Participation required completion of a health questionnaire and blood sample donation collected via venipuncture into Royal Blue Top trace element analysis tubes. Whole blood and plasma were both collected from participants when possible Two Agilent 8800 triple quadrupole ICP-MS/MS instruments connected to autosamplers were used to measure the following 11 heavy metals: aluminum, arsenic, cadmium, lead, mercury, platinum, silver, thallium, tin, titanium, and vanadium. Analytical methods utilized both helium and oxygen as discriminatory gases when operating in kinetic energy discrimination mode. Oxygen was used as a reactive gas for select elements. RIs were then established according to Clinical and Laboratory Institute Guidelines. **Results**: Three general reference value distributions were observed across the 11 heavy metals assessed: 1) variable concentrations with age (i.e. aluminum, arsenic, cadmium, lead, silver, thallium, tin, titanium, vanadium), 2) increasing concentrations with age (i.e. mercury), 3) mostly undetectable concentrations from 5 to 18 years (i.e. platinum). Thus, of the 11 heavy metals assessed, only mercury required age partitioning at 12 years due to statistically significant increases in reference value concentrations. No statistically significant sex-specific differences were observed. **Conclusion**: The current study reports accurate and robust RIs for 11 heavy metals in a healthy Canadian cohort of children and adolescents for the first time. The addition of heavy metals to the CALIPER database will expand its clinical utility and be of immense value to laboratories offering such testing in the assessment of toxic metal exposure in children using similar analytical methodologies.

B-244

Pipecolic Acid is an Inconsistent Biomarker of B6-Dependent Epilepsy

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Background: B6-responsive or pyridoxine-dependent epilepsy (PDE) is a rare, autosomal recessive disease, most often caused by defects in antiquitin, an enzyme involved in lysine catabolism. This enzyme deficiency leads to abnormal accumulation of alpha aminoadipic semialdehyde (AASA) and piperidine-6-carboxylate (P6C), a known inactivator of pyridoxal 5'-phosphate. Pipecolic acid (PA) is immediately upstream of P6C, and its levels are known to be affected by diet. Abnormal accumulation of these metabolites aid in the diagnosis of PDE and long-term therapeutic monitoring. B6 supplementation and lysine-restricted diets are primary strategies to minimize seizures. Clinical guidelines for therapeutic management of patients with PDE do not include target metabolite concentrations. PA is stable and abundant in blood compared to AASA or P6C; however, PA levels can be within the normal range for patients with PDE. Previous studies have not characterized the relationship between PA and the primary markers, AASA and P6C. The goal of this study is to compare AASA, P6C, and PA concentrations in a cohort of patients with PDE to assess trends and predictability. We hypothesize that PA may normalize at lower AASA and P6C concentrations. We aim to evaluate the utility of long-term monitoring of PA rather than AASA and P6C for patients with PDE. **Materials and Methods:** Data from the electronic medical records were collected for pediatric patients diagnosed with PDE with elevated plasma AASA/P6C performed in the Seattle Children's Hospital biochemical genetics labora-

tory. Metabolites are measured using liquid-chromatography tandem mass spectrometry. 305 samples from 43 patients were reviewed, the majority submitted from outside institutions. Patients were aged 0 – 19 years at the time of collection (median age: 61 months; 48.9% female). Each patient averaged 6.76 samples (range: 1-28 samples per patient). **Results:** AASA and P6C were abnormally elevated in all samples (normal <0.2 μM and <0.5 μM, respectively). 81% of patients and 62% of the samples had normal PA concentrations (n = 35 patients and 188 samples); normal PA is 0.1 - 4.2 μM for individuals >1 month of age. AASA and P6C have a linear relationship (r^2 = 0.75); in contrast, PA does not correlate with AASA or P6C. Longitudinal testing of patients demonstrates relative metabolite stability, however, concentrations vary significantly between patients. Inspection of a single case diagnosed and managed locally confirmed that therapeutic intervention did dramatically reduce metabolite concentrations, consistent with previous reports. **Conclusions:** Rapid diagnosis of genetic causes of seizures is critical for optimal patient care. The metabolites AASA and P6C correlate and are reliable biomarkers for PDE. The lack of correlation between PA and AASA or P6C suggests that there is significant risk of false negative results, even at the time of diagnosis. Lower AASA and P6C values likely reflect therapy; correlation to clinical symptoms and dosing was not feasible because nearly all patients were tested as part of reference laboratory services. Given the frequency of normal PA but persistent elevation of P6C and AASA, trending PA may have very limited value for long-term therapeutic monitoring.

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sFlt-1/PlGF Ratio is Superior to Hypertension and Proteinuria for Predicting Preeclampsia

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Background: Preeclampsia is a heterogenous, multisystem disorder characterized by hypertension and proteinuria. Due to the non-specificity of symptoms in preeclampsia, the use of prognostic biomarkers is important for early detection and monitoring. The ratio of the anti-angiogenic factor, soluble fms-like tyrosine kinase 1 (sFlt-1), to the pro-angiogenic factor, placental growth factor (PlGF), is associated with increased risk of preeclampsia. Here, we describe a clinical evaluation of the Roche Elecsys sFlt-1 and PlGF assays at the first North American site in which they were clinically implemented. **Methods:** sFlt-1, PlGF, and the sFlt-1/PlGF ratio were measured in serum samples from 153 pregnant females at Sunnybrook Health Science Centre (Toronto, ON). The mean patient age was 33.2 ± 5.1 years and the gestational age ranged from 20+0 to 40+3 weeks at time of blood collection. Preeclampsia diagnosis was verified by expert chart review. sFlt-1 and PlGF were measured on the automated analyzer cobas® e602 module using Elecsys immunoassay reagents (Roche Diagnostics) in the routine clinical diagnostic laboratory. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were assessed for different sFlt-1/PlGF ratio cut-offs (≥33, >38, ≥85, ≥110) and PlGF cut-offs (<12 ng/L, <100 ng/L). Furthermore, these same clinical performance parameters were used to compare the optimal sFlt-1/PlGF ratio to hypertension and proteinuria alone and in combination to predict preeclampsia within 7 days and 28 days. **Results:** Overall, 51 (33.3%) of pregnant females in our cohort were clinically diagnosed with preeclampsia. The median and interquartile range (IQR) for sFlt-1, PlGF, and sFlt-1/PlGF ratio were 3606 ng/L (2151-7222 ng/L), 194 ng/L (97-433 ng/L), and 20 (4-62), respectively. The sFlt-1/PlGF ratio cut-off of 38 exhibited the highest diagnostic accuracy of 90.2% (CI: 85.5%-94.9%) compared to the other sFlt-1/PlGF ratio cut-offs, although not statistically significant. The sFlt-1/PlGF ratio cut-off of 38 had a significantly higher diagnostic accuracy compared to both PlGF cut-offs, hypertension, and proteinuria for predicting preeclampsia. When specifically comparing the sFlt-1/PlGF ratio cut-off of 38 to hypertension and proteinuria to predict preeclampsia within 7 and 28 days, the sFlt-1/PlGF ratio was significantly superior, primarily due to the poor sensitivity of proteinuria and poor specificity of hypertension. Lastly, when compared to hypertension and proteinuria combined, adding sFlt-1/PlGF ratio as a third criterion (all three criteria must be met), the specificity and positive predictive value significantly improved, reaching 100% specificity and 100% PPV for predicting preeclampsia within 7 days and 28 days. **Conclusion:** Our study shows the utility of the sFlt-1/PlGF ratio at the first North American institution to adopt this test clinically, which serves a high-risk obstetrical unit. Of the sFlt-1/PlGF ratio cut-offs assessed, a cut-off of >38 was shown to have optimal clinical performance, superior to either hypertension or proteinuria alone. Furthermore, specificity and PPV improved when sFlt-1/PlGF ratio was combined with hypertension and proteinuria.

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Comparison of Cystatin C-based Equations with Measured Glomerular Filtration Rate in a Diverse Pediatric Population

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Background: Assessment of kidney function is essential for early detection, monitoring, and intervention of kidney damage. While the gold standard to assess kidney function is glomerular filtration rate (GFR), equations that estimate GFR from serum creatinine and Cystatin C (CysC) are less invasive routes to assess GFR. In pediatric populations, CysC-based equations provide a closer approximation as they are independent of body composition. Limited information is available on the performance of CysC-based equations in comparison with measured GFR (mGFR) with tracers other than iohexol. Furthermore, the use of race-corrected equations can cause an overestimation of the eGFR in groups such as African Americans. Therefore, the goal of our study was to evaluate how estimated GFR (eGFR), based on several CysC and creatinine-based equations, with and without race correction, relates to mGFR. **Methods**: A total of 160 subjects (age 4 months to 20 years; 51 non-Hispanic white, 69 Hispanic white, 29 non-Hispanic black, 7 Asian, and 4 unspecified) were retrospectively studied to compare the mGFR from multiple blood sample collection after intravenous tracer injection (Tc-99mDTPA) with eGFR using the CKiD, Schwartz CysC, MDRD, CKD-EPI creatinine, CKD-EPI 2012 CysC, and CKD-EPI CysC-creatinine equations. Linear regression analyses was performed to assess correlation between the mGFR and eGFRs. Percentage within the 10 and 30 % of mGFR was also evaluated. **Results**: The average mGFR for this cohort was 88.5mL/min/1.73 m^2. Equations with race correction gave an overestimated eGFR across all ethnic groups, especially in the Hispanic white and non-Hispanic black cohorts. The best correlations to mGFR were seen with no race-corrected equations CKiD and CKD-EPI CysC. **Conclusion**: Overall, CysC-based equations without race correction provide a good approximation of mGFR and a less invasive alternative to monitoring kidney function in pediatric population, irrespective of race/ethnicity.

		No race correction eGFR			Race corrected eGFR		
	Measured GFR	CKiD	CKD-EPI 2012 CysC	Schwartz CysC	CKD-EPI CysC-creatinine	CKD EPI creatinine	MDRD
Correlation coefficient	-	0.7197	0.6780	0.6650	0.7255	0.5631	0.5088
Slope	-	0.78	1.07	0.59	1.06	1.81	14.93
Intercept	-	21.77	2.06	22.95	27.21	5.15	-994.77
Within 10% error	-	30.6	17.5	29.4	11.2	5.0	7.5
Within 30% error	-	69.4	65.6	69.4	38.7	17.5	13.1
Mean (SD)	88.5 (35.9)	90.9 (30.0)	96.8 (37.6)	75.0 (25.0)	121.3 (37.5)	165.4 (50.8)	326.3 (276.1)
Mean (SD) Non-Hispanic white	88.2 (33.6)	91.4 (26.0)	102.2 (36.3)	77.3 (23.6)	121.8 (35.5)	159.0 (49.5)	288.0 (219.8)
Mean (SD) Hispanic white	84.0 (37.2)	92.4 (35.3)	96.2 (38.4)	74.8 (25.4)	120.9 (39.2)	164.3 (51.7)	345.2 (312.5)
Mean (SD) Non-Hispanic black	93.3 (32.2)	85.6 (23.2)	90.6 (36.8)	72.3 (26.8)	122.3 (34.9)	180.5 (46.2)	330.6 (230.6)
Total Bias	-	-2.4	-8.3	13.5	-38.8	-76.9	-237.8
Bias in Non-Hispanic white	-	-3.1	-14.0	10.9	-33.6	-70.8	-199.8
Bias in Hispanic white	-	-8.4	-12.3	9.2	-36.9	-80.3	-261.2
Bias in Non-Hispanic black	-	7.6	2.7	21.0	-29.1	-87.2	-237.4

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Postprandial inflammation and metabolic dysfunction in adolescents with obesity and insulin resistance.

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Background: The striking rise in adolescent obesity and insulin resistance is associated with lipid and lipoprotein abnormalities, which are integral to the increased risk of future Type 2 Diabetes (T2D) and cardiovascular disease (CVD). In addition to fasting lipids, postprandial dyslipidemia is an independent risk factor for CVD and is a better reflection of metabolic activity compared to fasting lipid markers. Obesity contributes to CVD via inflammatory stimulation, which is also associated with insulin resistance pathogenesis and obesity-related mortality. In addition, intestinal and lipid metabolism are intimately related and reportedly maladaptive in obesity and insulin resistance. The objective of the current study was to characterize the fasting

and postprandial profiles of inflammatory and metabolic markers in adolescents with obesity and insulin resistance, and to assess their role in the pathogenesis of postprandial dyslipidemia.

Methods: Participants were recruited from the SickKids Team Obesity Management Program (N=30; adolescents with obesity) or from the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) cohort of healthy adolescents (N=15; normal weight [NW] adolescents). All participants (12-<19 years) underwent a 6-hour oral fat tolerance test in the hospital investigational research unit. Analytes measured included cytokines (interleukin [IL]-6, -10, -18, -1β, tumour necrosis factor-α, interferon-γ, monokine induced by γ, and cluster of differentiation factor 163) and a complete metabolomics profile (>133 metabolites). Analytes were measured using an automated immunoassay system (Bio-Techne) and a combination of direct injection mass spectrometry (MS) with a reverse-phase liquid chromatography-MS/MS custom assay, respectively. Subjects with obesity and severe insulin resistance, obesity and mild insulin resistance, and those with NW were compared for all analytes using a two-way ANOVA.

Results: Adolescents with obesity and insulin resistance demonstrated significantly dysregulated fasting and postprandial profiles in cytokines and metabolites. Specifically, levels of IL-6 were significantly elevated in both obesity groups at fasting (P<0.001), which was maintained postprandially compared to the NW response (main effect of group: P<0.05). The between-group difference in postprandial responses observed in other studied cytokines did not reach, although few approached (i.e. tumour necrosis factor-α, IL-18), statistical significance. Further, acylcarnitines, amino acids, biogenic amines, and glycerophospholipids were significantly downregulated postprandially in obesity compared to NW subjects. For example, significantly reduced levels of fasting octadecadienylcarnitine and a blunted postprandial response was seen in obesity compared to NW subjects (group*time interaction P<0.001). Similar between-group differences were also observed postprandially in arginine, choline, and lysophosphatidylcholine acyl C14:0 (group*time interaction P<0.05). Key exceptions were observed in select analytes: levels of glutamic acid, alpha aminoadipic acid, lactic acid, and creatine were significantly elevated in both obesity groups compared to NW, at fasting and postprandially (group*time interaction P<0.005).

Conclusion: Adolescents with obesity and insulin resistance exhibit significant fasting and postprandial dysregulation of several inflammatory and metabolic markers integral to lipid metabolism. These data may offer novel subclinical biomarkers for early metabolic and cardiovascular diseases, such as postprandial dyslipidemia, in pre-diabetic adolescents. Future research should seek to determine the clinical utility of identified biomarkers and their predictive capacity for postprandial dyslipidemia and future CVD and T2D.

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Interference Assessment of Ketone Bodies on Laboratory Pediatric Creatinine Measurement

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BACKGROUND: Recent evidence demonstrated that a high proportion of children hospitalized for diabetic ketoacidosis (DKA) develop acute kidney injury (AKI), which is diagnosed and staged by measuring serum creatinine (Cr). However, the presence of ketone bodies (KB)—acetone, acetoacetate and β-hydroxybutyrate (BOHB) can interfere with Cr measurement in both enzymatic and Jaffe methods. This complicates the assessment of renal function when pediatric patients with DKA are transferred between centres that use different Cr methods. In order to understand the KB interference profiles and evaluate its impact on the diagnosis of AKI in children with DKA in our region, we undertook a multi-center study to compare the accuracy of Cr methods utilized at one pediatric tertiary care hospital and three general teaching hospitals. All methods were compared to the gold standard, liquid chromatography-tandem mass spectrometry (LC-MS/MS), in the presence of KBs.**METHODS:** Residual patient plasma pools at approximately 50, 100 and 250 uM Cr were spiked to final concentrations of acetone (0 to 50 mM), acetoacetate (0 to 20 mM), or BHOB (0 to 20 mM). Aliquots were distributed for testing by either one of two enzymatic assays (Ortho VITROS® 5600 (E1) or Roche cobas® C501 (E2)), or one of two Jaffe methods (Beckman Coulter UniCel DxC 800 (J1) or Siemens VISTA® 1500 (J2)), or Xevo TQ LC-MS/MS (Waters Corporation) in duplicate. The % difference in Cr was calculated for each method with each KB and interference was defined as exceeding ±15%.**RESULTS**: E1 and E2 were largely unaffected by the presence of KBs as the absolute % bias relative to Cr levels by LC-MS/MS for treatment groups were <15% (range, -12% to 8%). Similarly, J1 and J2 were largely unaffected by the presence of BOHB (range, -1% to -10%). The presence of acetone resulted in highly significant (P-value range, P<.01 to P<.0001) dose-dependent positive interference in both Jaffe methods, whereas the presence of acetoacetate resulted in highly significant (P-value range, P<.01 to P<.0001) dose-dependent positive and negative interference in J1 and J2, respectively. The absolute magnitude of interference by acetone and acetoacetate in J1 and J2 was inversely proportional to the Cr concentration. **CONCLUSION:** Compared to the enzymatic methods, the Jaffe methods are much more susceptible to interference by acetone and acetoacetate, especially at lower Cr values which are commonly seen in pediatrics. Interpretation of changes in Cr concentration in transferred patients can become ambiguous and true renal function unclear if different methods are used without awareness of method specific biases. Therefore, understanding the KB interference profiles of different Cr methods is crucial for improvement of DKA patient care. Standardization of all the Cr methods in a given region to an enzymatic method is recommended.

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Analytical and Clinical Evaluation of Whole Blood Bilirubin as a Screening Test for Neonatal hyperbilirubinemia

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Background: Neonatal jaundice is the most common condition that requires medical attention in newborns. Prior to discharge, all babies born at our centre are screened using total serum bilirubin (TSB), the current gold standard for hyperbilirubinemia assessment. However, whole blood bilirubin (TwB) analyzed by co-oximetry has recently emerged as a faster alternative requiring much lower sample volumes (45 uL vs ~250uL for TSB). The aim of this study is to determine the performance of TwB in comparison to TSB for neonatal hyperbilirubinemia assessment.**Methods:** We recruited 189 neonates with TSB ordered from both inpatients and outpatients at our centre. For each baby, bilirubin was measured both with the standard blood collection procedure for TSB (capillary blood from neonates by heel pokes with lithium heparin microtainer), as well as an additional drop via capillary rod for TwB testing. Both samples were sent to the lab for testing with TSB being measured by VITROS 5600® (Ortho Diagnostics), and TwB measured by ABL 90 FLEX PLUS (Radiometer). All enrolled patients provided written consent by their guardians. This study was approved by the Research Ethics Committee of our institute (H18-01979). Bilirubin measurements were compared via Pearson's correlation, two one sided t-tests (TOST), Passing-Bablok regression and Bland-Altman plots. The turn-around-time (TAT) from sample collection to the reporting of results, between these two bilirubin tests were also compared.**Results:** Of the 189 cases, both TSB and TwB were available in 168 patients (88.8%). The median age was 3 days with an interquartile range of 2 to 5 days and the oldest infant being 13 days. The Pearson correlation between measurements was 0.98, and the TOST procedure for ± 7 umol/L indicated no significant difference between the bilirubin measurements by these two methods. Passing-Bablok regression yielded an estimated slope of 1.11 (95% CI = (1.08, 1.15)) and an intercept of -23.5 umol/L (95% CI = (-31.7, -18.0)), while the Bland-Altman plots had levels of agreement of -32.3 and 35.4 umol/L, with a mean bias of 1.55 umol/L and slightly higher disagreement at higher bilirubin values. The mean TAT for the TSB and TwB was 51.86 minutes (SD = 29.57) and 20.98 minutes (SD = 9.6) respectively ($p < 0.001$). Applying the Bhutani nomogram 40th percentile (95th percentile) to categorize risks, the results by the ABL, in comparison to the VITROS, exhibited a sensitivity of 92% (72%) and 87% (93%) respectively. **Conclusion:** Whole blood bilirubin by ABL 90 FLEX PLUS blood gas instrument is an accurate and fast alternative to the current standard of care of TSB. Further assessment of the potential of TwB testing to shorten the length of hospital stay, and to reduce costs by a randomized controlled trial is ongoing.

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The Alteration of Biochemical Blood Examination Among Athletes Using Anabolic Androgenic Steroids

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Background: A growing number of athletes are using synthetic anabolic-androgenic steroids (AAS), comprised of testosterone and other derivatives, to enhance athletic performance and muscle mass. Over the years, numerous reports elucidated the side effects brought on by the illegal use of unprescribed AAS such as liver disorders and infertility. Consequently, AAS's recreational use has become a case of concern for the

general public's health worldwide and should be brought to more serious attention in Saudi Arabia. As of yet, AAS's effect on the hepatic and reproductive systems in Saudi athletes has never been studied. Here, we examined liver function and sex hormone tests in the blood of AAS users as compared to non -users. **Methods:** Fasting blood samples were collected from 16 athletes, 10 AAS users (cases) and 6 non-users (controls) to measure liver function parameters, specifically: ALT, AST, GGT, LDH, ALP, CK, total protein, albumin, direct and total bilirubin, and fertility hormones including luteinizing hormone (LH), follicle stimulating hormones (FSH), total testosterone, estradiol, and prolactin (PRL). Furthermore, a self-reported questionnaire was used to identify the type of AAS used, the dosage, and the length of the course prior to sample collection. **Results:** The results in AAS users were significantly increased in ALT ($p<0.001$), AST ($p<0.001$), CK ($p<0.05$), and LDH ($p<0.001$), while albumin and total bilirubin levels were significantly decreased ($p<0.001$ and $p<0.01$ respectively). However, for fertility hormones and among the same AAS users the following significant changes were obtained; increased levels of total testosterone ($p<0.05$), prolactin ($p<0.05$) and estradiol ($p<0.05$) while LH and FSH were significantly decreased ($p<0.01$ and $p<0.001$ respectively). **Conclusion:** Recreational AAS use outside the therapeutic frame induces biochemical changes and increases chances of liver damage and might cause infertility with chronic use in Saudi athletes.

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The Utility of Interleukin-6 in Managing the COVID-19 Hospitalized Patient

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Background The documented sequelae of COVID-19 caused by the SARS-CoV-2 virus frequently include acute respiratory distress, acute renal failure, and thrombosis. The pro-inflammatory events that precede these complications involve the actions of cytokines secreted by macrophages, fibroblasts, mast cells, and other cells of the vascular endothelium, in response to infections and tissue damage. Central to this acute inflammatory process is interleukin-6 (IL-6), a pro-inflammatory cytokine whose circulating levels in the blood may be measured using immunoassay methods. Increased levels of IL-6 have been associated with complications, morbidity, and mortality in hospitalized patients with COVID-19. The "cytokine storm" that precedes these worsening conditions may be due, in part, to the function of IL-6, which has been investigated as a prognosticator in the disease course of COVID-19. Therapeutic suppression of IL-6 production has also been employed to reduce the effects of a prolonged inflammatory state.

Methods Residual serum specimens from routinely ordered comprehensive metabolic profiles were obtained within one day of their collection from 64 randomly-selected hospitalized adult patients with active COVID-19 infection who had tested positive for the SARS-CoV-2 virus as determined by real-time polymerase chain reactivity (rt-PCR) using the Abbott m2000 Molecular System (Abbott Molecular Diagnostics, Abbott Park, IL). All serum samples had been stored at 2-8°C, and aliquots were prepared and stored below -20°C until their analysis for IL-6. Quantitative determination of IL-6 was performed using an immunoenzymatic chemiluminescent assay (DxI-800 Immunoassay System, Beckman Coulter, Chaska, MN), which has received emergency use authorization (EUA) by the United States Food and Drug Administration (FDA). Comprehensive review of medical records was performed by pathology medical residents to access and summarize the patients' medical history, stratify biometric and clinical laboratory data, and categorize outcome. All data acquisition and analysis were performed according to the research protocol approved by the Loyola University Institutional Review Board.

Results We found that increased IL-6, (>11.0 pg/mL) was associated with prolonged length of stay, respiratory complications, and overall mortality of patients hospitalized with COVID-19. The median IL-6 level was 29.6 pg/mL in patients who were hospitalized less than 5 days, and 77.0 pg/mL in patients who were hospitalized for 5 days or longer ($p<0.05$). Patients requiring mechanical respiration and intubation had median IL-6 levels of 164.5 pg/mL while those who did not require intubation had median IL-6 levels of 29.4 pg/mL ($p<0.05$). Among patients who expired, the median level of IL-6 was 1384 pg/mL, while among those who recovered from COVID-19, the median IL-6 level was 46.2 pg/mL ($p<0.05$). Increased IL-6 was found to be positively associated with other acute phase reactants, including increased c-reactive protein (98%) and ferritin (84%), and also with an increase in fibrin degradation product d-dimer (96%).

Conclusions Our analysis affirms that IL-6 measurement has utility in identifying the pro-inflammatory risk associated with the disease progression of COVID-19. Patients found to have increasing serum concentrations of IL-6 may be identified as requiring therapeutic intervention to reduce the associated risk of acute cytokine-mediated inflammation in the management of the hospitalized COVID-19 patient.

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Implementation of Newborn Screening Test in Dominican Republic

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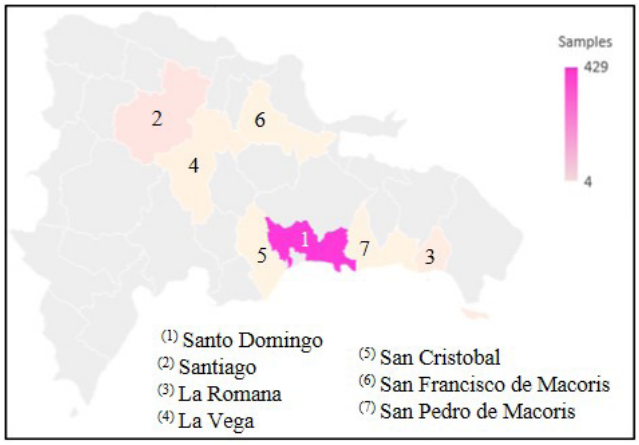
Background: Neonatal screening is designed to carry out a massive and universal search for a series of congenital diseases, potentially catastrophic and difficult to recognize clinically in the neonatal population. In turn, this may permit the avoidance of some physical and mental handicaps, as well as death. Our institution is the first private clinical laboratory to process neonatal screening test from heel samples in the Dominican Republic (DR). Methods: Blood samples were taken from the heel between 2 and 5 days after birth on filter paper and the samples were allowed to dry for 4 hours. Seven tests were included in the screening: thyroid stimulating hormone (TSH), 17-hydroxyprogesterone (17-OHP), phenylalanine (PKU), total galactose (TGAL), immunoreactive trypsin (IRT), biotinidase (BIO) deficiency and glucose-6-phosphate dehydrogenase (G6PD) deficiency. PerkinElmer's time-resolved fluorescence-based or immediate fluorescence-based assays were used. Results: A total of 504 samples were reviewed, with data covering the period from June 2019 to December 2020. For processed screening tests, apparently positive results were obtained in 13 tests for G6PD, 8 tests of IRT, 3 tests of TGAL, 2 tests of TSH, 1 test of PKU, 1 test of 17-OHP and all samples were apparently negative for BIO deficiency. Conclusion: Neonatal screening implementation is complex, and the diseases tested for vary greatly in different countries. In the DR, it might be important to prioritize G6PD and IRT testing in the newborn screening program. More support and promotion are needed at different levels to make newborn screening more widespread. In addition, it is important to strengthen follow-up in apparently positive cases and include confirmatory tests.

Figure 1. Geographic distribution of samples



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Whole blood cardiac troponin 'triaging' to improve early detection of myocardial injury at a pediatric hospital

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Background: Rapid cardiac troponin (cTn) testing is essential for identifying myocardial injury and is now the gold standard in adult hospitals. As children present with a different spectrum of etiologies of myocardial injury than adults, the importance of offering rapid turnaround times (TATs) for cTn testing is underappreciated in the pediatric population. The American Association for Clinical Chemistry recommends that point of care tests be used if laboratories cannot provide adequate TATs. At Alberta Children's Hospital in Calgary, Alberta, Canada, plasma specimens for Roche high-sensitivity cardiac troponin T (hs-cTnT) are couriered ~2 km for off-site testing at the rapid response laboratory at Foothills Medical Centre. Recent changes in hospital patient population, availability of healthcare resources such as rapid-dispatch couriers, as well as increased testing demand have resulted in longer TATs, nega-

tively affecting pediatric cardiac care. Our objective was to devise, implement and evaluate cost-effective pre-analytic and analytic solutions to improve TAT. **Methods:** Baseline TATs for specimen collection to result verification were reviewed. Candidate pre-analytic changes were identified in collaboration with affected hospital units (e.g. Pediatric Emergency Medicine, Pediatric Cardiology). Candidate point of care instruments for on-site cTn testing were identified. Comparison between point of care conventional cTn assay and hs-cTnT was conducted to determine if and how the conventional cTn assay could be used to improve the timeliness of clinical decision making. TAT pre- and post- implementation and diagnostic efficiency were evaluated to determine if the intervention was effective. **Results:** Target TAT for collect to verify for priority (STAT) cTn was <90 minutes. Baseline Median TAT for orders transported offsite was 103 minutes with approximately 34% of tests having a TAT >120 minutes. To decrease TAT, an order instruction was added to the clinical information system requiring a separate collection tube (to eliminate specimen aliquotting) and to disqualify serum separator tubes (to eliminate the clotting requirement). These changes had no impact on TAT. The Quidel Triage whole blood cTn-I test was evaluated as on site testing solution and compared against plasma hs-cTnT. Total imprecision was approximately 25%, and low end of the AMR sensitivity was not comparable to hs-cTnT. However, using a positive cutoff value of ≥0.02 µg/L, the Triage had a sensitivity of 91% and specificity of 100% to detect an hs-cTnT elevation above 52 ng/L (n=40). This point of care test with a median TAT of approximately 30 minutes was implemented for on-site rapid rule-in, and specimens continued to be sent for off-site hs-cTnT testing to confirm all positive and negative findings. A chartable comment was added to results indicating that a negative result does not exclude the possibility of myocardial injury, and that the ordering physician should in these cases wait for the hs-cTnT result. **Conclusions:** On-site point of care whole blood cTn testing can be used to rapidly confirm significant or late-presenting myocardial injury. Combined with simultaneous off-site confirmatory hs-cTn testing for rule-out, this workflow can provide an interim solution until on site point of care hs-cTn testing is available.

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Reference intervals for hs-cTnT, NT-proBNP and plasma lactate in Vancouver mothers near the time of delivery

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Background: Laboratory testing is a part of maternal care when delivery or health complications threaten the wellbeing of mom and/or baby. Symptoms of pregnancy and labour can often overlap with those of myocardial infarction and heart failure, and some pregnant women may have pre-existing cardiovascular conditions. Consequently, cardiac markers are used to help identify cardiovascular events, even during pregnancy. N-terminal pro b-type Natriuretic Peptide (NT-proBNP) increases by varying degrees throughout pregnancy and rises significantly in pre-eclampsia, while troponin is reported to be higher in pregnant women with hypertension. Similarly, identification of infection during labour and delivery is important to avoid maternal and neonatal morbidity. Elevated plasma lactate can serve as an indicator of infection. Despite the necessity of these biomarkers in acute maternal care, few reference intervals (RIs) are established during pregnancy, and even less exist for the period of labour and delivery. This study derived normal reference intervals for three acute care biomarkers: high sensitivity cardiac troponin T (hs-cTnT), NT-proBNP and lactate in healthy women near the time of labour and delivery. **Methods:** Healthy mothers aged 19 to 45, with healthy, uncomplicated, singleton pregnancies, delivering at BC Women's Hospital, were recruited for participation in the Pregnancy Reference Intervals for Safe Medicine (PRISM) study. Women who were in labour or were being admitted for scheduled C-section or induction of labour were asked to donate blood. Lithium heparin plasma was stored frozen until study recruitment was completed. A total of 233 samples were used for cardiac biomarker analysis. Specimens were then analyzed using clinical testing methods on the Ortho Vitros 5600 or Roche e601 platforms. Reference intervals were derived using previously published approaches, in line with CLSI recommendations, and included assessment of data distribution and transformation when needed, outlier detection, assessment of need for partitioning between labouring and non-labouring women, and non-parametric calculation of reference limits for partitions with > 120 values, or using the robust method when partitions were smaller. Analysis was performed using Microsoft Excel and MedCal (v19.2) software. **Results:** Reference intervals for hs-cTnT, NT-proBNP and lactate were determined. Hs-cTnT did not require partitioning and the overall 99th percentile reference limit was determined to be 7.50 ng/L. Partitioning between labouring and not in-labour women was required for NT-proBNP and lactate. For NT-proBNP, the upper RI limit calculated as the 97.5%tile was 192 ng/L for mothers who were in labour and 106 ng/L for mothers who were not yet inlabour. Lactate reference intervals were determined to be 0.83-3.38 mmol/L and 0.70-2.07 mmol/L for labouring and non-labouring groups, respectively. **Conclusion:** Local peripartum reference intervals have been derived using samples from contemporary Canadian mothers. Upper limits of NT-proBNP and plasma lactate are slightly higher in labouring mothers, and are also higher than limits used for non-pregnant women, while the upper limit of hs-cTnT was lower in this cohort, suggesting that use of pregnancy-specific RIs may improve lab test interpretation in the peripartum period.

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The Prevalence of Anemia and Obesity in Indian School Children: A Survey

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Background: Anemic children are prone to suffer from developmental delay as well as cognitive and behavioral disturbances. In India, the socioeconomic shift in the past few decades has led the transition to a sedentary lifestyle with poor dietary habits leading to an increased incidence of overweight and obesity in children and adults alike. Furthermore, obesity and anemia share some common links. In this survey, we have carried out a prevalence study of obesity and anemia in school-going children.

Methods: The study was carried out on 1675 children and adolescent participants aged 5-18 years after obtaining informed consent from their parents. Standing height (in cm) and weight (in kg) were recorded on a stadiometer and a Krups weighing machine, respectively. Body-mass index (BMI) was calculated using the formula, BMI=weight in kilos/ (height in meters)^2. The overweight and obese populations were determined from the CDC growth chart data, where BMI >97th percentile was considered obese. For assessing anemia, hemoglobin was measured using Drabkin's method. The statistical analysis were carried out using Stata and the R programming platform.

Results: A total of 1675 participants were surveyed, among which 60.5% were males and 39.5% were females. The difference in age between males and females (median age 10 years for both) was non-significant (p=0.70). However, they differed in height (median [IQR] boys 140 [125-158], girls 138 [122-153], p=0.01), waist circumference (median [IQR] boys 59 [52-68], girls 58 [51-65], p=0.02), waist-to-hip ratio (median [IQR] boys 0.87 [0.83-0.90], girls 0.85 [0.81-0.89], p<0.01) and hemoglobin levels (median [IQR] boys 12.8 [11.5-13.7], girls 11.9 [11.0-12.8], p<0.01). A total of 294 girls (44.4%) and 283 boys (29.7%) were anemic. There were significant differences between BMI within age groups for both boys (median [IQR] for 6-11 years: 22.77 [20.16-23.79], 12-14 years: 27.11 [27.06-27.19], 15-18 years: 29.75 [28.38-30.83], p<0.01) and girls (median [IQR] for 6-11 years: 23.38 [21.89-24.87], 12-14 years: 29.28 [27.39-30.98], 15-18 years: 30.11 [29.32-30.49], p<0.01). The highest percentage of anemia was observed in the 12-14 years age group in girls (54.2%) and 15-18 year-old boys (54.2%). Also, 4.4% of boys and 4.9% of girls were found to be obese, whereas 8.4% boys and 9.2% girls were overweight. Among the obese children, 28.2% were anemic, while 29.3% of overweight children were anemic (Pearson's chi-squared=7.68, p=0.02).

Conclusion: This study sheds light on the prevalence of obesity and anemia in Indian schoolchildren and adolescents, while also suggesting an association between the two conditions. Due to improvement in the socioeconomic status of the Indian population and sedentary lifestyle, the obesity epidemic has caught up even in lesser developed nations. This study points to the fact that though calorie consumption has increased due to better economic conditions, obese children are still nutritionally challenged which has become evident as high prevalence of anemia among obese children. Nutritional counselling, as well as lifestyle modification, should be advocated in school curricula to make an early impact.

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Abnormal lipoprotein particle profiles in children and adolescents with Cystic Fibrosis: the effects of modulator therapy

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Background: Cystic Fibrosis (CF) patients are encouraged to consume ~40% of total calories from fat to compensate for the high energy demands associated with this pathology. The reported association between high-fat diets and increased risk for cardiovascular disease (CVD) and type 2 diabetes has prompted the investigation of lipids abnormalities in the setting of CF. Prior studies have described dyslipidemia in CF patients using the traditional lipid biomarkers; however, there is a growing body of literature that supports the clinical utility of advanced lipid testing in predicting CVD risk. The goal of the study was to characterize lipoprotein particles in children and adolescents with CF and correlate the results with clinical findings.

Methods: A total of 98 serum samples were collected from 91 participants: 46 males, 45 females, 10.4±5.1 years of age. Most patients were homozygous or compound heterozygous for F508del (n=88), and 45 patients received CF transmembrane conductance regulator (CFTR) modulator therapies for at least 3 months. Eighty-four participants (92%) were pancreatic insufficient on the enzyme replacement therapy. 24-hour dietary recalls were collected from all patients. Particle number and size of low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL) were measured using the AXINON LipoFIT by NMR assay (Numares, Germany). Data analyses were performed in Prism software (La Jolla, CA). The study was approved by the appropriate IRB.

Results: When compared with established reference intervals, 79% and 43% of samples had low concentrations of HDL and large-HDL particles, respectively. In contrast, VLDL particle size and large-VLDL particle number were increased in 91% and 57% of samples, respectively. These abnormalities were similarly present in fasting (n=25) and non-fasting (n=73) specimens suggesting that these findings are not postprandial artifacts. Particle numbers of LDL, small-LDL, and large-VLDL as well as VLDL particle size were significantly higher ($p<0.05$) in pancreatic sufficient patients (n=6) compared to pancreatic insufficient (PI) patients (n=67); although, total and saturated fat intake was similar in both groups (35% and 11% of total calories on average, respectively). Interestingly, the use of CFTR modulator therapy appears to have a beneficial effect on lipoprotein profiles. PI patients on CFTR modulators for >3 months (n=41) had higher concentrations of HDL particles (31±5 vs 26±5 µmol/L, $p<0.0001$) and lower concentrations of atherogenic large-VLDL particles (4±2 vs 7±6 nmol/L, $p=0.001$) than PI patients not receiving the therapy (n=43). The small LDL-particle number was also lower in the PI "modulator" group (292±118 vs 348±159 nmol/L) although the difference was not statistically significant ($p=0.060$).

Conclusion: Our data support the clinical utility of lipoprotein particle analysis in the setting of CF. Children and adolescents with CF had low concentration of HDL particles and high concentrations of VLDL and large-VLDL particles, a pattern associated with increased risk for CVD and type 2 diabetes. Patients on CFTR modulators appeared to have healthier lipoprotein profiles compared to those without intervention. Long-term follow-up is necessary to evaluate the anti-atherogenic properties of modulators.

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Validation of venous blood 'short draws' in standard-sized anticoagulated tubes for pediatric testing

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Background: Minimizing blood collection is important in pediatrics because patients have lower blood volume than adults. Glass capillaries and microcollection containers allow open collection of small volumes, however venipuncture is usually preferred because specimens are less likely to be hemolyzed and are not exposed to air for long periods. Performing 'short draws' with standard sized anticoagulated tubes (e.g. 1 mL in a 3 mL tube) is sometimes used in pediatric phlebotomy. However this process is not commonly validated in clinical laboratories and the effect of concentrated anticoagulant may not be known for multiple analytes. Our objective was therefore to compare results for several common chemistry tests and the complete blood count (CBC) in 'complete' vs 'short' drawn anticoagulated venous blood specimens. **Methods:** Five volunteers had blood drawn in two identical anticoagulated evacuated tubes (BD vacutainers) per analyte class (e.g. lithium heparin plasma separator tube for general chemistry, K2-EDTA for HbA1c and CBC). One tube ('complete draw') was filled with 3 mL of blood, whereas the other tube ('short draw') was filled with 1 mL of blood from the same volunteer. All tubes were inverted 10X and plasma tubes were centrifuged at 1200 RCF for 10 minutes. Specimens were tested for 33 common chemistry analytes (e.g. sodium, bilirubin, total CO_2, glucose, TSH, cortisol) and the CBC. Paired T-tests were used to determine if results from short draws were significantly different than results from complete draws. Absolute differences were expressed as a percentage of total allowable error (TAE) set by the College of American Pathologists (CAP). **Results:** Total CO_2, sodium, total calcium, CRP, GGT, and TSH in short draws were significantly ($p<0.05$) different than in complete draws. As a percentage of TAE, results for short vs complete draws were as follows: total CO2 = -43%, sodium: -34%, total calcium = -14%, CRP = -2%, GGT = +9%, TSH = -2%. All other tests results were not significantly different in short vs complete draws. **Conclusions**: Short drawing venous blood in standard-sized anticoagulated tubes is likely valid to perform in pediatric patients for many common laboratory tests.

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The accuracy and clinical implications of point-of-care testing in children

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Background: Point-of-care testing (POCT) is commonly used at our institution to gather data quickly for sick patients. Central laboratory testing is considered the gold standard, but results often lag POCT by a significant time period. Thus, management decisions are made on POCT results. We aimed to determine whether POCT is an accurate and clinically appropriate method to measure electrolytes, glucose, and hemoglobin compared to standard laboratory testing.

Methods: We retrospectively reviewed 128 consecutive patients assessed in the emergency department or admitted prior to November 1, 2019 who had both POCT and laboratory electrolytes (+/- glucose, hemoglobin) performed within 4 hours of each other. A sample size of 128 was required to determine a sodium difference of 3 mmol/L for an effect size of 0.5 with 0.05 level of significance and 80% statistical power. Paired t-tests compared values between laboratory testing and POCT for each patient. Secondary kappa coefficient analyses looked at the agreement within clinically-determined normal ranges. Testing was performed on Radiometer ABL835 FLEX, Beckman Coulter DxC 800, and Sysmex XN-9000 analyzers.

Results: There were 56 males and 72 females; age range 0.01- 17.93 years. There were statistically significant differences between POCT and laboratory values for all electrolytes and hemoglobin, with POCT higher than laboratory values (Table). Within clinically-determined normal ranges, there was substantial agreement between POCT and laboratory for potassium, glucose, and hemoglobin and fair agreement for sodium and bicarbonate.

Conclusions: Our study highlights the importance of verifying abnormal POCT electrolytes and hemoglobin with laboratory testing. POCT values may, in some instances, mask clinically significant hyponatremia/hypokalemia or overestimate hypernatremia/hyperkalemia. Patients should be monitored with either laboratory testing or POCT but not both, as there is potential for incorrect treatment decisions with the latter. Our study is limited to the specific POCT/laboratory analyzer combinations used at our institution.

Table: Distribution and significance of the differences between POCT and laboratory values.

	Patient Count (n)	Mean Difference	Standard Error Difference	Minimum Difference	Maximum Difference	P Value
Sodium (mmol/L)	128	3.15	0.18	-5	14	<0.0001
Potassium (mmol/L)	128	0.23	0.04	-0.9	2.5	>0.0001
Bicarbonate (mmol/L)	128	3.13	0.16	-3.3	9.1	<0.0001
Glucose (mmol/L)	93	0.06	0.04	-1.3	1.3	0.12
Hemoglobin (g/L)	107	5.46	0.61	-20	47	<0.001

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Calcium and Magnesium Deficiencies are Associated with Imbalance in Oxidative Stress Biomarkers, and Angiogenic Growth Mediators among Ghanaian Women Who Developed Preeclampsia

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Background: Preeclampsia (PE) is a leading cause of maternal and perinatal deaths especially in resource-limited countries, like Ghana. Although the aetiology of PE is unknown, accumulated evidence indicates that poor placentation, imbalance angiogenic growth mediators (AGMs) and increased oxidative stress (OS) are major underlying factors. Micronutrients, such as calcium (Ca) and magnesium (Mg) are recognized to reduce PE development via endothelial cell control, optimal OS and a balanced AGM. However, in the Ghanaian clinical setting, these micronutrients are not routinely measured, and the link between OS biomarkers, AGMs and these micronutrients is yet to be explored. We evaluated the association between OS biomarkers, AGMs and micronutrients such as Ca and Mg.**Methods:** In this case-control study conducted at the Obstetrics and Gynaecology Department of the Komfo Anokye Teaching Hospital (KATH), Kumasi Ghana, 197 pregnant women clinically diagnosed PE who were purposively recruited as cases and 301 normotensive pregnant women (NTN-PW) as controls. Samples were collected and estimated for Ca, Mg, soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PIGF), vascular endothelial growth factor-A (VEGF-A), soluble endoglin (sEng), 8-hydroxydeoxyguanosine (8-OHdG), 8-epiprostaglandinF2-alpha (8- epi-PGF2α) and total antioxidant capacity (TAC). Statistical analysis was performed using the R Language for Statistical Computing version 3.6.0 and *p*-values less than 0.05 were considered as statistically significant. **Results:** PE women had significantly lower levels of Ca, Mg, PIGF, VEGF-A and TAC but higher levels of sFlt-1, sEng, 8-epi-PGF2α, 8-OHdG, sFlt-1: PIGF ratio, 8-epi-PGF2α: PIGF ratio, 8-OHdG: PlGF ratio and sEng: PIGF ratio compared to NTN-PW ($p < 0.0001$). Among the PE women, Ca and Mg correlated negatively with sFlt-1, 8epiPGF2α, 8-OHdG, sFlt-1/PIGF ratio, 8-epi-PGF2α/PIGF ratio, 8-OHdG/PlGF ratio

and sEng/PIGF ratio but positively with VEGF-A, PIGF and TAC ($p < 0.05$). Preeclamptic women had higher proportions of calcium deficiency (78.2% vs 14.2%; p<0.0001) and magnesium deficiency (72.1% vs 6.3%) compared to NTN-PW. The 4th quartile for sFlt-1, sEng, 8-epi-PGF2α, 8-OHdG, sFlt-1: PIGF ratio, 8-epi-PGF2α: PIGF ratio, 8-OHdG: PlGF ratio and sEng: PIGF ratio, and the 1st quartile for VEGF-A, PIGF and TAC were associated with increased odds of calcium and magnesium deficiency among PE pregnant women. **Conclusion:** Magnesium and calcium deficiencies are associated imbalance in AGMs and OS biomarkers among PE women. Serial and routine measurement of these micronutrients would allow the monitoring of poor placental angiogenesis while enabling an understanding of the triggers of increased OS and reduced antioxidant in PE. Thus, Magnesium and calcium supplementation would be essential for PE women.

Toxicology and Therapeutic Drug Monitoring

B-263

Three-way comparison of urine drug screening cups

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Background: Mass spectrometry (MS) is the gold standard for drug screening. At times, using MS for drug screening may result in longer turn-around-times and conversations with patients may be delayed. Therefore, implementing a rapid urine drug screen (UDS) cup to facilitate prompt therapeutic decisions and immediate discussion of results with patients is helpful to clinic workflows. The objective of this study was to evaluate the performance of three commercially available waived UDS cups. **Methods**: Residual urine samples from 50 patients undergoing targeted drug screening by liquid chromatography tandem MS (LC-MS/MS) were collected. Each urine sample was distributed between three UDS cups: Alere Drug Screen Test Cup, UScreen Drug Test Cup, and Rapid Response Multi-Drug One Step Cup. Drugs evaluated on the test strips and corresponding cut-offs (ng/mL) were amphetamine (AMP, 1000), barbiturate (BAR, 300), benzodiazepine (BZO, 300), buprenorphine (BUP, 10), cocaine (COC, 300), methadone (MTD, 300), methamphetamine (MET, 1000), morphine (MOR, 300), oxycodone (OXY, 100), and 11-nor-delta-THC-9-COOH (THC, 50). Results were read at 5 minutes by three observers and compared to the drugs identified as positive by LC-MS/MS with the corresponding cut-offs (ng/mL): AMP, 100; BAR, 200; BZO, 50; BUP, 5; COC, 150; MTD, 150; MET, 400; MOR 20; OXY, 40; THC, 20. For the adulteration study, residual urine samples were either diluted with water, treated with bleach or substituted with apple juice and results for creatinine, pH, oxidants and/or specific gravity were evaluated, as applicable. **Results**: Percent agreement between UDS cups and LC-MS/MS for positive (+) or negative (-) urine samples.

Test	Number of positive or negative patients by LC-MS/MS	Alere	UScreen	Rapid Response
AMP	positive (8)	**88%**	**88%**	**88%**
	negative (42)	100%	100%	100%
BAR	positive (3)	100%	100%	**67%**
	negative (47)	100%	100%	100%
BZO	positive (9)	100%	100%	100%
	negative (41)	100%	100%	100%
BUP	positive (23)	100%	100%	100%
	negative (27)	100%	100%	100%
COC	positive (13)	100%	100%	100%
	negative (37)	100%	100%	100%
MTD	positive (4)	100%	100%	100%
	negative (46)	100%	100%	100%
MET	positive (3)	100%	100%	100%
	negative (47)	100%	100%	100%
MOR	positive (12)	100%	100%	100%
	negative (38)	100%	100%	100%
OXY	positive (13)	**92%**	**92%**	100%
	negative (37)	100%	100%	**95%**
THC	positive (17)	**94%**	**94%**	100%
	negative (33)	100%	100%	100%

Conclusion: There were slight differences between the three rapid UDS cups for BAR, OXY and THC compared to LC-MS/MS results, and no observed differences between cups for adulteration results. The majority of UDS cup results showed good agreement deeming the UDS cup acceptable for clinical implementation.

B-264

Concordance of umbilical cord drug testing in multiple births

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Background: Non-medical drug use during pregnancy is estimated to occur in 4 – 6 % of U.S pregnancies. While meconium has traditionally been the gold standard for detection of drug-exposed newborns, umbilical cord tissue is an alternative specimen that has been gaining popularity due to its ease of collection, immediate availability, and similar detection window. Furthermore, by analyzing cord tissue, detection of infant medications is avoided. Previous studies have shown relatively high concordance of drug detection in meconium from multiple births, but little is known about concordance of drug detection in umbilical cords from multiple births. The study objective was to review the results of umbilical cord drug screening in multiple births to compare drug(s) and/or drug metabolite(s) detected and assess the frequency and potential cause of discrepancies. **Methods:** Data from 3,580 cords identified as twins or triplets from a national reference laboratory dataset and 260 cords from twins/triplets born at an academic medical center were deidentified and retrospectively reviewed. Qualitative results of all drugs/drug metabolites in a 47-drug panel and a separate cannabinoids assay were used to identify whether results were qualitatively equivalent for infants within each set. Any positive result in one infant with a corresponding negative result in the other infant(s) were considered 'mismatched.' Results from mismatched cords were further investigated to determine the semi-quantitative concentration of discrepant analytes in each cord. **Results:** In the reference lab dataset, 25.2% of cords were positive for one or more analyte on the 47-drug panel and 20.7% of cords were positive for cannabinoids. In this dataset, 32 of 1,038 sets of twins (3.4%) had mismatched results on the 47-drug panel. The incidence of mismatched results among

the individual drugs or metabolites tested was 0.09% (88 of 99,264 total results). The most frequent mismatches were found in opiates (n=46), with morphine (n=10) being the most mismatched of any single analyte. Within a drug class, individual mismatches did not always affect overall interpretation as the parent drug and/or associated metabolites were found in both twins. For opiates, 22 of the 46 mismatches had at least one concordant parent/metabolite associated with the mismatched analyte. Mismatches for cannabinoids in the reference lab dataset occurred in 6 of 737 sets of twins (0.8%), and five were attributed to detection of the analyte just above the cutoff in one twin and just below the cutoff in the other. In the academic medical center dataset, 57 of 260 umbilical cords (21.9%) tested positive for one or more drugs and/or metabolites. Of these, 4 mismatches (3.2%) were identified, including cannabinoids (n=2), phentermine (n=1) and oxycodone (n=1), all involving twins. Mismatches were identified in both monochorionic/diamniotic and dichorionic/diamniotic twins. All involved cases where the discrepant analyte was likely present in the negative twin but either slightly below reporting cut off or not able to meet chromatography quality criteria. **Conclusion:** Our data shows mismatched results of umbilical cord drug testing occur in less than 4% of twins and were not found to occur in triplets.

B-265

A Novel Dilute-N-Shoot Assay for Measuring Pain Management Drugs in Human Urine

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Background: Compliance to pain management contracts has become important over the years. For a doctor prescribing these narcotics to ensure that the patients are using the medications properly, periodic drug tests are ordered. These tests are used to make sure that patients are not diverting, abusing, or taking other drugs of abuse. Urine drug screens and confirmation tests are ordered to demonstrate that patients are complying with their contracts. Confirmation methods typically use some form of LC/MS to determine the concentration of each narcotic in the urine. These methods often require a fast analysis time, the ability to handle a large number of analytes and be fairly in expensive. We report on a dilute-n-shoot assay that uses filtration instead of centrifugation to remove protein and other materials from the urine samples. Filtration is done using a filter plate that contains a 0.2 µm filter. **Method:** A urine sample is incubated at 55° C for 15 min using beta-glucuronidase. The samples are allowed to cool to room temperature. Once the samples have cooled, they are spiked with internal standard. The Captiva filter plate wells are filled with a 0.1% formic acid in a 30:70 methanol/water solution. The amount of solution used will result in the final concentration of urine being diluted 20:1. This solution is used to precipitate the protein, as well as, any salts in the solution. The samples are filtered through the plate using a positive pressure manifold. The plate is then ready for LC/MS analysis. This sample preparation is used to prepare urine samples containing opioids, benzodiazepines, and other miscellaneous drugs. It is also used to prepare samples for containing ethyl glucuronide and butalbital. The opioids, benzodiazepines, and miscellaneous drugs are analyzed using a Raptor biphenyl column with a water/methanol/formic acid mobile phase. The total assay time is 6 minutes. Ethyl glucuronide analyzed using a C18 column with a water/acetonitrile/formic acid mobile phase, with a run time of 3.5 minutes. **Results:** The total sample preparation time takes less than 1 hour. The reproducibility is very good with CVs for the analytes being less than 10%. The total ion chromatogram showed no major interferences in the area where the analytes elute of the UPLH column. The LOQ met the needs of our laboratory, with most analytes having a LOQ of 10 ng/mL. Fentanyl, norfentanyl, buprenorphine, norbuprenorphine and 6-MAM have a LOQ of 5 ng/mL. Column reproducibility was good with more than 6000 injections being made on single column without significant loss in column performance. **Conclusion:** This dilute-n-shoot method was reproducible, accurate and precise. Proficiency test results have been acceptable, using this assay. We have seen a long column shelf life and an increase in productivity, due to the reduced sample preparation time. The assay LOQs did not change. We find this to be a reliable inexpensive alternate dilute-n-shoot method.

B-266

Investigating beyond antigen-antibody interference in urine drug immunoassay: Metronidazole inhibition of glucose 6-phosphate dehydrogenase

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Background: It is widely known that antibodies in immunoassays are susceptible to cross reaction with other analytes that could lead to erroneous results. However, interferences affecting the enzymes used in immunoassays are rarely reported in literature. This poster will present an investigation of a case of urine sample that triggered the error code 1350, indicating "Unable to calculate reads within absorbance range" in Architect c systems urine drug screen immunoassays. Repeat analysis of the sample in another instrument produced the same error code and samples from other patients did not trigger the error suggesting that this error is specific to the sample in question. A second urine sample was obtained from the patient after 12 hours from the initial collection and it was negative for any drugs tested by immunoassay. Review of the patient chart indicated that the patient ingested 3000 mg metronidazole few hours prior to the admission at the ED. Metronidazole is a synthetic antibiotic used for treating bacterial and protozoal infections. Physical examination of the urine is unremarkable. Review of the reaction graphs showed very low absorbance. Glucose-6-phosphate dehydrogenase (G6PDH) enzyme is used in the Architect urine drug screen immunoassay and some immunoassays where the production of NADH is monitored and quantified. Therefore, G6PDH inhibition by metronidazole was further investigated as the possible cause of the error code 1350. **Methods:** To determine if metronidazole is the cause of the error**,** a patient sample previously reported as positive for opiate was spiked with different concentrations of metronidazole. Aliquots of sample spiked with water was used as control. Samples were assayed using Architect Multigent Opiate assay per manufacturer instructions. Reaction graphs were also obtained from the instrument for comparison. **Results:** Urine sample spiked with water or 6.25 mg/mL of metronidazole did not trigger the error code 1350 while urine spiked with 25 mg/mL of metronidazole produced an error code suggesting that the drug interferes with the assay. Review of the reaction graphs from the sample spiked with 25mg/ml metronidazole showed very low absorbance at the designated reading time. Multigent Opiate assay is a homogenous enzyme immunoassay. The drug and drug-G6PDH conjugate competes for antibody binding. At higher drug concentration in the sample, the G6PDH produce more NADH while the reverse is expected if there is less drug in the sample due to the decrease G6PDH activity upon binding of the antibody to the drug-G6PDH conjugate. Therefore, if metronidazole binds with the antibody, the expected result should be higher absorbance due to higher production of NADH. However, the opposite was observed suggesting that the G6PDH activity was inhibited. **Conclusion:** G6PDH is an enzyme that is involved in oxidative stage of pentose phosphate pathway commonly used in automated immunoassays. Urine samples spiked with metronidazole (25mg/mL) replicated the error code and reaction graph characteristics observed in the patient sample in question.

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Evaluation of Concordance of Fentanyl Urine SEFRIA Immunoassay with LC-MS/MS Method

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Background: Synthetic opioids have been reported by the CDC as the main driver of drug overdose deaths in the United States. Urine drug screening by immunoassays play an important role in emergency medicine settings to quickly identify drug class and guide physicians to appropriate therapy for patients even though limitations on analytical specificity is well known. Fentanyl is one of the top opioids identified as the cause of overdose deaths, and traditional opioid screening assays cannot detect this synthetic opioid. We sought to evaluate the concordance of an FDA-cleared immunoassay assay, Fentanyl Urine SEFRIA, with our validated fentanyl LC-MS/MS assay. Fentanyl Urine SEFRIA utilizes artificial fragments of *E.coli* enzyme beta-galactosidase. The assay determines the qualitative concentration of fentanyl by measuring spectrophotometrically the conversion of chlorophenol red beta-D-galactopyranoside to chlorophenol red and galactose at 570 nm. This reaction happens when the enzyme donor-fentanyl conjugate combines with the enzyme acceptor and form an active beta-galactosidase. Binding of the antibody to enzyme donor-fentanyl conjugate inhibits complementation. **Method:** 200 fentanyl positive and 200 fentanyl negative samples determined by validated LC-MS/MS were randomly selected and frozen at -20°C until enough samples were collected. All samples were analyzed using Fentanyl Urine SEFRIA kit provided by Abbott Diagnostics on Abbott ARCHITECT c16000. Positive samples were defined as urine samples having fentanyl ≥2 ng/mL or with norfentanyl ≥8 ng/mL. Since the current LC-MS/MS method did not detect fentanyl analogs and SEFRIA has cross-reactivity to fentanyl analogs such as butyryl fentanyl and acetyl fentanyl, further analysis for these analogs will be investigated. **Results:** 3 samples categorized as false negative only had detectable norfentanyl. The SEFRIA immunoassay has 0.005% cross-reactivity at 20,000 ng/ml norfentanyl per package insert, which would explain the discrepant results. Excluding these three samples from analysis the specificity and sensitivity were 93% and 92%, respectively. Further investigation for presence of fentanyl analogs or other interfering substances in discrepant results are being investigated. **Conclusion:** In this study, the Fentanyl Urine SEFRIA immunoassay had a specificity and sensitivity of 93% and 92%, respectively, using a confirmatory method at 2 ng/mL cut-off. A recent publication showed that at

1 ng/mL cut-off, SEFRIA specificity and sensitivity were 80% and 95% respectively when also compared to an LC-MS/MS method. In our institution, all presumptive positives by urine drug screen immunoassay are automatically reflexed to confirmation by LC-MS/MS. False positive results observed in this study may be due to presence of fentanyl analogs not detected by our targeted mass spectrometry method or fentanyl concentrations below 2ng/mL. However, review of chromatograms of the false positive samples did not show any quantifiable fentanyl or norfentanyl. In our current workflow, false negative fentanyl would not be reflexed to confirmatory testing if no other drug was positive by immunoassay screen. LC-MS/MS results of false positive samples in this study show that all samples were positive for at least one other drug. For future studies, investigation of false negative results could include analysis of samples that contain potential inhibitors of beta-galactosidase or inhibition of formation of active beta-galactosidase.

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The New Clinical Chemistry Acetaminophen Assay for the ARCHITECT and Alinity Instruments

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OBJECTIVE: To present the performance results of the new Acetaminophen assay on the ARCHITECT and Alinity c instruments.**METHODOLOGY:** The new Acetaminophen assay on both instruments utilizes the same enzymatic/colorimetric methodology as the previous ARCHITECT Acetaminophen assay. Briefly, acetaminophen is hydrolyzed to *p*-aminophenol and acetate. Next, *p*-aminophenol reacts with 2,5-dimethylphenol in the presence of manganese to generate a chromophore that is measured at 660 nm. **RESULTS:** The table below displays the performance characteristics of the new Acetaminophen assay relative to the previous ARCHITECT assay. The interference results represent only a select number of endogenous/exogenous interferents evaluated at 3 acetaminophen concentrations (5, 30, 150 µg/mL). No significant interference (all within ±7.5% or ±1.50 mg/dL) was observed at the indicated interferent concentrations.

Characteristic	**Previous Assay Performance Characteristics**				**New Assay Performance Characteristics**			
Imprecision	**N**	**Mean (µg/mL)**	**Total**		**N**	**Mean (µg/mL)**	**Within-Lab**	
			SD (µg/mL)	**%CV**			**SD (µg/mL)**	**%CV**
	80	10.1	0.29	2.9	120	5	0.5	9.1
	80	36.7	0.47	1.3	120	15	0.2	1.3
	80	112.2	1.49	1.3	120	51	0.4	0.7
	80	201.0	2.6	1.3	119	73	0.6	0.8
	80	320.2	4.7	1.4	120	84	0.6	0.7
					120	227	1.3	0.6
					120	278	2.0	0.7
					120	362	2.6	0.7
Method Comparison:	**Previous Assay vs. Comparative Method**				**New vs. Previous**			
	N		88		N		119	
	R		0.9998		R		0.9993	
	Equation		[Previous] = 1.064 [Comparative] + 1.1		Equation		[New] = 1.042 [Previous] – 0.034	
	Range (µg/mL)		5.7 – 356.5		Range (µg/mL)		4 – 356	
On-Board Stability (days)	8				12			
Calibration Stability (days)	1				7			
Interferent	**[Interferent]**				**[Interferent]**			
Conjugated Bilirubin (mg/dL)	2				14			
Unconjugated Bilirubin (mg/dL)	2				28			
Hemoglobin (mg/dL)	200				570			
Human Triglycerides (mg/dL)	-				933			
Intralipid (mg/dL)	200				2000			
N-Acetylcystine (mg/L)	1500				1663			

CONCLUSIONS: The new Acetaminophen assay provides an option for Acetaminophen testing on both Abbott platforms and displays enhanced performance characteristics relative to the previous assay.

B-269

Modernized standards for evaluation of Drugs of Abuse/Toxicology assays as demonstrated by the Cocaine assay on the Alinity c analyzer from Abbott Laboratories

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Background: The Drugs of Abuse (DOA) Clinical Chemistry assays are used as a screening tool for commonly abused drugs. Qualitative applications provide results from urine samples as positive or negative, while semiquantitative applications provide an approximate concentration. Positive screening results on both applications must be confirmed by a reference method such as GC/MS or LC-MS/MS. Abbott's DOA panel of assays deliver accurate, quick, reliable results, and provide clinicians a faster result at lower cost than a traditional reference method.Current expectations by the FDA have impacted how new DOA assay submissions are evaluated for performance and accuracy. New analysis methods in precision[1], accuracy[1], recovery[1], cross reactivity[1], and spike recovery are being utilized to assess drugs of abuse (DOA) assay performance that more accurately represent the clinical utility of the assay. Additionally, sensitivity is no longer being evaluated as these assays are semiquantitative only and should not be interpreted as quantitative. The Alinity c Cocaine assay executed verification studies based on these new expectations as part of a recent submission.

Objective: To demonstrate the analytical performance of the Cocaine assay on the Alinity c system, highlighting the modernized DOA performance evaluation methods. **Methods:** Key performance testing including precision via cutoff analysis, accuracy compared to the reference method, recovery/dilution linearity, and spike recovery, were assessed per Clinical Laboratory Standards Institute (CLSI) protocols and/or FDA guidance[1]. **Results:** The observed results for precision, accuracy, recovery/dilution linearity and spike recovery of the Alinity c Cocaine assay are summarized in the table below.

Study	Cutoff (ng/mL)	Results Summary
Precision	150 and 300	Qualitative and Semiquantitative: 4 samples spiked below the cutoff remained negative with 100% accuracy.4 samples spiked above the cutoff remained positive with 100% accuracy.
Accuracy to LC-MS/MS	150	Qualitative and Semiquantitative:% Overall Agreement = 99.00%
	300	Qualitative and Semiquantitative:% Overall Agreement = 98.00%
Dilution Linearity / Accuracy by Recovery	150 and 300	Qualitative: N/ASemiquantitative:% Recovery was between 95.7% and 111.4%. $R^2 = 0.9981$
Spike Recovery	150	Qualitative – No ± 2SD overlap between spiked samples and cutoff calibrator and samples spiked to 112.5 ng/mL remained negative and samples spiked to 187.5 remained positive with 100% accuracy.Semiquantitative – sample recovery from 98.3% to 110.9%
	300	Qualitative – No ± 2SD overlap between spiked samples and cutoff calibrator and samples spiked to 225 ng/mL remained negative and samples spiked to 375 remained positive with 100% accuracy.Semiquantitative – sample recovery from 109.5% to 115.4%

Conclusion: The Alinity c Cocaine assay demonstrates acceptable assay performance utilizing modernized DOA screening assay performance evaluations standards.1.Lias, C.H. (2016, July 26). FDA Regulation of Drugs of Abuse Tests SAMHSA DTAB Meeting. Retrieved from https://www.samhsa.gov/sites/default/files/meeting/documents/dtab-fda-regulation-drugs-abuse-tests-clearance.pdf

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Identification of novel macrovancomycin complexes using laboratory developed methods

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Background: Vancomycin is a glycopeptide antibiotic used to treat Gram-positive bacterial infections such as methicillin-resistant Staphylococcus aureus (MRSA). Therapeutic drug monitoring is recommended to optimize efficacy and minimize nephrotoxicity. Vancomycin is typically 50-55% protein-bound and total vancomycin concentrations in serum are used to guide dosing. Recently we identified several patients with unexpected vancomycin pharmacokinetics, abnormally prolonged elimination half-life, and evidence of treatment failure. Preliminary laboratory investigations suggested that the vast majority of measurable vancomycin in these patients' serum was associated with large molecular weight complexes (termed macrovancomycin) and presumably not bioavailable. However, laboratory methods used for troubleshooting (polyethylene glycol(PEG)-precipitation to nonspecifically precipitate large molecular weight complexes and 30kDa centrifugal filters to remove complexes >30kDa from serum) have not been validated for this purpose. The aim of this study was to 1) validate the use of PEG-precipitation and 30kDa centrifugal filter devices as methods to identify macrovancomycin and 2) to establish reference intervals (RIs) for both methods to differentiate abnormal macrocomplexes of vancomycin from normal vancomycin-protein binding.

Methods: Residual waste serum samples from clinically-ordered vancomycin were obtained over a two week period to reflect the reference population (n=122 samples from 87 unique patients, age 3-84 years, 35 female, 52 male). Residual serum from three patients identified as having unexpected vancomycin pharmacokinetics were also obtained. Serum vancomycin was measured using VANC3 reagent on a Cobas 8000 c502 (Roche Diagnostics, Inc. Indianapolis, IN). All samples were treated using a Millipore Centrifree 30 kDa centrifugal filter (MilliporeSigma, Burlington, MA) and PEG (MilliporeSigma) precipitation (patient serum:25% PEG solution (1:1)) followed by centrifugation. Vancomycin was measured before treatment (total) and in the soluble fraction after PEG-precipitation (soluble) or the filtrate after 30kDa filtration (filtrate). The % PEG-precipitated vancomycin was calculated as [(total-soluble)/total] x 100. The % >30kDa vancomycin was calculated as [(total-filtrate)/total] x 100. Assay accuracy in the post-treatment sample matrices was assessed by measuring recovery of spiked vancomycin in (n=3) control serum pools after PEG-precipitation/filtration. Central 95th % RIs and 90% confidence intervals (CIs) were calculated for the reference population using EP Evaluator software.

Results: Total vancomycin concentrations ranged from 5.2-32.1 mcg/mL. The RI (90% CI) for % PEG-precipitated vancomycin was 0-16% (0-0%,12-19%). The RI (90% CI) for % >30kDa-complexed vancomycin was 6-35% (3-7%,32-41%). For the three patients with unexpected prolonged elimination half-lives (F/63y, M/76y, F/74y), the total serum vancomycin results were 17.4, 15.6, 16.6 mcg/mL. The corresponding %-PEG precipitated vancomycin results were 94%, 88%, 82% and the % >30kDa-complexed vancomycin results were 99%, 98%, 86%, respectively. Vancomycin recovery studies to rule out matrix effects from PEG and filtration demonstrated 104-107% recovery in PEG-treated serum and 95-102% recovery in filtrates.

Conclusion: Macrovancomycin, vancomycin associated with large molecular weight complexes, was identified in three patients with unexpected vancomycin pharmacokinetics using PEG-precipitation and 30kDa centrifugal filtration. The % PEG-precipitated vancomycin and % >30kDa vancomycin results in patients with macrovancomycin were >5-fold and >2-fold above the established upper reference limits, respectively, suggesting that these laboratory methods are suitable for identifying macrovancomycin complexes in serum.

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A Novel UPLC-MS/MS Method for Plasma Antibiotics for Clinical Research

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Background: A clinical research method for a large panel of antibiotic drugs in plasma was developed; azithromycin (5-500 ng/mL); ciprofloxacin and clindamycin (0.1-10 µg/mL); amoxicillin, ampicillin, cefotaxime, chloramphenicol and linezolid (0.5-50 µg/mL); cefazolin, cefepime, ceftazidime, cefuroxime, flucloxacillin, meropenem and sulbactam (1-100 µg/mL); daptomycin and piperacillin (2-200 µg/mL). **Methods:** Matrix matched calibrators and QCs were prepared using in-house stocks and pooled plasma. Samples (50 µL) were treated with internal standard in methanol. A water/methanol/ammonia gradient was used with a Waters ACQUITY UPLC BEH C18 2.1 x 1.7 µm, 100mm column on a Waters ACQUITY UPLC I-Class FTN and Xevo TQD mass spectrometer utilizing polarity switching in a 5-minute run. **Results:** No system carryover was observed following analysis of plasma samples containing the highest concentration calibrators. Analytical sensitivity investigations indicated precise quantification (≤20% CV, ≤15% bias) at concentrations equal to or lower than the lowest concentration calibrator. Total precision and repeatability were assessed (3 pools, 5 replicates, 5 days; n=25) and determined to be ≤10% RSD. Linearity experiments determined the method provided first or second order polynomial fits over the ranges analyzed; additionally, each run met acceptance criteria (coefficient of correlation ≥ 0.995, determined concentrations of calibrators ±15% of nominal, ±20% in the case of the lowest calibrator). Post-column infusion experiments demonstrated analytes eluted in regions free of major ion suppression or enhancement. Evaluation of matrix effects at low and high concentrations indicated compensation by the internal standard. Addition of high concentrations of several endogenous and exogenous materials did not affect quantification. EQA schemes for antibiotics were limited, however samples were analyzed when available. **Conclusion:** This quantitative method for clinical research demonstrates very good precision with minimal matrix effects and allows for the multiplexing of a large panel of antibiotics in plasma in a short run time. For Research Use Only. Not for use in diagnostic procedures.

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Analytic Specificity of Three FDA-Cleared Urine Fentanyl Immunoassays

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Background: Fentanyl use and misuse has increased substantially in the last 15 years. Routine urine drug screening for illicit use or compliance testing requires analyte-specific immunoassays, which are relatively new to the market. Given the potential medicolegal implications of false positive drug screening results, a thorough evaluation of immunoassay sensitivity, specificity, and accuracy is necessary prior to implementation.

Methods: 58 deidentified patient urine specimens were analyzed by LC-MS/MS for fentanyl and norfentanyl (limit of detection 0.5 ng/mL), as well as 3 different fentanyl

immunoassays with identical calibrator cutoffs (1.0 ng/mL fentanyl; Immunalysis SEFRIA, ARK Fentanyl I, ARK Fentanyl II). The sensitivity, specificity, and accuracy of the 3 immunoassays were calculated relative to the reference method (LC-MS/MS).

Results: The sensitivity of the 3 immunoassays was equivalent (~96%). However, specificity and accuracy for the SEFRIA, ARK I, and ARK II for the assays differed substantially (33%/56%, 73%/79%, 97%/97%, respectively), in the 58 samples tested. False positives tended to occur in patients taking certain drugs such as labetalol and trazodone, though some methods were more affected than others (SEFRIA>>ARK I>>ARK II).

Conclusion: The ARK Fentanyl II immunoassay exhibited the best sensitivity, specificity, and accuracy compared to LC-MS/MS in the samples tested. In samples identified as false positives, a review of the patient medication lists often identified labetalol or trazodone, consistent with package insert specificity data (SEFRIA). False positives are most likely to occur with the SEFRIA fentanyl immunoassay, especially when screening patient populations more likely to be prescribed these drugs (e.g. pre- and post-partum hypertension, outpatient behavioral health). When practical, validation activities, even for FDA-cleared assays, should utilize real patient samples containing drugs which may cause false positives.

B-273

Clinical Laboratory Contribution To Better Psychotic Patient Outcomes By In-Lab Drug Monitoring

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Background: Clozapine is an effective antipsychotic medication, used primarily to treat schizophrenia and bipolar disorder. Management of these illnesses is a challenge, however, with treatment failure and relapse being far too common. Mental health professionals have limited quantitative insight into antipsychotic drug treatment, mostly because rapid drug monitoring has not been available. Lacking objective information, prescribers are less likely to identify the cause of complicated courses of antipsychotic treatment and make the correct treatment decision. This problem creates the potential for discontinuing an otherwise promising drug, adding another antipsychotic, or increasing the dosage above the recommendation, exacerbating the risk of side effects and iatrogenic lapses in adherence.**Response**: Our hospital sends out around 80 clozapine orders per month. Because of the need to provide greater access to clozapine levels, we evaluated a relatively new method (Saladax Biomedical, Bethlehem, PA) on our Beckman Coulter AU5800 instruments.**Performance Standard (PS)**: The therapeutic range for clozapine is 350-600 ng/mL (trough value). Concentrations below 350 are associated with treatment failure. Concentrations about 1000 or greater cause toxicity. 30% is a reasonable allowable error for this drug. Thus: PS1: 105 ng/mL at 350 ng/mL; PS2: 300 ng/mL at 1000 ng/mL**Results**: Precision: At the low concentration of 130, random error (RE) was 46. At the medium concentration of 369, RE was 59. At the high concentration of 1098, RE was 282. Each of these are acceptable.

Linear Range: The linearity was tested from 84.3 to 1367 ng/mL. Allowable error for linearity was ½ total allowable error, or 15%. Actual data: slope 1.025, y-intercept 0.7, for a total observed error of 1.9%, which easily passes.Method Comparison: The clozapine assay was compared to an LC-MS/MS method. Passing-Bablok regression statistics were used. The Systematic Error was 52 at 350 ng/mL, and 105 at 1000 ng/mL, both of which meet the allowable error goals.

Conclusions: This new clozapine method is acceptable for use. Deploying this method in our laboratory will provide values in < 2 hours rather than 3-5 days, greatly enhancing the laboratory's contribution to the care of these patients.

B-274

Validation of a SPME-GC-MS/MS method for measuring serum pentobarbital on a Thermo Scientific TSQ Duo tandem mass spectrometer and comparison to a GC-MS method

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Background: Pentobarbital is a short-acting barbiturate often utilized in the clinical setting for medically-induced coma and reduction of intracranial pressure following traumatic brain injury. In a suspected brain death event it is imperative that the patient's serum pentobarbital level is below the therapeutic level prior to performing a brain death examination. Serum pentobarbital levels for these events were a frequently-requested STAT order in our facility, but STAT turnaround time was difficult to achieve as the test was referred to another laboratory. To facilitate quicker turnaround time, our laboratory developed a method using GC-MS/MS and solid-phase microextraction (SPME). The advantages of the SPME method are simple sample preparation and no introduction of organic solvents into the column or system.

Objective: Our goal was to determine the performance specifications of a SPME-GC-MS/MS method for measuring serum pentobarbital and to compare the method to the established gold-standard GC-MS method.

Methodology: The following parameters were evaluated to validate the SPME-GC-MS/MS method: Linearity, lower limit of the measuring interval (LLMI), matrix effect, carryover, accuracy, dilution, precision and interferences.

Results & Conclusions: Linearity was determined to be 1.0 - 64.0 µg/mL; dilution studies enabled measurement up to 320 µg/mL. No issues due to matrix effects were detected. Carryover was determined to be less than 0.5%. Pharmaceuticals that are frequently co-administered with pentobarbital were tested for interference at concentrations above the CLSI recommended level; no clinically significant interference was observed. Inter-day and intra-day precision studies conducted at two different concentrations (low and high) yielded CVs < 10%. Accuracy was assessed by method comparison using patient samples and spiked samples to an established reference GC-MS method (n=40); comparison to the reference method yielded the following statistics: slope = 0.998, intercept = 0.06045, R^2 = 0.9830. Also of note, sample preparation time due to use of SPME was reduced from approximately 65 minutes for a traditional GC-MS method to approximately 15 minutes for the SPME method. The performance characteristics of our SPME-GC-MS/MS method were shown to be clinically comparable to the gold-standard GC-MS method.

B-275

Investigation of the Absorbance Interference Flag in a Urine Oxycodone Screening Assay

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Background Oxycodone is a semi-synthetic opioid prescribed for pain management in patients with moderate to severe pain. In the US, the drug can be prescribed as Oxaydo, OxyCONTIN, Roxicodone, and Xtampza ER or in combination with aspirin (Percodan) or acetaminophen (Percocet). Opioid misuse, abuse, and addiction can lead to overdose and death. According to Drug Abuse Warning Network (DAWN), there has been a surge in oxycodone-related deaths in the US. Oxycodone compliance should be routinely monitored for pain management patients. In our facility, urine oxycodone can be screened using an enzyme immunoassay and quantified via an LC-MS/MS assay, the gold standard method. In the qualitative assay, oxycodone labeled with glucose-6-phosphate dehydrogenase (G6PDH) competes with the free drug from urine samples, binds to a fixed amount of oxycodone antibody. The unbound G6PDH labeled oxycodone converts nicotinamide adenine dinucleotide (NAD) to NADH and generates light, which is subsequently measured spectrophotometrically at 340 nm. Although rare, we were unable to report 45 samples (0.04%) in a two-year period due to an absorbance error flag. Absorbance error occurs when the concentration or activity value is beyond the measuring range of the instrument detector. Herein, the aim of this study was to investigate the reasons for this absorbance error. **Methods** Thirty patient samples with the absorbance error were collected and stored under refrigeration after all the clinically ordered tests were completed. Urine oxycodone was retested with the Roche cobas c502 analyzers (Roche Diagnostics, Indianapolis, IN, USA). Bilirubin, urobilinogen, ketones, ascorbic acid, glucose, protein, blood, pH, nitrite, and leukocytes were semi-quantitated using the Multistix 10sg dipsticks (Siemens, Tarrytown, NY, USA). Medications and medical history of the patients were also reviewed.**Results** After the initial absorbance error, analysis is repeated. All specimens repeated the absorbance error on day of original testing. Three were grossly hemolyzed, and 17 had cloudy appearances. Most of the specimens had abnormal colors (brown, orange, and red) and a substantial amount of sediment after centrifugation (required before analysis). No remarkable information was found from the medication list and the medical history of the patients. Eight of the patients had an oxycodone confirmation LC-MS/MS test ordered at the same time when the screening test was ordered. Interestingly, only one of the patients had discrepant oxycodone results, meaning a presumptive positive result from the screening test but a negative result from the confirmation test. **Conclusion** In summary, the abnormal color and the presence of sediments, not removed by standard centrifugation, might be the reason for the absorbance error. The immunoassay may still provide useful information even in the presence of discoloration and sediments. Manually diluting the samples with absorbance error might mitigate the issue. However, it also increases the risk of producing false-negative results by diluting a sample with near borderline concentration. Therefore, a confirmation test is recommended for accurate and specific quantification of oxycodone.

B-276

Development of an automated nanoparticle assay and Bayesian-based tool for rapid busulfan pharmacokinetic analysis

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Background: Busulfan is an alkylating agent used in allogeneic hematopoietic stem cell transplantation for various malignant and non-malignant disorders. Therapeutic drug monitoring of busulfan is common, as busulfan exposure has been linked to veno-occlusive disease, disease relapse, and failed engraftment. The authors developed an automated immunoassay, along with stable calibrators and controls, and quantified busulfan in sodium heparin plasma.**Methods:** The study evaluated a homogenous nanoparticle immunoassay, the MyCare™ Busulfan Assay Kit (Saladax Biomedical, Inc.), for precision, sensitivity, linearity, and accuracy on Beckman Coulter 480 analyzer; it compared the method with two mass spectrometry methods (LC-MS/MS and GC/MS), using remnant patient samples (n=231).**Results:** The method uses 10 µL of sample and time to first result is approximately 10 minutes with a throughput of 480 samples per hour. The coefficients of variation for repeatability were ≤ 4.7% and within-laboratory precision were ≤ 6.1%. The linear range was 187-2,000 ng/mL, samples up to 6,000 ng/mL can be measured with sample dilution. Measured mean values deviated by ± 4% from assigned values. Comparison between validated mass spectrometry methods resulted in an intercept of 16 ng/mL a slope of 0.95 and correlation coefficient R ≥ 0.99. Results can be entered directly into cloud-based clinical decision support tools, such as InsightRX, that use model-informed precision dosing (MIPD) to improve busulfan target attainment. Recent work has demonstrated that Bayesian-based precision dosing significantly improves the percentage of patients on target and reduces variability in the spread of target attainment (%CV) compared to weight-based dosing or conventional NCA calculations (Shukla et al, 2020). Additionally, MIPD supports sparse sampling, overall improving patient safety and requiring fewer samples. **Conclusion:** The MyCare Busulfan Assay coupled with the InsightRX software can provide accurate, high throughput and rapid turnaround of results in a hospital setting.

B-278

Anti-Infliximab Antibody Dynamics as a New Predictor of Loss of Response to Infliximab

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Background: The inflammation modulator infliximab (IFX), a therapeutic antibody for therapy of inflammatory bowel disease (IBD) and rheumatic disorders, has proven to be a powerful tool to achieve remission and improve life quality. However, about a third of IFX-treated IBD patients develop anti-drug antibodies (ADA) that are associated with secondary loss of response to IFX. Until today, no consensus therapeutic algorithm has established itself in clinical practice with IFX, and high-frequency therapeutic drug and immunogenicity monitoring (TDIM) data on individual patients are scarce. This study evaluated the performance of individual ADA dynamics rather than threshold values for prediction of secondary loss of response (sLOR) to IFX therapy.

Methods: Patients diagnosed with IBD on IFX maintenance therapy with a proactive TDIM policy were enrolled in the study by a German outpatient clinic. Information about remission status and laboratory parameters, including concentrations of ADA, IFX, C-reactive protein (CRP) and fecal calprotectin (FC), were analyzed retrospectively. For a defined time period starting from the first ADA-positive TDIM visit, slopes of ADA and IFX (S_A and S_I) concentrations as dynamic indices, and parameter medians (ADA_{median}, IFX_{median}, CRP_{median}, FC_{median}) as static indices were calculated and analyzed for correlations with total IFX discontinuation and sLOR.

Results: 500 visits from 38 IBD patients (28 Crohn's disease, 10 ulcerative colitis) were available for analysis. The median duration of IFX maintenance follow-up was 68.2 weeks. Significant differences for outcomes total IFX discontinuation ($p = 0.01$) and sLOR ($p = 0.004$) were found upon grouping by S_A (ADA-0 = non-detectable ADA, ADA-down = negative S_A, ADA-up = positive S_A). Longer sLOR-free survival was observed for patients in the ADA-down group compared to the ADA-up group ($p = 0.02$). S_A was confirmed to be a significant risk for sLOR via Cox proportional hazards regressionsLOR ($p = 0.002$, HR = 1.209). ROC analysis yielded a sLOR prediction sensitivity of 83.3% and a specificity of 93.5% for a S_A threshold of 2.0 $AU \cdot mL^{-1} \cdot week^{-1}$.

Conclusion: We suggest S_A as a new and straightforward diagnostic index that may help to decide whether continuation of IFX therapy is reasonable after ADA emerge. Proactive TDIM until ADA emerge enables early intervention. After the first ADA-positive visit, only one more control visit after approximately 17 weeks sufficed to predict sLOR well.

B-279

A positive test result for the alcohol biomarker phosphatidylethanol can still be used to underpin short term abstinence.

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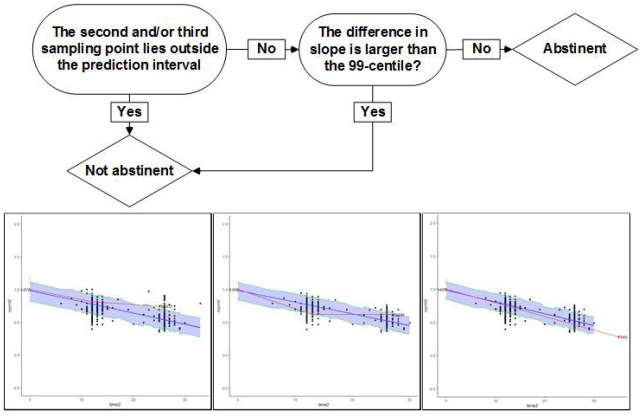
Introduction: Amongst the direct biomarkers for alcohol intake phosphatidylethanol (PEth) becomes more and more valued. PEth increases the sensitivity of uncovering alcohol consumption and monitoring abstinence. PEth has a half-life of 7 - 8 days. Upon chronic and excessive alcohol use PEth values are easily over 300 ng/mL. Hence, it can take weeks for PEth values to drop below the decision limit for monitoring abstinence (20 ng/mL). The question arises whether abstinence can be confirmed based on two consecutive PEth positive results. **Methods:** A large scale PEth monitoring study was set up. Over 500 participants agreed to stay sober for one month and took during this period 3 finger prick samples via self-sampling at home using volumetric absorptive micro sampling devices (VAMS, Mitra®, Neoteryx). PEth 16:0/18:1 was quantified using a validated liquid chromatography - tandem mass spectrometry method. A population-based algorithm capable of predicting abstinence with 95% probability was set up by fitting a linear mixed effect model to discern patterns in PEth elimination over time while accounting for intra- and interindividual variability in PEth scores. The latter was further incorporated in a 2-step decision tree (see figure), looking (i) whether or not PEth-values fall outside the prediction interval, and (ii) whether the slope between two PEth values is compatible with abstinence. Validation of this decision three was based on data of 74 people reporting to drink alcoholic beverages, alcohol free beverages or having eaten liquor-containing sweets. **Results:** Allowing a cut-off of "4 units spread over 14 days", the sensitivity and specificity of the decision tree was 89%. This is a realistic cut-off compared to other reports on detectability of a single dose intake of ethanol. **Conclusion:** Monitoring the decrease in PEth, while the latter is still positive, can underpin claims of abstinence upon short term monitoring.



B-280

Development and Utility of Antifungal Drugs Measurement Using Liquid Chromatography Tandem Mass Spectrometry

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Antifungal drugs (voriconazole, posaconazole, and itraconazole) are often used in the management of organ transplant patients. However, in those patients, their clearance is often variable and thus therapeutic measurements are helpful in attaining the narrow therapeutic levels. Additionally, there is wide intra-individual variability in their metabolism. Excessive levels lead to both hepatic and nephrotoxicity. Testing was being performed as a send-out at a distant reference laboratory with prolonged turnaround

time. This study describes the development and utility of an LCMSMS-based assay for the measurement of voriconazole, posaconazole, and itraconazole and its active metabolite hydroxyitraconazole.

Methods: An LC-MSMS (Waters Acquity TQD) method using a C18 column; binary mobile phases (0.1% formic acid in acetonitrile and in water respectively) were used to develop an assay for the combined measurement of serum voriconazole, posaconazole, itraconazole and its active metabolite hydroxyitraconazole. Standards and their deuterated forms D3 and D4 were used. Serum samples (50 microliter) and internal standards were extracted into methanol and injected into the LC-MSMS. Imprecision, accuracy, analytical measurement range, carryover and sensitivity studies were conducted as per in-house protocol. Interference studies were conducted for hemolysis, icterus, and lipemia as well as for various antibiotics (cyclosporine, tacrolimus, sirolimus, everolimus, ketoconazole, amoxicillin, and azithromycin) likely to be used in combination with the candidate antifungal drugs. Correlation studies with a reference laboratory was also performed.

Results: LC-MSMS based methodology was developed. Accuracy was 92.8%, 95.0%, 94.6%, and 92.5% for voriconazole, posaconazole, hydroxyitraconazole, and itraconazole respectively. Intra- and inter-run imprecision was <2.7% and <5.4% at 8-9 micg/mL, <2.9 % and < 5.1% at 1.95-2.02 micg/mL and <5.9% and 4.9% at 0.22-0.25 micg/mL respectively for all dugs. Analytical measurement range was determined as 0.05 to 10.0 micg/mL for all drugs. A dilution of 1:2 was validated allowing a reportable level up to 20 micg/mL. Carryover was negligible at <1.1 %. No significant interference was observed for lipids, bilirubin, hemoglobin, cyclosporine, tacrolimus, sirolimus, everolimus, ketoconazole, amoxicillin, and azithromycin. Correlation with a reference laboratory-based LC-MSMS methodology was within the total allowable error of 20% for all drugs. Review of reportable results since introduction of the assay showed values ranging from <0.05 to 9.93 micg/mL with 24.6% being subtherapeutic and 10.3% toxic levels for voriconazole and ranging from <0.05 to 5.04 micg/mL with 18.9 % being subtherapeutic for posaconazole. For itraconazole reported values ranged from <0.05 to 3.93 micg/mL, and from <0.05 micg/mL to 4.32 micg/mL for hydroxyitraconazole with 8.3% being subtherapeutic for itraconazole and hydroxyitraconazole. Turnaround time was less than 48 hours with a STAT option of less than 24 hours.

Discussion: Rapid, reliable assay for the combined measurement of the antifungal drugs voriconazole, posaconazole, and itraconazole and its metabolite hydroxyitraconazole was developed. The assays provided an improvement in turnaround time and thus rapid intervention in the highly critical organ transplant patients. The high percentages for subtherapeutic and toxic levels observed indicated the intraindividual variability and supported the need for the rapid measurements. In conclusion; an LC-MSMS based methodology was developed with acceptable performance characteristics and turnaround time.

B-281

Oral Fluid as a Viable Matrix for Detecting Drug-Drug Interactions

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Background: Drug-drug interactions (DDIs) are a substantial concern for many patients and monitoring for DDIs is critical to preventing adverse drug events (ADEs) and potential hospitalization, particularly for those under treatment for multiple conditions and/or under the care of multiple providers. Detection of drugs and other interacting substances (e.g. foods and/or supplements) in biological fluids provides a direct means to assess what patients have ingested, providing an objective detection of potential DDIs that may be missed by traditional drug reconciliation programs or pharmacy staff. Typically, urine is used to assess ingested drugs; however, oral fluid (OF) has been increasingly evaluated as a useful alternative matrix in clinical and forensic toxicology, drug monitoring programs and telehealth settings. We recently began offering an OF test for detection of common CYP-mediated interactants in a pain management, addiction treatment and behavioral health population. Given the lack of prevalence data for many of these interacting substances in OF, we conducted a post-release evaluation of the ability to detect these substances and identify potential DDIs in this matrix. **Methods:** Over 180 drugs in oral fluid were monitored through LC/MS/MS testing. Briefly, OF samples collected with a commercial collection device were processed via automated SPE and extracts were diluted without drying and injected onto an LC-MS/MS system. A modified C18 column was used to chromatograph compounds and qualitative detection was via scheduled multiple reaction monitoring using electrospray with polarity switching. Several non-compliance categories of drug were monitored including behavioral health, antibiotic, and cardiovascular compounds as well as food/supplement ingestion markers. Over 3500 patient samples were processed to determine potential ADEs (including severity) and category of analytes involved. **Results:** Through objective detection of ingested compounds, we observed that greater than 40% of the patients tested through this program exhibited at least one potential DDI. Most of these interactions were classified as moderate with 13% of interactions reported classified as severe or contraindicated. Behavioral health, cardiac, and anti-depressant drugs were observed most frequently for severe interactions. Resultant ADEs most often identified were cardiac related (arrhythmias or other cardiovascular complications). The majority of contraindicated ADEs were from the presence of alcohol with a secondary drug, most frequently with opiates that would ostensibly result in potential increased opiate release. **Conclusions:** Detection of potential DDIs in OF via LC/MS/MS provides valuable information to clinicians, particularly when treating patients not able or willing to disclose all medications taken, patients being treated for multiple conditions or patients seeing multiple providers. Many of the potential ADEs observed in this cohort were for drugs not routinely monitored in OF compliance testing; testing for these other classes of drugs is important for pain and/or behavioral health specialists to help prevent ADEs in their patients. These findings further expand the applicability of OF testing and when combined with the ease of collection and sample handling, provide further justification for the use of this matrix in the clinical toxicology, drug monitoring and telehealth settings.

B-282

Screening and Semi-Quantitation of 212 Fentanyl Analog Compounds by Orbitrap Exploris 120.

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According to the National Forensics Laboratory Information System the number of fentanyl-related reported cases increased from 945 cases in 2013 to 71,341 cases in 2017 while case load has risen minimally over this same time span. Deaths attributed to synthetic fentanyl analogs rose 10 % from 2017 to 2018 and are thought to be likely driven by illicitly manufactured fentanyl analogs. Here we present a method for fentanyl screening and semi-quantitation in urine for all 212 fentanyl analog compounds contained in the FAS Kit and Emergent Panels V1-V3. Sample preparation includes simple 20X dilution in water, quantitation by Full HRAM MS scan, and confident confirmation by retention time, accurate m/z, isotope pattern matching to calculated theoretical isotope pattern, and matching experimentally collected MS2 spectra to an in-house MS2 spectral library.Method:A chromatographic method of 15.5 minutes was used for the analysis of fentanyl and fentanyl analog compounds using a Thermo Scientific™ Vanquish Flex™ UHPLC system consisting of a binary pump, a column compartment, and an autosampler. The separation was performed on a Thermo Scientific™ Accucore™ Phenyl Hexyl column (2.1 mm x 100 mm, 2.6 µm) maintained at 40 °C. Mobile phases consisted of 2 mM ammonium formate in water with 0.1 % formic acid for mobile phase A and a mixture of 2 mM ammonium formate in methanol: acetonitrile (50:50 v:v) with 0.1 % formic acid for mobile phase B. Compounds were detected on a Thermo Scientific™ Orbitrap Exploris 120™ mass spectrometer equipped with a Thermo Scientific™ OptaMax™ NG ion source with a heated electrospray ionization probe. Full MS scan was used for screening and semi-quantitation while targeted MS2 by data dependent analysis was used for confirmation. A targeted mass inclusion list containing 212 fentanyl compounds with expected retention times and accurate m/z was used. Results: Data from the 22 mixes were processed in Thermo Scientific™ TraceFinder™ 5.1 software to determine Limit of Detection (LOD), Limit of Quantitation (LOQ), and Limit of Identification (LOI) for each compound. LOD is defined as the lowest detectable concentration in which % RSD for peak area was < 15 % for three replicate injections. LOQ is defined as the lowest concentration in the calibration curve giving an average % bias between nominal and back calculated concentration within ± 20%, a % CV below 20% for 3 replicate injections of calibrators, and a correlation coefficient of ≥ 0.99 r2 for all compounds. LOI was defined as the lowest concentration where all three replicate injections have the correct identification with an isotope and MS2 match score of > 90 and 80 % respectively. A majority of the fentanyl analogs had a LOD or LOQ of 2.5 ng/mL with ≥ 75 % of the compounds having a LOQ and LOI of 10 ng/mL or better. The limit of detection for a majority of the compounds was 0.5 ng/mL with ≥ 75 % of the compounds having a LOD of 1.0 ng/mL or better.

B-283

Stability Evaluation of Thiopurine Metabolites in Whole Blood, Washed RBCs and Sample Extracts

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Background: Monitoring thiopurine metabolites (TM) is helpful to optimize dosing for thiopurine drug therapy in the treatment of inflammatory bowel disease and acute

lymphoblastic leukemia. Therapeutic monitoring of TM is complicated by poor stability of the drugs in solvents and biological fluids. We evaluated stability of 6-Thioguanine (6-TG) and 6-Methylmercaptopurine (6-MMP) in whole blood (WB) stored at 4°C, in red blood cells (RBCs) separated from WB samples stored at -70°C and in sample extracts stored at -20°C and at 4°C. **Methods:** 6-TG and 6-MMP were analyzed using LC-MS/MS method. In brief, RBCs were separated from WB collected from TM positive donors, 6-TG and 6-MMP conjugates were hydrolyzed; 6-TG was analyzed in its native form; 6-MMP was converted to 6-MMP imidazole. *Stability in WB*: Aliquots of WB samples (n=5) were processed within 24 hours of the blood draw, while separate aliquots of the samples were stored prior to processing for 7 and 14 days at 4°C. *Stability in RBCs*: RBCs were separated from aliquots of WB samples (n=12) and washed; multiple aliquots of RBCs were stored at -70°C and processed on 4 occasions over 5 months. *Post-extraction stability at* -20°C: Samples (n=15) were analyzed on the day when they were prepared; the remaining aliquots were stored at -20°C for 2 weeks and then analyzed. *Post-extraction stability at* 4°C: A set of sample extracts (n=39) was stored in a 96-well plate in an autosampler compartment at 4°C and analyzed on 3 consecutive days.

Results:

			6-TG %change as compared to Day 1			6-MMP %change as compared to Day 1		
No.	**Type of Stability**	**No. of Samples (n)**	**Mean %**	**Median %**	**Central 90% of distribution**	**Mean %**	**Median %**	**Central 90% of distribution**
1	Whole Blood for 7 days at 4°C	5	-6.8%	0.0%	-31.3 to 5.6%	-4.3%	0.0%	-17.5 to 0.4%
	Whole Blood for 14 days at 4°C		11.2%	16.2%	-4.4 to 19.7%	31.7%	31.2%	18.4 to 45.8%
2	In RBCs for 3 months at -70°C	12	4.7%	7.0%	-14.1 to 18.1%	6.2%	2.4%	-27.1 to 5.7%
3	Post-Extraction Stability for 2 weeks at -20°C	15	-1.0%	-0.3%	-5.0 to 1.8%	-1.0%	-2.2%	-5.8 to 2.9%
4	Post-Extraction Stability for 3 days at 4°C	39	2.0%	1.7%	0.6 to 4.1%	1.0%	1.0%	0.3 to 2.2%

Conclusions: Based on the results, WB samples should be processed in less than 7 days of storage at 4°C. 6-TG and 6-MMP were stable in washed RBC samples when stored at -70°C for up to 3 months. The sample extracts were stable at -20°C for up to 2 weeks and up to 3 days at 4 °C.

B-284

Follow Up Study of Positive Screening Fentanyl Results from Abbott Architect Urine Immunoassay

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Background/objective: Clinicians of the psychiatry service (both inpatient and emergency) in our hospital use Fentanyl tests, which provide clinical data on patients in need of treatment for substance use disorder and to assess sources of possible overdose. It has been reported in the literature and observed at our institution that certain medications may increase the possibility of false positivity in the fentanyl screening test results. In order to get better understanding of our screening test and provide clinicians the best quality of service, we followed up with all screening positive Fentanyl from this group with quantitative confirmation test from October 2019 to January 2021. The goal of this study is to find out: a. the confirmatory positive rate of the initial screening positive patients; b. whether we can design a cost-effective testing algorithm to provide clinicians accurate Fentanyl test results.

Methods and Material: Fentanyl screening test: Abbott Architect (Immunalysis) Fentanyl qualitative screening test is used to detect fentanyl in patient urine samples on Architect c4000 with positive cutoff at >/=1.00ng/mL. Fentanyl Confirmatory test: quantitative confirmation test is performed at reference laboratory Labcorp using LCMS methodology (Sciex Model 5500). The positive cutoff is Fentanyl >/=0.5 ng/mL and/or Nonfentanyl >/=0.5 ng/mL. Confirmation test order algorithm: It is initiated by clinicians on inpatient and emergency psychiatry services. They will request a confirmation test to be added to the same urine sample whenever they get a Fentanyl screening positive result. **Results**: From October 2019 to January 2021, 39 Fentanyl positive urine results have been reported from the patient of this clinical group and confirmation test was subsequently added on each sample. 18 of 39 were confirmed positive and 21 of 39 were confirmed negative. Among the 18 confirmed positive samples, 13 had initial screening result above 2.00 ng/mL while 5 had initial result between 1.00 and 1.97ng/mL. All 21 confirmed negative results had initial screening results below 2.00ng/mL (1.00 to 1.99). The overall confirmed positive rate of all screening positive patients from this clinical group is 46.2%. Meanwhile, confirmed positive rate is 100% among patient samples with initial screening results >/=2.00ng/mL and only 19.2% among initial screen results between1.00-1.99ng/mL.**Conclusion and Discussion:**Our study indicated that Abbott Architect (Immunalysis) Fentanyl assay with cut off at >/= 1.00 ng/mL is prone to producing false positive results among samples in this patient group with initial screening results between 1.00 and 2.00ng/mL. While the confirmative rate will be improved if positive cutoff were moved up to 2.00ng/mL, the false negative rate would have also increased significantly as 5 out of 18 true positives would have been falsely identified as negative. As such, the confirmatory test may be necessary for clinicians to properly assess and guide care for patients who may have either knowingly or unknowingly consumed fentanyl. However, the most cost effective and clinically sufficient way to conduct the confirmatory test should be focusing on samples with positive screening result <2.00ng/mL for patients from this group.

B-285

Urine drug screening in the era of designer benzodiazepines: results of three immunoassay platforms and comparison to LC-QTOF-MS

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Background: We investigated the presence of designer benzodiazepines in 35 urine specimens obtained from emergency department patients undergoing urine drug screening on clinical indication. All specimens screened positive for benzodiazepines while confirmatory testing using a 19-component liquid chromatography-tandem mass spectrometry (LC-MS/MS) panel showed no benzodiazepines at detectable levels.

Methods: All specimens were obtained from emergency departments of a single US Health system (Michigan). Following clinically ordered drug screening using Abbott ARCHITECT *c* assays and lab-developed LC-MS/MS confirmatory testing, urine specimens were screened for amphetamines, barbiturates, benzodiazepines, cocaine, methadone, opiates, and THC using assays from two additional manufacturers (Roche cobas c502, Siemens Dimension Vista). Specimens were then further screened by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS); high-resolution mass accuracy m/z and retention time data were queried to identify presumptively positive species.

Results: Following benzodiazepine detection using the Abbott ARCHITECT (cutoff 300 ng/mL using oxazepam calibrators), benzodiazepines were subsequently detected in 28/35 and 35/35 urine specimens, respectively, using Siemens (cutoff 200 ng/mL, lormetazepam calibrators) and Roche (cutoff 100 ng/mL, nordiazepam calibrators) assays. Semi-quantitative analysis of the 7 specimens with concentrations below the Siemens assay cutoff revealed approximate cumulative benzodiazepine concentrations of 143-196 ng/mL. Concurrently detected species using Abbott screening assays included THC (26/35), cocaine (10/35), opiates (7/35), amphetamines (6/35), and methadone (2/35); Roche and Siemens assays failed to identify amphetamines in one sample, while Roche assays identified amphetamines and methadone individually in two additional samples. LC-QTOF-MS showed the presumptive presence of at least one designer benzodiazepine (i.e., benzodiazepines or benzodiazepine analogs with no approved medical use) in 30/35 specimens: flubromazolam (12/35), flualprazolam (11/35), flubromazepam (2/35), clonazolam (4/35), etizolam (9/35), metizolam (5/35), pyrazolam (1/35). Two or three designer benzodiazepines were detected concurrently in 12/35 samples. Nitrazepam, a benzodiazepine used internationally but not US FDA-approved, was presumptively identified in one specimen. Among the five patients who showed no benzodiazepines by targeted LC-MS/MS or LC-QTOF-MS, one stated use of Xanax (alprazolam); clinical presentations for the others were not conclusive. Designer benzodiazepines were detected exclusively in the absence of any

prescribed benzodiazepines, suggesting screening assays were cross-reactive with the former. Among the designer benzodiazepines detected, including triazolobenzodiazepines and thienodiazepines, multiple have uncharacterized cross-reactivity with the screening assays used: flualprazolam (Roche); flubromazolam, flualprazolam, flubromazepam, clonazolam, etizolam, metizolam, and pyrazolam (Siemens and Abbott).

Conclusion: Urine benzodiazepine screening assays from three manufacturers were cross-reactive with multiple non-US FDA-approved benzodiazepines, suggesting the utility of screening methods for detecting designer benzodiazepine use. Clinical and forensic toxicology laboratories using traditionally designed LC-MS/MS panels may fail to confirm the presence of non-US FDA-approved benzodiazepines detected by screening assays, risking inappropriate interpretation of screening results as false-positives. As prescribed and unprescribed benzodiazepine use grows, laboratories should consider expanding their confirmatory test panels to include non-US FDA-approved benzodiazepines and/or performing untargeted screening with structural identification (e.g., LC-QTOF-MS) to ensure appropriate interpretation of drug screening results.

B-286

Dextromethorphan and Guaifenesin Induced Acute Renal Failure

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Case History: A 17-year-old healthy male presented to an outside facility with abdominal pain and oliguria. He was hypertensive (163/99 mmHg), acidotic and uremic. Foley catheter was placed which resulted in ~10 mL brown to red urine. Urinalysis demonstrated hematuria and proteinuria. He was given Pepto-Bismol (Bismuth subsalicylate) and 1500 mL of normal saline, and then transferred to our center. On arrival, he was afebrile, awake, alert with mild tenderness to palpation in the lower abdomen. He received hydralazine, acetaminophen and morphine as needed. He denied taking NSAID, illegal drugs or any other over-the-counter medications. He was taking asenapine for his anxiety and depression. His subsequent clinical course, laboratory and biopsy findings did not fit with the available history. He subsequently admitted taking 25-30 tablets of Mucinex and other undisclosed cold medicines to get "high" following initiation of hemodialysis. He attested that he felt a "good buzz" after the ingestion.**Materials and Methods:** Chemistries were performed using a Vitros 5600 analyzer. Urinalysis was performed by Iris analyzer. Immunoassays and gas chromatography-mass spectrometry (GC-MS) were used for drug screening. Pathology studies of renal biopsies included light, immunofluorescence, and electron microscopy. Dextromethorphan and guaifenesin were quantified by NMS laboratories using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results and Discussion: The patient presented with findings of acute renal failure. The evaluation for an etiology was unremarkable. Serum enzyme immunoassay, twelve drug screen panel was positive for acetaminophen and opiates, and negative for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, phencyclidine, propoxyphene, salicylates, and tricyclic antidepressants. Over the next 36 hours his BUN rose rapidly from 36 to 97 mg/dL, and serum creatinine from 3.27 to 10.16 mg/dL. He remained anuric throughout this period with an indwelling Foley catheter. He received a total of 3 sessions of hemodialysis over the next 48 hours. He started to make urine after four days of complete anuria and recovered quickly to be discharged on the eighth day.

The kidney biopsy showed significant acute tubular injury. The glomeruli showed no cellular proliferations, necrosis, or crescents. There was no evidence of inflammatory infiltrates, vasculitis, or immune mediated glomerular injury. The electron microscopy confirmed tubular injury with no evidence of glomerular pathology.

After patient's admission of abusing over-the-counter medications, an expanded urine drug screen by GC-MS was performed; dextromethorphan and guaifenesin were detected. Blood presumptively collected 48-72 hours post-ingestion showed guaifenesin and dextromethorphan concentrations at 0.13 mcg/mL and 290 ng/mL respectively. Despite the significant time lapse between ingestion and specimen collection, the dextromethorphan concentration was still in the toxic range. Given approximate half-live of <6 hours for both dextromethorphan and guaifenesin, the peak concentrations of these drugs would have been significantly higher when the patient had presented to the outside facility. We attributed his renal injury to toxicity from the ingested drugs by exclusion of other causes.

Conclusions: The case represents rare findings of acute renal failure caused by ingestion of guaifenesin and dextromethorphan. It also highlights the drug abuse of common cold medications by teenagers.

B-287

Status of antioxidants in relation to heavy metals induced oxidative stress in patients with Polycystic ovarian syndrome (PCOS)

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Background: Polycystic ovary syndrome (PCOS) is a major concern for women health as it is the most common endocrine disorder in women of reproductive age. It is estimated that 1 in 15 women are affected by this syndrome worldwide. PCOS is marked by hyperandrogenism, anovulation, menstrual abnormalities, and polycystic ovaries. It is linked with insulin resistance, dyslipidemia and increased risk of type 2 diabetes mellitus, metabolic syndrome, obesity, and endometrial carcinoma. Heavy metal exposure seems to have devastating effect on the humans. For their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants/human carcinogens that are known to induce multiple organ damage, even at lower levels of exposure. Despite a long history of studies on PCOS, its etiology is still unknown. Oxidative stress has been recognized to play a central role in the pathophysiology of PCOS.

Methods: Owing to the existence of correlation between oxidative stress and PCOD, present study evaluated levels of antioxidants (glutathione and SOD) and heavy metals in 56 women patients diagnosed with PCOS and 50 women who were diagnosed negative with PCOS (Control group).

Results: There was no significant changes in the sociodemographic characteristics between the two groups. Levels of serum As, Cd, Pb, Hg increased and serum GSH and SOD levels diminished significantly in PCOS group compared to control at $P<0.001$. SOD was negatively correlated with As & Pb at $P<0.05$. Additionally, PCOS group exhibited a strong negative correlation between GSH and As ($P<0.01$), GSH and Pb ($p<0.05$) and GSH and Hg ($P<0.01$). Furthermore, As correlated positively with increased levels of Cd, Pb and Hg among PCOS women. A significant positive correlation was determined between Pb & Cd and Cd & Hg at $P<0.001$.

Conclusion:

Based on the results, we conclude that there is considerable oxidative stress in PCOD patients compared to healthy control and apparently the heavy metal toxicity contribute significantly to the generated oxidative stress.

B-288

Analytical Validity and Clinical Utility of Homogenous Mobility Shift Assay Method for Vedolizumab Bioactivity and Precision Dosing in Inflammatory Bowel Diseases

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Background: Therapeutic drug monitoring (TDM) of Vedolizumab (VDZ) can improve outcomes in inflammatory bowel disease (IBD). Our objective was to establish the performance characteristics of the Homogenous Mobility Shift Assay (HMSA) method as a precision dosing tool for VDZ in IBD.

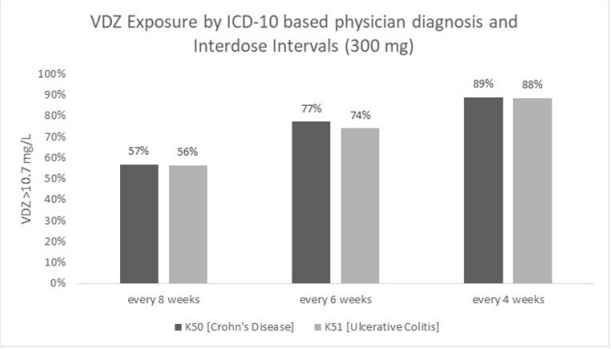
Methods: Validation testing was conducted in a clinical laboratory accredited by the American College of Pathologists (Prometheus Laboratories, San Diego) using liquid chromatography size exclusion HMSA. The evaluated performance characteristics consisted of linearity, accuracy, precision, specificity, and lower limit and upper limit of quantification using spiked human serum. Following validation and implementation in the clinical laboratory, estimates of VDZ exposure associated with higher likelihood (Odds Ratio = 4.0) of endoscopic remission (>10.7 µg/mL) was assessed using de-identified samples, as established during clinical validation (J Crohns Colitis 2019 13:963-969).

Results: The reportable range was 1.6 µg/mL LLOQ (precision = 10.6% CV; accuracy = 89.0% recovery) to 40 µg/mL ULOQ: (precision = 5.3% CV; accuracy = 99.1% recovery). Intra-day, inter-day precision (CV) and accuracy (% recovery) across the reportable range were 3.4%, 6.9% and 100.7%, respectively. The HMSA method was specific with interfering substances yielding <8% error. The method was linear (slope=0.978; intercept=-0.016; R^2=0.999). In practice, over a one-year period (01/2020 to 12/2020), VDZ levels from 1695 specimens from IBD patients receiving 300 mg VDZ every 8 weeks yielded a mean VDZ of 14.8 µg/mL (SD=12.9 µg/

mL) with 57% of patients presenting exposure above the threshold (>10.7 µg/mL). As presented in Figure 1, inter-dose interval shortening from every 8 weeks (n=1695) to every 6 weeks (n=299) and 4 weeks (n=525) resulted in higher likelihood of VDZ levels above the threshold.

Conclusion: Our data indicate the VDZ-HMSA method is validated to report VDZ concentrations with acceptable consistency to be used for optimal TDM dosing relative to the endoscopic remission threshold.

Figure 1: VDZ Exposure in Relation to Interval Dosing and ICD-10 Diagnosis



B-289

Adalimumab Serum Levels in Relation to World Health Organization Standardization in Clinical Gastroenterology Practice

J. M. McFarland, H. Law, M. Schwalbe, O. McLachlan, J. Ho, A. Jain, T. Dervieux. *Prometheus Laboratories Incorporation, San Diego, CA*

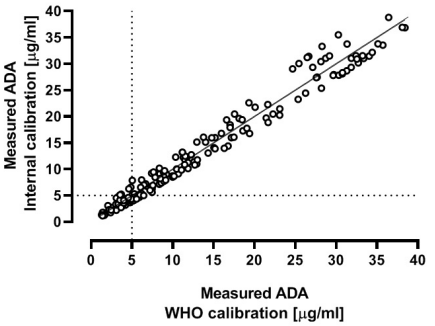
Background: Proactive therapeutic drug monitoring (TDM) of Adalimumab (ADA) is emerging as important to prevent the formation of anti-Adalimumab antibodies (ATA) in inflammatory bowel disease (IBD). Our objective was to evaluate performance characteristics of Homogenous Mobility Shift Assay (HMSA) standards against the 1st international World Health Organization (WHO) standard for ADA bioactivity.

Methods: Data collection was conducted in a clinical laboratory accredited by the American College of Pathologists (Prometheus Laboratories, San Diego) using liquid chromatography size exclusion HMSA. ADA WHO Standard (17/236) was purchased as lyophilized powder and re-suspended in human serum. ADA concentrations measured by in-house and WHO calibration methods were compared using 31 de-identified specimens. ADA levels in relation to ATA formation were evaluated from a de-identified clinical specimen database submitted for TDM. Statistical analysis consisted of regression analyses and determination of interval likelihood ratio (ILR). Longitudinal changes in ATA titers (log normalized) were analyzed using linear mixed effect models.

Results: Between run (n=6) precision was 6.2% (median CV; range 3.1-12.5%) and 4.2% (range: 0.9-10.4%) for ADA specimens measured using in-house and WHO calibration, respectively. As presented in Figure 1, ADA levels measured using the in-house calibration method (mean [SD]: 16.0±9.7 µg/ mL) were comparable to those recovered using the WHO calibration method (mean 15.9±9.9 µg/ml) (Deming's slope 1.03 CI95%: 1.01-1.04). In a population of 84,540, ADA levels determined from 61,491 subjects (40±17 years, 53% female) recovered a mean ADA of 10.0±8.0 µg/ mL with 16.2% patients presenting detectable ATA (>1.7 U/ml; mean 1.9±7.6 U/ mL). ADA levels <1.6 µg/mL associated with a 9.9-fold higher probability of ATA (ILR=9.85 CI 95%: 9.54-10.2). In contrast, ADA exposure >10 µg/mL resulted in negligible likelihood of ATA.

Conclusion: Calibration of the ADA-HMSA to WHO may facilitate harmonization of TDM in the clinical gastroenterology practice for assessing ATA relative to accurate ADA levels.

Figure 1: Deming's slope of Measured ADA by HMSA Method using In-house vs. WHO Calibration
A Total of 31 Specimens Across the Dynamic Range of the Analytical Method were Processed 6 Consecutive Times



B-290

Immunoassay for the Detection of Norfentanyl in Urine on Beckman Coulter AU480, AU680, AU5800, and DxC 700 AU Clinical Analyzers

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Background:The LZI Fentanyl assay is a homogeneous enzyme immunoassay for the qualitative detection of norfentanyl in urine on the Beckman Coulter AU480, AU680, AU5800, and DxC 700 AU clinical analyzer platforms. The assay is based on competition between norfentanyl in the sample and fentanyl-labeled glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent. The assay is available at a 5 ng/mL cutoff using norfentanyl as the target analyte.

Advantages: Norfentanyl is more abundant in urine and has a longer half-life than fentanyl, the parent compound.[1] Studies by Cummings et al., and Feng et al., found that urinary norfentanyl concentrations were higher than fentanyl concentrations.[2,3,4] A study by Feng et al., found that having even low cross-reactivity to norfentanyl can improve the sensitivity of the assay.[3] A study by DePriest et al., concluded that inclusion of norfentanyl in the test panel produced an increase in detection rates for parent drug use by 42.1%.[5]

Precision: The 510(k) precision study was performed in qualitative mode (one-point calibration against the cutoff calibrator) on the Beckman Coulter AU680. Further validation studies were performed on the Beckman Coulter AU480, AU5800, and DxC 700 AU. The following average Standard Deviation (SD) and % Coefficient of Variation (% CV) were found across all four analyzer platforms. For Qualitative Precision, the within-run precision had an average SD of 3.0 mAU and average % CV of 4.8 %. The total precision had an average SD of 3.5 mAU and average % CV of 5.6 %.

Cross-reactivity:Cross-reactivity studies were performed on the Beckman Coulter AU480, AU680, AU5800, and DxC 700 AU. The following representative cross-reactivity profile from 510k studies were performed on the AU680 clinical analyzer: 100.0 % cross-reactivity to norfentanyl, 156.25 % cross-reactivity to fentanyl, 71.4 % cross-reactivity to acetyl fentanyl, 142.8 % cross-reactivity to acryl fentanyl, 142.8 % cross-reactivity to butyryl fentanyl, 81.9 % cross-reactivity to furanyl fentanyl, 142.8 % cross-reactivity to methoxyacetyl fentanyl, 142.8 % cross-reactivity to thienyl fentanyl.

Method Comparison:Method comparison studies were performed in qualitative mode on the Beckman Coulter AU480, AU680, AU5800, and DxC 700 AU. Clinical samples containing norfentanyl were confirmed by LC/MS. Across all analyzers, there was 100% agreement with all confirmed positive results and >82.0 % agreement with confirmed negative results. Discrepant samples contained levels of fentanyl that contributed to the false positive result due to >100% cross-reactivity with fentanyl.

Conclusion:The LZI Fentanyl Enzyme Immunoassay produces accurate and reliable results across the Beckman Coulter AU480, AU680, AU5800, and DxC 700 AU clinical analyzer platforms. The

assay is sensitive for the detection of the major fentanyl metabolite, norfentanyl, as well as the parent, fentanyl, with low interference from other opiates.

References:1.Silverstein, J.H. et al., *Anesth. Anal.* (1993).2.Cummings, O.T. et al., *Journal of Analytical Toxicology* (2016).3.Feng, S. et al., *Journal of Analytical Toxicology* (2021).4.Baselt R.C. et al., Disposition of Toxic Drugs and Chemicals in Man, 10th edition (2014).5.DePriest, A. et al., *Journal of Analytical Toxicology* (2010).

B-291

Development and validation of trace metals in human urine by inductively-coupled plasma mass spectrometer

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Background: Trace metals play important roles in biological processes, both as essential components and toxins. Monitoring the body status of trace metals thus has become an important function of many clinical, industrial, and government laboratories. Labor policies demand increasingly accurate methods for determining trace metals. Therefore a clinical diagnosis needs to have a rapid and thru method for determination. The objective of this work is the Development and validation of a rapid ICP/MS method for the determination of trace metals (chrome, cadmium, nickel, manganese) in human urine for clinical practice.

Methods: This method was developed on an Agilent Technologies - 7700 Series ICP-MS. Among the instrumental parameters used to develop the method of quantification of inorganic elements by ICP-MS, was the use introduction system Agilent ASX-520; use of collision gas (He = 5.0) in the reaction/collision cell; and monitoring of isotopes of the studied elements. The extraction procedure is a simple dilution of human urine with a solution of ETDA, Triton, Butanol, and internal standard solution.

Results: The linear range achieved was between 0,5 and 100 µg/L for cadmium, nickel, and manganese, and 2,5 and 100 µg/L for chrome. The limit of detection was 0,1 µg/L for cadmium and manganese, 0,4 µg/L for chrome, and 0,01 µg/L for nickel. The limit of quantification was 0,5 µg/L for cadmium and nickel, 0,8 µg/L for chrome, and 0,4 µg/L for manganese. The inter and intra assay-precision for cadmium was in a range of 1,0 to 3,3%, and 0,4 to 4,44, respectively; for chrome, 0,7 to 2,9%, and 0,4 to 1,8%; for nickel, 1,15 to 5,5% and 0,5 to 4,7%, and, for manganese, 1,4 to 6,1% and 0,4 to 8,8%. The method was robust for all the metals studied since a large number of samples were analyzed in 3 months in a large laboratory located in Belo Horizonte, Brazil.

Conclusion: In conclusion, the method has been developed and validated successfully, and can be applied in clinical routine for determination of trace metals in human urine.

B-292

Investigating the impact of rheumatoid factor on vancomycin measurement in serum separator tubes by Roche® Diagnostics Kinetic Interaction of Microparticles in Solution (KIMS) assay

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Background: The objective of this study was to determine whether significant positive interference by rheumatoid factor is observed for vancomycin measurement in BD Vacutainer® Serum Separator Tubes (SSTs) with the Generation 3 Roche Diagnostics kinetic interaction of microparticles in solution (KIMS) assay. Vancomycin is a glycopeptide antibiotic used to treat bacterial infections. Therapeutic drug monitoring is useful in achieving therapeutic doses and avoiding and/or confirming suspected vancomycin toxicity. Serum specimens containing rheumatoid factor (RF) are known to cause positive interference on different vancomycin immunoassays including, fluorescence polarization immunoassay, enzyme multiplied immunoassay technique (Roche Diagnostics, Laval, QC) and particle-enhanced turbidimetric inhibition immunoassay technique (Siemens Healthcare Diagnostics Inc, Tarrytown, New York), while plasma specimens are unaffected. Currently, it is not known if RF interferes to the same degree with vancomycin measurement in serum in newer generation immunoassays such as VANC3 KIMS (Roche Diagnostics, Laval, QC).

Methods: Residual patient specimens from routine laboratory testing for RF were used to prepare RF-positive and RF-negative pools. Specimens exceeding 300 kU/L RF were selected for positive pools whereas specimens with less than 10 kU/L (RF cutoff) were selected for negative pools. Pooled specimens were centrifuged at 1200 xg and RF concentrations were measured by RF-II assay (cobas®) on the Roche c 702 cobas®. Pools were divided into 2 aliquots, and frozen at -20 °C until use. Vancomycin in saline (50 mg/mL) was spiked into corresponding RF positive and negative plasma and serum specimens at two concentrations (12-15 and 40-45 mg/L). Serial dilutions of vancomycin spiked RF pools were generated by dilution with vancomycin spiked RF negative specimens. Plasma and serum specimens were measured in triplicate by VANC3 KIMS and RF-II assay on the Roche c 702 cobas®. Mean, SD, and %CV were calculated for each group. RF interference for vancomycin was considered significant if the measured vancomycin in RF-positive specimens deviated greater than 2 mg/L for specimens spiked with 12-15 mg/L or greater than 10% for specimens spiked with 40-45 mg/L relative to RF-negative specimens.

Results: The RF concentrations in RF-positive plasma and serum pools were 480 and 340 kU/L respectively. Vancomycin values in RF-negative plasma specimens were 15.6 ± 0.1 mg/L and 45 ± 0.4 mg/L. Relative to RF-negative pools, no significant change in vancomycin recovery was observed in any plasma specimens containing RF. In the high RF-positive pools vancomycin levels were 15.0 ± 0.5 (RF = 488.3 ± 1.3 kU/L) and 46.9 ± 1.2 (RF = 484.6 ± 11.9 kU/L). The recovered vancomycin in RF-negative serum specimens was 12.6 ± 0.1 mg/L and 41.8 ± 0.8 mg/L. Relative to RF-negative pools, no significant changes in vancomycin recovery were observed in any of the serum pools containing RF. In the high RF-positive serum pools, vancomycin levels were 12.6 ± 0.2 (RF = 331.5 ± 3.5 kU/L) and 41.0 ± 0.6 (RF = 332.0 ± 4.5 kU/L).

Conclusion: Our data suggests that RF concentration up to 480 kU/L in plasma and 340 kU/L in serum specimens, do not significantly impact vancomycin measurement by KIMS assay.

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The Detection of Fentanyl in Umbilical Cord Tissue and Maternal Perinatal Fentanyl Exposure

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Background: The rate of Neonatal Abstinence Syndrome (NAS) has been growing due to the increasing use of opioids in recent years. Fentanyl is a common pain medicine used in the obstetric management of labor. Chronic exposure to fentanyl prior to birth can potentially result in NAS. Umbilical cord presents a unique opportunity for drug testing and potential identification of NAS. However, data to guide the selection of an appropriate cut-off for umbilical cord fentanyl detection to distinguish illicit substance exposure from therapeutic in-hospital administration of fentanyl is currently lacking. The specific aim of this study was to determine if the currently available cutoff for fentanyl in umbilical cord (500 pg/g) was appropriate to distinguish illicit fentanyl exposure from therapeutic in-hospital administration of fentanyl

Methods: A secondary analysis of the medical records and umbilical cord toxicology results of births occurring at Charleston Area Medical Center Women's and Children's Hospital (CAMC) between October 01, 2018 and November 15, 2018 were analyzed for perinatal administration of fentanyl and the detection of fentanyl in the corresponding umbilical cord specimens. Umbilical cord tissue segments were collected according to standard protocol and shipped to United States Drug Testing Laboratories (Des Plaines, IL) for analysis. The study was approved by the CAMC Institutional Review Board (IRB Number 19-634).

Results: The study included 62 subjects who met the inclusion criteria. Of these 62 subjects, 37 received fentanyl prior to delivery. The amount of fentanyl administered ranged from 6.7 mg up to 392 mg. The total duration of fentanyl administration ranged from 0.5 hours to 80 hours with the duration of fentanyl administration prior to birth ranging from 0.3 hours up to 11.7 hours. Sixty-two (62) umbilical cord specimens were received for analysis and only 1 umbilical cord specimen was reported positive. In the 1 positive case, fentanyl was not administered during labor and delivery. Excluding the single screen and confirm positive specimen, the mean b/b_0 for the cases that received fentanyl prior to delivery was 91.3% ± 10.6% and the mean b/b_0 for the cases that did not receive fentanyl was 98.2% ± 6.5%. An independent samples *t* test showed a mean b/b_0 difference of 6.92 (95% CI: 2.54, 11.30) and this difference was statistically significant [$t(58.9) = 3.158$, $p = 0.003$]. Linear regression analysis of the total fentanyl delivered pre-delivery and b/b_0 of the fentanyl immunoassay test result for the corresponding umbilical specimen was -0.087 ($R^2 = 0.349$).

Conclusion: This study demonstrated that umbilical tissue is a suitable specimen type for the detection of fentanyl. Additionally, this study has demonstrated that the cutoff selected adequately identifies illicit fentanyl use while not flagging cases where fentanyl was administered by the hospital prior to birth.

B-297

To Report or not to Report: The Clinical Implications of Clozapine Metabolite Reporting

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Background: Clozapine (CLO) is a second-generation atypical antipsychotic medication that is primarily converted to Norclozapine (NCLO) via Cytochrome P450 metabolism. Both CLO and NCLO have the potential to cause distinct concentration-dependent adverse drug reactions (ADRs) and though therapeutic drug monitoring of

CLO is recommended, it is unclear if routinely reporting both analytes is beneficial. This study provides a retrospective data review to evaluate the expected inter- and intra-individual variation of CLO and NCLO to allow the development of analytical quality targets as well as quantify the frequency that NCLO may be present in higher concentrations in patients, thus contributing to unexplained ADRs if unmeasured. **Methods:** Data for CLO and NCLO results collected 8 – 12 hours post-dose as well as associated blood work (e.g. CBC, renal and liver function tests) were obtained from the Laboratory Information System from January, 2016 to April, 2020. Our laboratory reports both CLO and NCLO individually based on a Liquid Chromatography Tandem Mass Spectrometry method validated against Clinical Laboratory Standards Institute (CLSI) guidelines. The analytical imprecision (CV_a) for CLO, NCLO and the CLO/NCLO ratio was derived from 12 months of quality control data and averaging the %CV of two levels of QC results. The intra- (CV_i) and inter-patient (CV_g) biological variation data for CLO, NCLO, and the CLO/NCLO ratio were calculated based on the most recent two quantifiable CLO and NCLO results in the dataset. **Results:** A total of 6990 patient results for CLO and NCLO were obtained from 862 patients in the dataset. No statistically significant correlations were found between raw CLO or NCLO results and liver or kidney function tests or CBC results. A total of 186 (22%) patients showed a CLO/NCLO ratio less than 1.0, thus have higher concentrations of NCLO than CLO in the blood. The CV_a was calculated to be 5.0%, 7.4% and 5.9% for CLO, NCLO, and the CLO/NCLO ratio, respectively. From 505 patients with greater than one measurement in the dataset, the CV_i was calculated as 20.1%, 14.2%, and 9.7% while the CV_g was calculated as 14.3%, 14.5% and 10.6% for CLO, NCLO and the CLO/NCLO ratio, respectively. The high %CVs for each of CLO and NCLO likely relates to changes in dose, or differences in time between dose and sample collection. Interestingly, the ratio of CLO to NCLO remains more stable in patients, suggesting that when a patient has a ratio less than 1.0 (i.e. more NCLO than CLO), that remains stable over time and if only measuring CLO clinically, ADRs related to NCLO could be missed. **Conclusion:** There is broad variability in the ratio of CLO to NCLO between patients, yet the intra-individual variability is lower. Reporting both CLO and NCLO allows interpretation of concentration-dependent ADRs for both analytes which could be missed in up to 22% of patients were NCLO not reported.

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Pharmacokinetic properties of melphalan in lymphoma patients receiving conditioning for stem cell transplantation

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Background: High-dose melphalan is a standard conditioning regimen used for autologous and allogenic stem cell transplantation (ASCT/allo-SCT) in patients with lymphomas and a variety of hematological malignancies. Melphalan dosing is most often calculated based on body surface area (BSA) which causes high inter-individual variability in clinical responses. Excessively high dose and adverse drug reactions (ADRs) such as mucositis, nausea, vomiting and diarrhea or suboptimal dose causing graft rejection are common. In this study, we quantified melphalan concentrations using a chromatography tandem mass spectrometry (LC-MS/MS) method in plasma from lymphoma patients undergoing conditioning for ASCT. Pharmacokinetic (PK) parameters of melphalan in patients receiving doses based on BSA was investigated.

Methods: 47 patients undergoing conditioning for ASCT were treated with a 5-minute IV push of melphalan + gemcitabine. Plasma samples were collected at 6 timepoints (0, 0.5, 1, 3, 5 and 7-10 hours) after infusion. Patient samples, calibrators or QC were precipitated with MeOH containing 5% ZnSO4 and melphalan-d8 internal standard (IS). The supernatant was diluted 1:5 with mobile phase A (0.1% formic acid in MeOH) and analyzed by LC-MS/MS using a Poroshell 120 EC-C18 column (Agilent) on a Agilent 1200 HPLC and 6410 LC-MS/MS with an electrospray ionization source. Melphalan and IS ions were detected by multiple reaction monitoring with transitions (m/z) 305.1->194.3 and 313.1->254.4 respectively. Melphalan concentrations in patient samples were quantified against a 6-point calibration curve (0.025-5 µg/mL). PK parameters including AUC and clearance were determined using Phoenix-8.1 WinNonlin (Certara) software and group statistics were performed in Excel version 16.0.

Results: The LOD of the method was 0.025 µg/mL and the assay was linear to 5 µg/mL. Within-run imprecision CV was 1.54% and 2.68% (0.5 and 2.0 µg/mL in-house prepared QC) and the between-run imprecision CV was 5.11% and 6.4%. The doses for patients included in the study ranged from 200-510 mg (380 ± 54 mg). Peak concentrations of melphalan ranged from 10.1-20.6 µg/mL at 5 minutes post-infusion and drug was cleared (<0.025 µg/mL) from circulation from all patients by 10 hours post-infusion. Concentration time plots for melphalan indicated bi-phasic elimination. Accordingly, a two-compartment PK model was used to evaluate individual drug exposure and clearance. AUCs were highly variable between patients 14.4±3.1 hr•µg/mL (range; 6.1-20.9, CV=21.4%). The mean clearance rate of the drug was 27.7±7.7 L/hr, consistent with published reports, but ranged from 15.6-50.1 L/hr (CV=27.9%).

Conclusion: This study demonstrates dosing melphalan based on BSA leads to marked inter-individual variation in melphalan exposure. This variation can be explained in part by pharmacogenomic and renal function differences between patients demonstrated by significant differences in drug clearance rates between patients. These PK differences likely contribute to high number of reported ADRs in this patient population. Additional, PK guided dosing studies will be helpful in achieving a more uniform conditioning treatment for ASCT.

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