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P102

Current status of laboratory reference intervals during pregnancy

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Objectives: Accurate interpretation of lab results during pregnancy requires availability of gestation-specific reference intervals (RIs), but few laboratories establish or provide pregnancy RIs. This study assesses current availability and quality of pregnancy RIs for multicultural Canadian populations and identifies gaps to inform future RI studies.

Design and Methods: We conducted a literature review of pregnancy RI studies for chemistry, immunoassay, and hematology tests, published between 1988-2018. Eighty publications were included. Sample size, study design, cohort demographics, partitioning, analytes studied and the RIs were summarized and compared between studies.

Results: Over half of studies used a cross-sectional design, over a third were conducted in Asia, and few included multicultural populations. Only 8 were North American, 4 of which included ≥120 individuals. RIs during labour/delivery (N = 4), or post-partum (N = 15) were underrepresented. Various instruments and vendors were represented. A broad range of biomarkers were studied, thyroid hormones being the most common. Reported RIs varied between studies, making it difficult to generalize results. For example, similar trends in WBC RIs were seen with gestational age in Caucasian and Asian cohorts but differed in African cohorts; while studies consistently found that in pregnancy albumin RIs differ from non-pregnant values.

Conclusions: Review of the literature illustrates difficulties in using published pregnancy RIs for Canadian women. Heterogeneity of the derived RIs, differences in study cohorts and missing information for some gestational time-points highlight the need for RIs established using large, ethnically diverse reference groups during pregnancy, labour/delivery, and post-partum.

Key Words: Reference intervals, pregnancy, perinatal, literature review

P104

Assessment of urine samples with the fully automated biochip analyser Evidence MultiSTAT for the screening of multiple drugs of abuse and creatinine from a single sample under twenty minutes

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Objectives: Multi-drug detection increases the screening capacity. Biochip Array Technology (BAT) allows the simultaneous detection of multiple drugs of abuse and creatinine in less than twenty minutes from a single sample, when applied to the fully automated benchtop biochip analyser Evidence MultiSTAT. This study reports the assessment of urine samples using this application.

Design and Methods: Simultaneous competitive chemiluminescent immunoassays on a biochip surface on the Evidence MultiSTAT were employed (21 different drug types can be detected simultaneously). Sampling 25µl of urine against a cut-off sample the results are qualitative.

Authentic urine samples (n = 98) were obtained from known drug users (Jacksonville Homeless refuge). All screening data was correlated against liquid chromatography/mass spectrometry (LC/MS) results from an independent laboratory. In addition, negative urine samples were spiked with certified reference material (n = 100).

Results: Authentic samples screening: a total of 10 different drugs were present (samples were positive for 1 or more drugs). Most of the samples were positive for benzoylecgonine/cocaine and tetrahydrocannabinol (THC), two samples positive for alpha-pyrrolidinopentiophenone (alpha-PVP). Negative for synthetic cannabinoids. Creatinine >20mg/dL in all samples indicating absence of sample dilution. Correlation against LC/MS: the overall percentage agreement was >90% for all assays except for THC. THC assay: 88% agreement (1 false negative and 25 false positive, the latter below the LC/MS cut-off however there were lower levels of THC confirmed present).

Conclusions: Data indicate applicability of the Evidence MultiSTAT platform to the rapid multi-drug screening (<20 minutes) of urine samples and favourable agreement with LC-MS.

P105

Do I really need fT3 and fT4? An overutilization change strategy to reduce free thyroid hormone testing

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Objectives: To reduce unnecessary free thyroid hormone testing, St. Michael's Hospital implemented a laboratory reflex algorithm that comprised of reflexed free thyroxine (fT4) with high TSH, and reflexed fT4 and free triiodothyronine (fT3) with low TSH. Reflexive testing resulted in minimal reduction due to clinically unmeaningful fT3 tests that were added-on. The current project aimed to further reduce inappropriate testing by optimizing the TSH reflex and ordering requisitions.

Design and Methods: Receiver operating characteristic (ROC) curves were used to assess suitability of using TSH reference intervals (0.4-5.5mIU/L) as reflex cut-points. To reduce inappropriate testing, two change ideas were implemented: 1) the current reflex was modified to reflex fT3 only when TSH was low and fT4 was normal, and 2) a reflex

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independent 'TSH only' order option was made available.

Results: ROC curve analysis identified comparable cut-points to the current TSH reference range (0.4mIU.L and 5.7mIU/L) and confirmed the appropriateness of using the reference range to initiate the reflex. The 'TSH only' option reduced reflexive testing by 23% (n = 2151 vs 1665/month). A 16% reduction (230 vs 192/month) of reflexed fT4 was observed, with only 2 laboratory add-on calls assessed as a balancing measure. A 30% increase in reflexed fT3 was observed (n = 117 vs 153/month) due to an error in the 2nd tier of the reflex.

Conclusions: Laboratory-based reflex algorithms may require hospital-specific modifications to achieve maximal reduction in unnecessary testing. A pre-implementation test environment and post-implementation result monitoring is important to identify unintended outcomes.

P106

Postprandial dyslipidemia in obese and insulin resistant adolescents: Evidence for impaired response of intestinal glucagon-like peptides and abnormal bile acid profile

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Objectives: Obesity and insulin resistance, increasingly prevalent in adolescents, commonly associates with postprandial dyslipidemia. Gut peptides (glucagon-like peptide (GLP)-1, GLP-2) and bile acids are secreted in response to fat ingestion and modulate dietary fat absorption and triglyceride-rich lipoprotein (TRL) output. We hypothesize that the GLP-1, GLP-2, and bile acid response to a high-fat meal is impaired in obese, insulin resistant adolescents and associates with postprandial dyslipidemia.

Design and Methods: Normal weight (NW; n = 15), obese, mildly insulin resistant (OB-MIR; n = 20), and obese, insulin resistant (OB-IR; n = 10) adolescents underwent an oral fat tolerance test with serum/plasma collected up to 6 hours. The serum lipid/lipoprotein profile was measured by nuclear magnetic resonance spectroscopy, plasma gut peptides by ELISA and serum bile acids by mass spectrometry. Postprandial responses were assessed by area under the curve (AUC), incremental AUC, and two-way mixed ANOVA.

Results: OB-IR adolescents exhibited reduced fasting HDL particle size (8.49 ± 0.07 nm) and exaggerated postprandial large TRLs (AUC: 59.7 ± 11.9 nmol-h/L) compared to NW (9.29 ± 0.09 nm ($p < 0.001$) and 16.8 ± 3.42 nmol-h/L ($p < 0.001$), respectively). Postprandial GLPs were blunted in OB-IR adolescents and inversely correlated with postprandial dyslipidemia. Postprandial total bile acids were lower in OB compared to NW adolescents, specifically lithocholic acid (AUC: 217 ± 77.4 nmol-h/L vs. 486 ± 89.1 nmol-h/L), a potent stimulator of GLP-1 secretion.

Conclusions: Postprandial GLP-1, GLP-2, and bile acids were blunted in response to a high-fat meal in OB-IR adolescents. However, it remains unknown if these postprandial metabolic changes are a cause or consequence of impaired glucose and lipid metabolism in an obese state.

P107

Evaluation of Beta-Hydroxybutyrate Assay on the Nova StatStrip Meter

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Objectives: To evaluate POC Beta-Hydroxybutyrate (BHB) assay on the Nova StatStrip meter including validity of different sample types and stability on whole blood (WB) samples.

Design and Methods: Day-to-day precision was examined with aqueous QC materials. Linearity was tested for the analytical range of 0.1- 7.0 mmol/L. Spiked WB was used for precision at high values. POC BHB results from twenty-five patient serum samples were compared to those from an in-lab assay (Randox/Architect, Abbott).

Results: The CV for POC BHB was 38.8% and 5.42% at 0.145 mmol/L and 3.15 mmol/L respectively; although within run CV was 0% at 0.1 mmol/L using vendor's QC materials. CV was 6.89% at 5.9 mmol/L using spiked WB. Mean linearity was $R^2 = 0.99$. The Passing-Bablok fit is $Y = 0.79x + 0.21$, indicating a positive bias for POC BHB levels < 2.5 mmol/L but negative bias at > 2.5 mmol/L compared to the Randox assay. Mean BHB in plasma was 0.08 mmol/L lower than that in WB. Mean BHB was lower by 0.09 mmol/L after 2 hours at RT on WB; neither however were statistically significant.

Conclusions: Precision of BHB on the Nova StatStrip was acceptable for results up to 7 mmol/L. WB, plasma and serum are all acceptable for this POC BHB assay and BHB remains stable in WB for up to 2 hours. Clinicians need to be aware of the positive bias at the reference cutoff of 0.6 mmol/L and negative bias at the clinical decision cutoff of 3 mmol/L, when making therapeutic decisions.

P108

Alkalinity and specific gravity do not interfere with proteinuria detection using three commercial dipsticks

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Objectives: Urinalysis dipsticks are widely used to detect proteinuria using pH-indicator dyes. Dipstick package inserts caution laboratories that alkalinity causes false positive urine and that high specific gravity leads to false negative protein results. The aim of this study was to determine whether pH and/or specific gravity affect urine protein detection using urinalysis dipsticks containing tetrabromphenol blue from three different vendors.

Design/Methods: The pH interference experiments used three urine pools that were progressively alkalinized with 1M NaOH, Na_2CO_3, or $NaHCO_3$; electrodes were used to monitor pH change. Urine aliquots were measured in triplicate using iChem 10SG, iChem Velocity, and Multistix 8SG dipsticks. Specific gravity interference was assessed with three urine pools spiked with human albumin that were mixed with NaCl; aliquots were measured for protein by dipstick analysis from the three vendors. Urine electrolytes and total CO_2 (TCO_2) were measured on all samples (Roche cobas 8000). Fresh patient urines (N = 25) were assayed to assess physiological TCO_2 and pH.

Results: Urine pH alone did not affect protein results unless pH > 12.5 (NaOH alkalization). False positive protein occurred at pH 9.9 and when TCO_2 > 97 mmol/L (Na_2CO_3 alkalization; $P < 0.05$). False positive protein occurred at pH 7.7 when TCO_2 was > 160 mmol/L ($NaHCO_3$ alkalization; $P < 0.05$). Patient urine did not exceed pH > 8.5 or TCO_2 > 86 mmol/L. NaCl elevated specific gravity and caused false negative protein detection when urine ionic strength > 1300 mmol/L ($P < 0.05$).

Conclusions: Tetrabromphenol blue dipsticks provide a robust marker of proteinuria provided that pH, TCO_2 and ionic strength are within physiological limits.

P110

Evaluation of two point of care Hemoglobin A1C devices in a hub and spoke laboratory model

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Objectives:

Testing percent glycated hemoglobin A (%HbA1c) is critical for diabetes management, and generally performed using high volume instruments by hub laboratories. However, pediatric patients could benefit from point of care (POC) %HbA1c testing. Two Health Canada approved POC %HbA1c devices were evaluated for implementation into pediatric endocrinology clinics.

Design and Methods:

The Roche Diagnostics cobas b101 and Siemens Medial Solutions Diagnostics DCA Vantage report %A1c by latex agglutination inhibition immunoassay; and were evaluated using CLSI guidelines. Precision was assessed using low (~5 %HbA1c) and high (~9.5 %HbA1c) level QC material(s). Accuracy was determined with National Glycated Standards program (NGSP) reference material. Linearity was

determined by dilution of patient specimens. Patient comparisons (N = 20) were performed versus the Roche Tina-quant Hemoglobin A1cDx Gen.3 reference assay using the Roche c513, a high-volume instrument. $p < 0.05$ was considered significant.

Results:

Within-run and between-run precision spanning low-high QC material for the b101 was 1.8-2.4 %CV and 3.2-4.3 %CV, respectively. Likewise, within-run and between-run precision of the DCA Vantage was 1.4-7.3 %CV and 1.9-3.8 %CV, respectively. The mean absolute bias versus NGSP material was -1.0 and +0.1 %HbA1c, for the b101 and DCA Vantage, respectively. The linear range of b101 and DCA Vantage was 4.2-12.4 and 3.9-13.3 %HbA1c, respectively ($p < 0.05$). Patient samples correlated well among assays ($p < 0.05$). However, the b101 and the DCA Vantage were negatively biased versus c513 (-0.6 and -0.2 %HbA1c, respectively), especially at >10 %HbA1c.

Conclusion:

Chiefly, laboratories must communicate and consider clinically relevant analytical discrepancies among POC %HbA1c assays and reference assays in hub

P112

The relationship between transcutaneous and serum bilirubin is affected by skin tone

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Objectives: Neonates are susceptible to hyperbilirubinemia and jaundice that can precede life-threatening encephalopathy. Fortunately, pathological jaundice is preventable by phototherapy. The newborn screening program in our healthcare region monitors for hyperbilirubinemia by measuring total serum bilirubin (TSB) or transcutaneous bilirubin (TcB). TcB measurement is interpreted using nomograms to direct high risk neonates towards follow-up TSB measurement and phototherapy. This study aimed to determine if there is an effect of skin tone on the relationship between TcB and TSB in neonates.

Design and Methods: Data was collected between 2014-2018 and included an objective skin tone assessment (i.e. light, medium, dark), and concurrent TcB and TSB measurements. TcB was measured by public health nurses using Drager JM-105/JM-103 meters, and TSB was measured by chemistry analyzer. Skin tone assessment was performed using standard makeup swatches. The TcB and TSB relationship was described by linear regression and bias plots.

Results: In neonates of light (n = 1424) or medium (N = 1156) skin tone, TcB values were positively biased versus TSB results until the measured TSB was greater than 256 (95% CI: 253-259) or 270 umol/L (95% CI: 268-273), respectively. TcB values from dark skin-toned neonates (n = 359) demonstrated a robust positive bias versus TSB measurements until the measured TSB was greater than 317 umol/L (95% CI: 305-333). The median absolute and relative biases' were distinct among light (+9 umol/L, 4%), medium (+21 umol/L, 10%), and dark (+57 umol/L, 26%) skin tones ($P < 0.05$).

Conclusion: TcB meters demonstrate differential bias based on skin tone. Specific nomograms may be required for multicultural populations.

P113

A mathematical approach to assess feasibility of common reference intervals across analytical platforms: Evidence from CALIPER pediatric reference intervals database

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Objectives: Clinical decision-making relies on accurately established reference intervals (RIs), which have been traditionally lacking in the pediatric population. By developing pediatric RIs, the Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER) project has addressed this gap. The aim of the current project was to develop and apply a mathematical approach to assess the appropriateness of common pediatric RIs across multiple analytical platforms.

Design and Methods: RIs from the CALIPER database for 33 chemistry assays were compared across 7 analytical platforms (Abbott ARCHITECT, Beckman DxC, Beckman AU, Roche Cobas, Roche Modular, Siemens Vista, and Ortho Vitros). Acceptable bias between instruments was specified as half the total allowable error as indicated by Institute of Quality Management in Healthcare. Instrument-specific RIs were compared by assessing the difference between the lowest and highest instrument limit for both lower and upper reference limits. Harmonization was recommended when there was acceptable bias.

Results: Twenty-two analytes (ALT-ACT, ALP, apoA1, apoB, AST, AST-ACT, C3, C4, cholesterol, cholinesterase, GGT, HDL-cholesterol, IgG, IgM, lipase, phosphate, prealbumin, total protein, transferrin, triglyceride, urea and uric acid) exhibited significant bias across analytical platforms, suggesting the need for platform-specific RIs. The remaining eleven analytes demonstrated acceptable bias, resulting in a recommendation one common RIs (albumin-G, albumin-P, ALT, direct bilirubin, calcium, creatinine, haptoglobin, IgA, iron, magnesium, and total bilirubin).

Conclusions: This is the first study to compare pediatric RIs across analytical platforms and propose common RIs which can lead to consistent result interpretation.

P114

Finding the 'right size' with Best Practices in Medicine (BPiM): An audit and feedback approach to address over and under laboratory test utilization

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Objectives: Laboratory test underutilization and overutilization both contribute to serious healthcare outcomes. To address this, the BPiM project focuses on exploring utilization rates of laboratory testing to 'right size' test ordering by increasing or decreasing as appropriate.

Design and Methods: Test ordering data for thyroid stimulating hormone (TSH) was retrospectively collected over a 3-month period. Data collection, analysis and output was automated using Python computer programming. Personalized scorecards were subsequently distributed to staff practitioners across 13 divisions in the Department of Medicine. Scorecards included information on: total test orders, cost, abnormal results, and repeat tests within 90 days. Practitioners were invited to complete an online learning activity. This educational intervention consisted of a test guideline refresher in conjunction with an interactive self-reflective survey. Subsequently, test ordering data was prospectively collected over the same time period, and new scorecards were distributed.

Results: After the first TSH audit cycle, there was a 30% response rate for educational activities and a decreasing trend in total test orders (n = 2707 vs 2440; $p = 0.07$). A significant increase in percent abnormal test results (17% vs 37%; $p < 0.001$) supported a change towards appropriate test ordering practices. A decrease in 90 day repeat test results was observed with the internal medicine practitioners (average n = 3.4 vs 1.5; $p = 0.01$). Scorecards for 25-hydroxyvitamin D and rheumatoid factor will be assessed in early 2019.

Conclusions: Right sizing laboratory test utilization with a multilevel, interdisciplinary, continuing professional development framework is an effective approach to implementing best practices in test ordering.

P115

Fecal calprotectin: evaluating its importance in monitoring Inflammatory Bowel Disease

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Objectives: Investigate if fecal calprotectin (FC) levels exhibit a concentration-dependent relationship to inflammatory bowel disease (IBD) and compared to traditional biomarkers (CRP/leucocytes).

Endoscopy, the current gold standard for characterizing IBD, directly assesses ongoing mucosal inflammation. This procedure is expensive, invasive and associated with significant patient discomfort and risk of patient harm. Currently used laboratory tests (e.g. total leucocyte count and C reactive protein (CRP)) are objective but non-specific markers for IBD. Calprotectin (50mg/kg cutoff), a Ca^{2+}/Zn^{2+}-binding protein produced mainly by neutrophils, represents a sensitive/specific marker of IBD.

Design and Methods: 26 Crohn's and Ulcerative Colitis patients were recruited following approval from the Hamilton Integrated Research Ethics Board. Heparin and EDTA tubes were utilized for CRP and leucocyte measurements respectively. FC was measured using ELISA principle. Biopsies taken throughout bowel locations were performed within 3 weeks of blood-work and each site was assigned either inflamed/non-inflamed site status. Data analyzed using Excel™(2016) and Prism™(2010) software (ANOVA*$p < 0.01$).

Results: We grouped patients according to inflamed sites observed during biopsy (0, 1-2, 3-5 and >5 sites). We observed a significant concentration-dependent rise in FC measurements (expressed mg/kg) in each group compared to 0 site (89,$p < 0.01$). Respective concentrations and significance: group 1-2 (607 ± 18.7p< 0.01), group 3-5 (1391 ± 207,$p < 0.001$) and group >5 (1664 ± 433,$p < 0.001$).

Conclusions:

FC had a concentration-dependent relationship with biopsy results for IBD and considerably better clinical utility when compared to traditional biochemical and cellular markers of IBD in blood. In most patients with active IBD, CRP was higher than 0 site group but exhibited no concentration-dependent relationship.

P116

Evaluation of cyclosporine, tacrolimus and sirolimus immunoassays on Roche Cobas e801 analyzers

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Objectives: In this study, performance of cyclosporine, tacrolimus and sirolimus immunoassays on Roche cobas e801 analyzer and Abbot Architect was compared with an LC-MSMS assay by measuring precision and patient comparison.

Design and Methods: De-identified residual whole blood EDTA specimens were used (cyclosporine (n = 55); tacrolimus (n = 30); sirolimus (n = 30)). The concentrations of each immunosuppressant were determined by (i) LC-MS/MS method (cyclosporine: AB SCIEX Triple Quad 3000 with Agilent 1100 LC; tacrolimus & sirolimus: Agilent LC1100 with 6410B MS) developed in the Calgary Laboratory Services. (ii) Elecsys assay on Cobas® e801 immunoassay analyzer. (iii) Abbot Architect chemiluminescent micoparticle immunoanalyzer (CMIA) for tacrolimus and sirolimus (iv) CEDIA cyclosporine Plus assay reagent kit from Thermo Fisher Scientific on a Cobas 601 analyzer for cyclosporine. Precision was determined with QC fluids. Data was analyzed by Analyse-it.

Results: Between-day precision goals met manufacturer specifications for each assay. The following Passing Bablok regression equations describe the relationship between different methods. Cyclosporine: Elecsys Cobas 801 = 1.373 + 1.015 LC-MS/MS(r = 0.961); CEDIA Cobas 601 = -11.33 + 1.084 LC-MS/MS (r = 0.931); Elecsys Cobas 801 = 9.882 + 0.9314 CEDIA Cobas 601 (r = 0.942); Tacrolimus: Elecsys Cobas 801 = -0.2133 + 1.063 LC-MS/MS (r = 0.994); Architect = -0.15 + 1.083 LC-MS/MS (r = 0.992);Elecsys cobas 801 = -0.09791 + 0.9791 Architect (r = 0.990); Sirolimus:Architect = 0.4099 + 1.288 LC-MS/MS (r = 0.974); Cobas 801 = 1.101 + 1.136 LC-MS/MS;Cobas 801 = 0.892 + 0.8426 Architect (r = 0.987).

Conclusions: The cyclosporine and sirolimus immunoassays had positive systematic biases compared to LC-MS/MS that were reduced on the e801 analyzer from the Passing Bablok regression analysis. The Elecsys tacrolimus assay on Cobas 801 had good agreement with both LC-MS/MS and Architect methods.

P117

Paediatric reference intervals for 17 Roche Cobas 8000 e602 immunoassays in the CALIPER cohort of healthy children and adolescents

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Background: The diagnostic utility of laboratory tests in paediatric medicine relies heavily on the availability of appropriate reference intervals (RIs). The Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER) has established a comprehensive database of covariate-stratified RIs for many laboratory tests using a large, healthy cohort. Here, we extend the testing to Roche immunoassays and report, for the first time, comprehensive paediatric RIs for 17 endocrine and special chemistry markers.

Design and Methods: A total of 601 healthy children and adolescents (1 day-<19 years) with no history of chronic illness and/or pregnancy were recruited and serum samples analyzed for 17 immunoassays on the Roche cobas 8000 e602 Immunoassay Analyzer. Age and sex-specific trends were assessed via Harris & Boyd and outliers were identified using the Tukey or adjusted Tukey method for Gaussian and non-Gaussian distributions, respectively. RIs and corresponding 90% confidence intervals were calculated according to Clinical and Laboratory Standards Institute guidelines.

Results: Reference values for all analytes measured required age partitioning, particularly during early life and throughout adolescence. Of the 17 analytes measured, 8 required sex partitioning, including ferritin, TSH, TT3 and all fertility/sex hormones, except prolactin.

Conclusion: This is the first study to determine robust paediatric RIs for Roche immunoassays. RIs were similar to those published by CALIPER on other analytical platforms, highlighting the reproducibility of age- and sex-specific trends observed across the paediatric age range. The RIs established in this study will improve the accuracy of test result interpretation and clinical decision-making in laboratories utilizing Roche immunoassays.

P118

Analytical evaluation and human factors assessment of two point of care hemoglobin A1c devices

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Objectives: Point of care (POC) Hemoglobin A1c (HbA1c) testing has been shown to lead to improved glycemic control in diabetic patients. This study evaluated the analytical performance and human factors (HF) features of two POC HbA1c systems: Roche cobas b101 and the new Alere Afinion2.

Design and Methods: Precision and accuracy were assessed using

remnant EDTA whole blood patient samples and two different reagent lots for each POC system. Repeatability consisted of 20 replicates in a single run. Within-laboratory precision consisted of 5 replicates per run, 1 run per day, for 5 days. Split-sample analysis (n = 40) compared the POC systems against a Roche Tina-quant Gen 3 central laboratory assay. A mixed methods HF assessment evaluated performance factors pertaining to device usability, interface and system design.

Results: Repeatability for both systems was < 2.0% at both concentrations tested (6.6% and 8.5%). Within-laboratory precision for the Afinion was < 2.0% at concentrations of 6.6% and 8.3%. Within-laboratory precision for the cobas was 2.5% at a concentration of 6.6%, and < 2% at a concentration of 8.3%. Split sample analysis revealed a bias of -0.5% between each POC method and the Roche central laboratory assay. Bias between the two Afinion lots was -0.04%; bias between the two cobas lots was 0.6%. The cobas device screen rated higher in the HF assessment; the Afinion had more user-friendly reagent cartridges and shorter testing times.

Conclusions: The Afinion POC HbA1c system showed better analytical performance than the cobas system. Each system had its own set of commendable HF features.

P119

Prevalence of confirmed human anti-animal antibody interference in the Beckman Coulter, Inc. (BCI) Access® luteinizing hormone (LH) assay

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Objectives: Luteinizing hormone (LH) measurement plays an important role in evaluating reproductive function in adults and in diagnosing early/delayed puberty in children. LH measurement by immunoassay is inherently subject to human anti-animal antibody interference (HAAA) which can lead to erroneous results and ultimately impact clinical decision-making. The objective of this study was to determine the prevalence and clinical impact of HAAA when using the BCI Access® LH assay.

Design and Methods: Serum samples collected for physician-ordered LH measurement over a 5-year period (December 2013- May 2018; n = 12,016) were analyzed using both the BCI Access® and Roche Elecsys® LH assays. Samples with possible HAAAs were identified when LH values differed by > 30% between methods. Possible HAAA samples were re-assayed on the BCI Access® LH platform following treatment with a heterophile blocking reagent (Scantibodies HBR); a decrease of > 30% confirmed the presence of an HAAA. Clinical misclassification was assessed by comparing the BCI Access® and Roche Elecsys® results according to age- and sex-specific reference intervals.

Results: HAAA was confirmed in 3.3% of patient samples (n = 405). The prevalence of HAAA was 1.9% (n = 186/9,699) in adults and 9.1% (n = 219/2,407) in the pediatric population. In specimens with confirmed HAAA, 56% (n = 225/405) of all patients would have had a change in clinical classification due to HAAA.

Conclusions: The prevalence of HAAA in BCI Access® LH assay in our patient population was 3.3%. Over half of the HAAA cases could have been clinically misclassified due to the erroneous results.

P120

Method development and validation of tranexamic acid by LC-MS/MS

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Objectives: Tranexamic acid (TxA) is a drug used during cardiac surgery to prevent blood losses. Dosing and i.v. administration practices are not standardized. The plasma levels 10-100 μg/mL are reported to be in the therapeutic range and higher levels are linked to increased incidence of adverse effects, most notably seizures with increased mortality. The aim of this study was to develop, optimize and validate an in-house liquid chromatography – tandem mass spectrometry (LC-MS/MS) method for TxA measurement in serum.

Design and Methods: The analytical method development was carried out in several steps including sample preparation, and optimization of chromatography and tandem mass spectrometry parameters. Method validation including day-to-day precision with 4 QC levels, limit of detection, sample stability, carryover, and concentration-signal linearity was carried out.

Results: A fast, simple and efficient LC-MS/MS method for analysis of TxA is serum was developed. The run time was 7 minutes with the total time of one hour needed for sample preparation and analysis on LC-MS/MS. The method precision was acceptable (%CVs = 10.5-12.6%) with no sample carryover observed. The samples were stable in refrigerator for up to 7 days. The method was linear in the concentration range 1-1000 μg/mL. The matrix effect on the analytical sensitivity was negligible and the lower limit of detection was 0.5 μg/mL.

Conclusions: Rapid and simple LC-MS/MS method for analysis of tranexamic acid was developed and validated. Different chromatography and mass spectrometry combinations needed to be assessed before method was optimized.

P121

Harmonized lipid reporting across Canada: Current variability and proposed harmonized lipid report

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Objectives: Despite published Canadian Cardiovascular Society (CCS) guidelines for dyslipidemia management, lipid reporting across Canadian laboratories remains highly variable. The CSCC Harmonized Reference Interval (hRI) Working Group aims to harmonize laboratory test reporting, interpretation, and support clinical implementation. This study assessed current lipid reporting practices and proposed common adult and pediatric lipid reports.

Design and Methods: The hRI Working Group disseminated a survey in November 2018 to the Canadian laboratory community to assess adult and pediatric lipid reporting. A common adult lipid report was proposed based primarily on the 2016 CCS guidelines. Pediatric lipid report recommendations were based primarily on decision limits derived from Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) reference data.

Results: The survey showed variability in adult lipid interpretative comments and the referenced source of decision limits (most prevalent: 50.0% published guidelines, 14.6% manufacturer package insert, 6.3% historical). 38% respondents included a reference to the 2016 CCS Guidelines in interpretative comments. Decision limits for pediatric lipid reporting exhibited substantial variation (e.g. LDL-C upper decision limit range: 2.7-4.5 mmol/L, CV: 15.6%). 50% respondents reported they were interested in implementing a harmonized lipid report. Common adult and pediatric lipid reports were subsequently proposed, including interpretive comments and flagging limits for total cholesterol, LDL-C, HDL-C, triglycerides, non-HDL-C and apoB.

Conclusions: Assessment of current lipid reporting practices supports the need for harmonized lipid reporting. Proposed common adult and pediatric lipid reports align with current clinical recommendations for dyslipidemia. Harmonized lipid reporting aims to promote laboratory harmonization and improve patient care.

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Storage stability of serum angiotensin converting enzyme in refrigerated and frozen conditions

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Objective: Pre-analytical factors such as sample storage and transport may lead to an elevation in serum angiotensin converting enzyme (ACE), leading to misdiagnosis and mismanagement of patient with sarcoidosis. Manufacturer's recommendations for ACE storage condition is vague (i.e. reportedly stable for at least 1-week refrigerated and several months frozen) with no recent supporting studies. This study defines the acceptable stability for ACE by investigating the appropriate storage temperature and duration.

Design and methods: Serum ACE levels were analyzed from 20 healthy and 47 patient samples using Trinity Biotech reagent on Roche Cobas 501. For refrigerated conditions, sample were tested up to 2 weeks at 4oC. For frozen conditions, aliquots were measured up to 8 weeks at -20°C. A freeze/thaw sub-study was performed at the 1-week period. Clinical significance was determined by comparing the bias with the total allowable error goals of 18% (biological variation). Statistical significance was determined using repeated measure ANOVA on ranks.

Results: ACE levels was 39.6 ± 12.9 IU/L (mean ± standard deviation) for healthy volunteers and 49.6 ± 42.5 IU/L for patients (RI: 13-57 IU/L). The bias was <6% up to 2 weeks at 4°C. The biases were 10-12% for 1, 2, and 4 weeks frozen, while the bias was 21% at 8 weeks frozen. One freeze/thaw cycle at 1 week resulted in a 6% average bias.

Conclusions: We have demonstrated storage of serum ACE is acceptable for 2 weeks at 4°C and up to 4 weeks at -20°C. Our findings are significantly different from the package inserts and suggest the need to verify manufacturer's information.

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Recruitment for pregnancy reference intervals for safe medicine (PRISM)

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Objectives: This study trialed a methodology for the creation of laboratory reference intervals for healthy pregnant women representative of the BC population. Recruitment, consenting and blood sampling were piloted and success monitored at the labour/delivery timepoint.

Design and Methods: Women were consented before hospital admission, on-site during early labour, or prior to C-section by study personnel or nurses. Eligibility was assessed using an intake questionnaire, chart review and a post-partum survey following hospital discharge. Recruitment tracking occurred through weekly and monthly recruitment and eligibility reports and process adjustments. Descriptive statistics were used to summarize cohort characteristics to assess similarity between the study cohort and BC population and identify recruitment and sampling challenges.

Results: Over 12 months, 408 women consented to participation. Blood was obtained from 94%; 204 samples prior to labour/C-section, 179 samples from labouring mothers. Up to 15% of samples experienced pre-analytical issues (ex. hemolysis, underfilling, mislabelling). Of all consented women, 151 were excluded based on health history, delivery or post-partum complications. Information for 55 mothers remains missing/pending. The final cohort includes 120 pre-labour and 81 in-labour women. Average maternal age was 33.3 yrs (pre-labour group) and 31.8 yrs (in-labour group). Average gestational age was similar in both groups (39 weeks).

Conclusions: PRISM has demonstrated strategies for successful and rapid recruitment of pregnant women for RI studies. Future work to improve participant pre-screening and quality of collected samples may decrease exclusion rates. The current strategy can be applied to RI studies at different time-points in pregnancy.

P124

Anti-Nuclear Antibody Test Utilization Patterns in Alberta

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Background/Objective: Antinuclear antibody tests (ANA) are ordered on patients suspected of an autoimmune disease (AD). Restricting ANA to patients with high pretest probability could reduce test volumes, increase positivity rates and appropriate specialist consultations, but may miss/delay diagnoses of evolving AD. To develop more effective strategies/policies, we assessed ANA utilization patterns in Alberta.

Design/Methods: ANAs were performed in five laboratories employing computer-aided or manual indirect immunofluorescence microscopy, or solid phase addressable laser bead immunoassay. Data was retrospectively obtained from three laboratory information systems.

Results: Approximately, 1/40 Albertans had an ANA done at annual costs approaching $900,000. This rate is inconsistent with the incidence of AD in Alberta (2.6/1000). Most ANA were ordered by community physicians (76%) as compared to rheumatologists (6%) and other specialists (25%), which indicates widespread use. Increase in ANA positivity may be attributed to gradual expansion of the International Consensus of ANA Patterns at one laboratory. Furthermore, 6% of patients had a repeat ANA within 6 months.

Conclusions: There appears to be overutilization of ANA in Alberta which may lead to unnecessary expenditures and referrals. These findings will lead to specific ANA ordering indications, while observing downstream consequences of costs and impact on detection of AD.

Antinuclear antibody (ANA) utilization in Alberta (2014-2017)

	2014	2015	2016	2017
Total ANA tests	92,118	94,681	92,183	92,185
ANA positivity rate	13.3	14.5	17.0	21.9
ANA per 1000 population	24	25	24	24
Cost ($ Canadian)	896,893	920,939	899,150	892,118

P125

Enzyme-free and wash-free localized isothermal signal amplification for cell surface imaging

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Objectives: Signal amplification often requires enzymes and wash steps. Enzymes can limit reaction temperature, restrict reagent storage conditions, and increase cost. Wash steps are laborious and complicates technical procedures. We introduce our technique for localized detection at room temperature without wash steps or enzymes.

Design and Methods: We designed inert DNA oligonucleotides consisting of four hairpins and two strands conjugated to polyclonal antibodies. In the presence of a specific cell surface protein, the antibodies bind to adjacent proteins and bring the conjugated strands together to form an initiating complex. This complex initiates the hybridization of each hairpin to form a long, nicked DNA polynucleotide anchored to the cell surface protein. Fluorophores retained within the polynucleotide provide amplified, localized fluorescence. After optimization of our technique, we applied our technique to the detection of HER2 cell surface protein on live HER2+ cells. Cells were seeded onto glass chambers and imaged after adding 100 μL of our DNA oligonucleotide mix solution.

Results: Localized fluorescence was detected on SK-BR-3 (HER2+) in 30 minutes at room temperature (Fig 1) and not on MDA-MB-231 (HER2-).

Conclusions: Our technique may be a simple alternative to immunohistochemical assays.

P126

Investigating potential interferences in pediatric creatinine measurements methodologies amongst pediatric renal transplant recipients

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Objectives: Both Jaffe and enzymatic methods are routinely used to measure creatinine. The Jaffe method is favoured for its economy but is more susceptible to interferences. Small changes in creatinine in pediatric populations, especially post-transplant, can have important consequences. An increase of more than 10% from the baseline would trigger an investigation of renal function and potentially a biopsy of the transplanted kidney. Anecdotal evidence suggests an unknown interferent is present in plasma of post-renal transplant patients, when examined by the Jaffe method, but not using the enzymatic method. Our objective was to document this interference and determine if the immunosuppressive drug, tacrolimus, is responsible for this difference between the two methods.

Design and Methods: A retrospective chart review study was done for 23 pediatric patients (3-18 years old) post renal transplantation. Patients' demographics and laboratory results were collected from August 2017 to February 2019.

Results: Difference between Jaffe and enzymatic method for measuring creatinine in our population exists with intra-individual differences that range from 0 to 54%. Largest differences were noted in patients who are less than 5 years old (n = 4). Jaffe method yielded higher measurements than the enzymatic method in all cases. We were unable to demonstrate a correlation between tacrolimus levels and the difference between Jaffe and enzymatic in this study (n = 231, $r^2 = 0.15$, Spearman's test).

Conclusions: Using the Jaffe method for creatinine measurements resulted in higher values compared to the enzymatic method in post renal transplant patients. It does not appear to be attributable to tacrolimus use.

P127

Reference interval harmonization across Canada: An indirect data mining approach to analyze large inter-provincial data

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Objectives: Reference interval harmonization across Canadian clinical laboratories is an important step towards improved clinical decision, as they support accurate and consistent test result interpretation. The CSCC Harmonized Reference Interval (hRI) Working Group aims to assess the feasibility of developing common RIs through a combination of direct analysis of reference data from healthy Canadians and indirect analysis of outpatient data from across Canada. This study assessed inter-provincial data for electrolytes through an indirect data mining approach to provide evidence supporting Canada-wide harmonization.

Design and Methods: Anonymized data were collected by LifeLabs (Ontario and British Columbia, Roche Cobas) as well as DynaLIFE (Alberta, Siemens ADVIA) from January-December 2017. Provincial, age and sex differences were assessed using the Harris & Boyd method and appropriate partitions were established. Outliers were identified (Tukey method), and monthly instability was assessed using a generalized additive model. Provincial and Canada-wide indirect RIs were estimated using the Arzideh method (CCLM 2007;45,1043-1057) and compared to direct reference intervals established from the Canadian Health Measures Survey (CHMS)(Clin Chem 2015;61,1049-1062).

Results: Estimated Canada-wide indirect RIs were generally consistent with direct RIs previously published from CHMS on healthy Canadians and those reported by other international initiatives (e.g. AHRIA, NORIP). Provincial partitioning was not required for most electrolytes.

Conclusions: This study demonstrated minimal regional and instrument differences across analytes and supports the development and use of hRIs for electrolytes across Canada. It remains important to assess additional analytes using both direct and indirect approaches, and to develop implementation support tools.

P128

Analytical performance of the Beckman Coulter high-sensitivity troponin (hsTnI) assay in Barricor plasma: a multi-instrument and cross-laboratory validation

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Objectives: To evaluate the recent Health Canada approved Beckman Coulter Access hsTnI assay to determine 1) assay robustness across multiple instruments/ laboratories, 2) analytical performance characteristics, and 3) equivalence to other hsT methods.

Design and Methods: Lithium-heparin Barricor plasma was used to assess precision, linearity, sensitivity, and comparability to conventional AccuTnI+3 and other hsTn assays. Studies were conducted at 3 laboratories using two DxI800s and one Access2.

Results: The LOBs and LODs were lower on the DxI800 at 0.27 and 0.90ng/L, compared to 2.9 and 3.2ng/L on Access2. Both showed excellent linearity across the measuring range. Total precision was slightly better on Access2 with CVs of 6.1% at 3.6 ng/L, 7.6% at 9.3, and 3.3% at 40.6, compared to DxI800 of 9.6% at 5.1, 7.3% at 12, and 3.3% at 44.9. Within-run precision on DxI800 was 4.7% at 5ng/L, 3.9% at 11.5, and 3.6% at 45.6. Good agreement was found in method comparison to AccuTnI+3 across all laboratories (slope = 0.966, r = 0.993, n = 305). However, Access2 showed a -18% bias versus DxI800 that was most significant at concentrations <150 ng/L with worse agreement <40ng/L (slope = 0.8732, r = 0.863, n = 185) on both instruments. At values <150ng/L, there was good agreement with

Abbott hsTnI (slope = 1.017, r = 0.932, n = 96), but poor agreement with the Roche hsTnT assay (slope = 1.687, r = 0.589, n = 56).

Conclusions: The Beckman hsTnI assay showed robust analytical performance across different laboratories/instruments. Excellent precision and sensitivity make it suitable for rapid clinical pathways. However, caution should be exercised before utilizing identical clinical parameters on both platforms.

P129

Expanding the reporting range of ferritin to facilitate the acute diagnosis of hemophagocytic lymphohistiocytosis: How high can we go?

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Objectives: Hemophagocytic lymphohistiocytosis (HLH) is characterized by an aggressive activation of the immune system that can lead to widespread tissue damage. Ferritin concentrations >10,000μg/L occur almost exclusively in HLH, therefore the ability to report ferritin >10,000μg/L is important in its diagnosis. In our laboratory, a recent switch to the Roche Diagnostics Tina-quant Ferritin Gen.4 reagent resulted in a decrease in the clinical reportable range (CRR) from 100,000μg/L to 8,000μg/L. This study aimed to support the acute diagnosis of HLH by expanding the CRR of our current assay via dilution verification.

Design and Methods: Discarded patient plasma samples ($N = 13$) with ferritin concentrations near the upper limit of the analytical measuring range (1,000ug/L) were identified. Manual and on-board dilutions were performed using the current assay's diluent (9% NaCl), and manual dilutions were performed with the previous assay's diluent (Roche Diluent Universal). Results were analyzed using bias plots and assessed for significance using the Student's t-test ($p < 0.05$).

Results: On-board dilutions with NaCl produced less variability and significantly smaller deviation from the original results when compared with manual dilutions ($p < 0.016$). Using the 50-fold on-board dilution (maximum on-board dilution) or the 100-fold manual dilution with NaCl, 95 and 85% of results were within the target total allowable error of the assay, respectively. A new reporting workflow was therefore developed.

Conclusions: The CRR of the Tina-quant Ferritin Gen.4 reagent can be increased to 50,000ug/L with a 50-fold on-board dilution and results between 50,000 and 100,000ug/L may be reported when clinically requested. This wider, analytically acceptable CRR has improved patient care.

P130

Pre-analytical contamination of pediatric urine drug screening samples with tributoxyethylphosphate (TBEP)

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Objectives: At our laboratory, pediatric urine samples for drug testing undergo untargeted screening by gas chromatography-mass spectrometry (GC-MS). Recently, three pediatric urine samples collected in-hospital were found to contain a large peak identified by our GC-MS library as tributoxyethylphosphate (TBEP). We therefore investigated the most common pediatric urine collection modes to determine whether we could identify the source of TBEP.

Design and Methods: Sterile pediatric and newborn urine collection bags, diapers, sterile cotton balls, and a sterile urine collection container were obtained from one of the units where TBEP-containing samples were collected. Patient sample collection was simulated using clinical laboratory reagent water (CLRW) by washing the urine collection bags and container, by aspirating CLRW from the diaper, or by washing the cotton balls and squeezing the CLRW out. Samples were extracted and analyzed using an Agilent 6890 GC coupled to a 5973 Mass Selective Detector using our patient sample protocol.

Results: Tributoxyethylphosphate was detected only in water squeezed from the cotton balls. Recently, we discovered that the supplier of sterile cotton balls to hospitals in our city had switched approximately 3 months previously. Repeating the extraction using samples from both previous and current sterile cotton balls confirmed that TBEP was only in cotton balls from the new supplier.

Conclusions: Using GC-MS analysis, we were able to identify the cotton balls used to collect pediatric and newborn urine samples as the source of TBEP, highlighting the importance of pre-analytical variables and their potential impact on comprehensive drug screening results.

P131

Detection of cannabinoids in dried blood spots by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

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Objectives: Dried blood spots (DBS) have become a specimen of interest for toxicology testing as they are an ideal specimen type for out-of-lab collection. With the legalization of marijuana, there is interest in monitoring cannabinoids in blood to correlate with degree of impairment. The objective of this study is to develop a method to detect cannabinoids and their metabolites in DBS.

Design and Methods: Spiked blood spots containing the target compounds tetrahydrocannabinol, 11-nor-9-carboxy-THC, 11-hydroxy-THC, cannabidiol, cannabinol, and tetrahydrocannabivarin were extracted with 10uL (1ug/mL) deuterated internal standards (Cerilliant) and 990uL methanol. Extracts were dried and the residue re-dissolved. Cannabinoids were detected using an ultra-high performance liquid chromatography system (Waters Acquity UPLC) coupled to a triple quadrupole mass spectrometer (Waters Xevo-TQ-XS) with an injection volume of 30uL. Reversed-phase chromatography was performed with a biphenyl column (2.7um particles, 100 x 2.1mm) with gradient elution using Mobile A (5mmol/L ammonium formate, pH 3.0) and Mobile B (0.1% formic acid in acetonitrile) at a flow rate of 0.350 mL/min. Analytes were detected using multiple reaction monitoring (MRM) with two MRM transitions per analyte via polarity switching electrospray ionization.

Results: All target compounds were selectively detected and separated over 9 mins. The isobars cannabidiol and tetrahydrocannabinol were monitored using two identical MRM transitions and the compounds distinguished by retention time. Within-run precision of QC material gave CVs of <20% for all analytes except 11-nor-9-carboxy-THC and tetrahydrocannabivarin. All analytes were linear up to 400ng/mL whole blood.

Conclusions: Our preliminary results show that DBS can be used to detect several cannabinoids and metabolites simultaneously.

P132

Influence of ethnicity on laboratory reference intervals: Evidence from the CALIPER cohort of healthy children and adolescents

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Objectives: Accurately established pediatric reference intervals (RIs) are essential for correct laboratory test interpretation and clinical decision-making. While studies have reported ethnic differences, many findings are conflicting and there is a lack of robust evidence to determine the need for partitioning by ethnicity. The objective of this study was to assess the influence of ethnicity on biochemical markers in the Canadian Laboratory Initiative in Pediatric Reference Intervals (CALIPER) cohort.

Design and Methods: A total of 52 biomarkers were measured in serum samples from the CALIPER cohort of healthy children and adolescents (5-<19 years). Biomarker concentrations were compared between four major Canadian ethnic groups (i.e. Black, Caucasian, East Asian, and South Asian, determined based on identical parental ethnicity). Results were verified using an additional 500 healthy pediatric samples with equal sample size across ethnicities. Ethnic-specific RIs

were established for biomarkers demonstrating significant ethnic differences that exceeded biological and analytical variation (i.e. reference change value).

Results: Of 52 biomarkers, 18 demonstrated large statistically significant ethnic differences. These results were verified in a second study for 7 of the 18 biomarkers including 25(OH) vitamin D, amylase, ferritin, follicle-stimulating hormone (FSH), immunoglobulin A (IgA), IgG, and IgM. Ethnic-specific RIs were established for these seven biomarkers.

Conclusions: The majority of biomarkers did not differ between four ethnically diverse pediatric groups, ruling out ethnicity as a major covariate for most routine chemistries or immunoassays. However, reference interval partitioning was necessary for 7 biochemical markers showing clear ethnic-specific differences.

P133

Validation and evaluation of infliximab assay in IBD

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Objectives: Inflammatory bowel disease (IBD) is a group of intestinal inflammatory disorders and biological therapy such as monoclonal antibodies against TNF-α has revolutionized the therapeutic paradigms of IBD. One such drug is infliximab (IFX) which targets circulating and cell surface TNF-α. The measurement of IFX trough levels and anti-drug antibodies has been utilized to evaluate clinical efficacy, safety and cost of the drug. The aim of this study was to validate an assay kit for IFX measurement in IBD patients.

Design and Methods: RIDASCREEN® (R-Biopharm AG) IFX ELISA kit was validated for precision, linearity, limit of detection (LoD) and compared with IDKmonitor® (Immunodiagnostik) and LISA TRACKER (Theradiag) ELISA kits. For method comparison, twenty-one IBD patients on IFX were recruited and trough serum samples were collected. Serum was stored at -20°C until use.

Results: RIDASCREEN® assay showed excellent precision at mean concentrations of 6.2 μg/mL and 10.8 μg/mL with %CV of 5.8% and 5.5%, respectively. The assay was linear in the measuring range 0.5 – 12.0 μg/mL, with LoD being 0.5 μg/mL. The comparison showed good correlation with IDKmonitor® ($r = 0.7950$) and LISA TRACKER ($r = 0.9076$). Two comparison methods showed negative bias towards RIDASCREEN® assay with -34.90% (-2.60 μg/mL) for LISA TRACKER and -32.00% (-2.48 μg/mL) for IDKmonitor®.

Conclusions: The assay displayed acceptable analytical performance with good precision and linearity within the measurement range of the assay. This study further demonstrated that ELISA based IFX test is convenient and reliable method for detecting IFX concentrations at trough in IBD

P134

Evaluation of Ortho ALTV formulation on the Vitros 5600: Change to Caliper ALT reference ranges

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Objectives: Ortho's new formulation for alanine aminotransferase (ALT) testing, ALTV, was evaluated in this study. Noting both a negative bias on method comparison, as well as a significant difference in manufacturer-stated adult reference ranges, undertook transference of Caliper pediatric reference ranges for the new formulation.

Design and Methods: Evaluation of precision, accuracy, method comparison, linearity, and interference studies were carried out as recommended by CLSI guidelines. For reference interval transference, left-over specimens from select outpatient children were identified (only glucose ordered, glucose normal) and tested using ALTV and, in some cases, ALT formulations.

Results: Precision, accuracy, linearity, and interference studies demonstrated performance within acceptable limits. Over two unique ALTV lot numbers/calibrations, Passing-Bablock analysis generated an equation of line: $y = (-8.188) + 0.9788x$. Bland-Altman analysis revealed a mean negative bias of ALTV of -9.07% for values >50 U/L. However, for ALTV values ≤ 50 U/L, the mean negative bias was -41.51%, exceeding allowable difference. Using outpatient pediatric specimens (age 1 year and up) we observed successful transference of Caliper Ortho ALT reference ranges on this population ($n = 26$), compared these to current reference ranges, and demonstrated successful transference of reference ranges translated using the equation generated by the Passing-Bablock analysis ($n = 86$).

Conclusions: In our evaluation, the ALTV formulation necessitates a downward adjustment of adult and pediatric reference ranges. Although subject to further refinement, the Caliper ALT ranges successfully transferred when the upper limit was lowered to 35 U/L for ages 1-13, to 33 U/L for 14-19 year-old males, and to 31 U/L for 14-19 year-old females.

P135

Performance evaluation of EPOC for point-of-care testing of blood gases, electrolytes and 5 common chemistry analytes for use in hospital surgical and ICU units

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Objectives: To evaluate the performance of the EPOC Blood Analysis system against the Rapidpoint 500 Blood gas analyzer.

Design & Methods: Precision assessment was conducted on 2-3 levels of QC, 10 runs per level, for each analyte. Venous or arterial whole blood samples collected in balanced heparinized syringes were obtained from 38 patients (15 female, 23 male) predominantly (61%) from the surgical and ICU units. Method comparisons were performed (using Deming regression) for all analytes on the EPOC System.

Results: The EPOC system showed clinically acceptable precision for all analytes. Coefficients of variation at medical decision points included: pH 0.18%, PO2 9.04%, PCO2 1.97%, Calcium 2.47%, Chloride 1.55%, Potassium 1.06%, Sodium 0.48%, Lactate 1.14%, Glucose 1.39%, Creatinine 4.43% and hematocrit 1.74%. Our method comparison analysis is summarized in Table 1. Mean Biases for all the analytes were within total allowable error limits. High concordance (82-97%) based on reference intervals was observed between EPOC and Rapidpoint500, HemataSTAT-II and Sysmex XN-10 for the measurement of blood gases and electrolytes, Hematocrit and Total hemoglobin respectively. Creatinine showed a relatively lower concordance (79%) between the ARCHITECTc8000 and EPOC.

Conclusions: The EPOC system is fit for use in the surgical and ICU setting.

Table1: Method Comparison Experiment results

Analyte	Comparator Method	Slope (95% CI)	Intercept (95% CI)	Mean bias	Total Allowable Error
PCO2	Rapidpoint500	0.856 (0.789-0.924)	6.42 (2.47-10.38)	-1.44 (-2.67%)	5.7%
PO2	Rapidpoint500	1.052 (1.010-1.094)	-1.44 (-5.69-2.82)	2.08 (3.51%)	
pH	Rapidpoint500	0.920 (0.875-0.965)	0.59 (0.26-0.02)	0.009 (0.12%)	
Calcium	Rapidpoint500	1.168 (1.037-1.299)	-0.18 (-0.33--0.03)	0.009 (0.79%)	2%
Chloride	Rapidpoint500	1.129 (1.036-1.222)	-11.9 (-21.4--2.5)	1.2 (1.2%)	1.5%
Potassium	Rapidpoint500			-0.14 (-3.37%)	5.61%

		1.000 (0.951-1.050)	-0.14 (-0.35-0.07)		
Sodium	Rapidpoint500	1.015 (0.857-1.173)	1.48 (-23.22-20.26)	0.57 (0.41%)	0.73%
Lactate	Rapidpoint500	1.042 (0.992-1.091)	-0.33 (-0.51--0.16)	-0.21 (-7.88%)	30.4%
Glucose	Rapidpoint500	1.000 (0.976-1.025)	-0.28 (-0.53--0.03)	-0.28 (-3.22%)	8.87%
Creatinine	Architect c8000	0.974 (0.928-1.020)	12.0 (-0.2-24.2)	7.5 (4.2%)	8.87%
Hematocrit	HemataSTAT -II	1.083 (1.020-1.146)	-4.7 (-6.9--2.5)	-1.8(-5.8%)	3.97%
Total Hemoglobin	Sysmex XN-10	1.27 (1.039-1.215)	-12.0 (-21.5--2.6)	1 (1%)	4.19%

P137

Comparison of fractionated urinary catecholamines to urinary metanephrines in the diagnosis of pheochromocytoma

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Objectives: Pheochromocytoma is a catecholamine secreting tumor arising from the adrenal medulla that may cause lethal hypertension and is often diagnostically challenging. While literature suggests that urinary Catecholamines (UCats) or Metanephrines (UMets) are sufficient for initial screening, some studies have suggested preference for UMets leading to both tests being often ordered simultaneously, thus increasing unwarranted testing. The objective of this study was to perform an audit comparing UCats results to those of UMets in screening for pheochromocytoma.

Design and Methods: UCats results by LC-MS/MS were compared to UMets results (HPLC/ECD) over a two-year period. Only patients who had both tests simultaneously were included in this study (n = 109). We subsequently reviewed all clinical information relating to the diagnosis of pheochromocytoma, other diagnoses and medication history.

Results*:* Two out of 109 patients were confirmed as having a pheochromocytoma. One had elevated UCats and the other had elevated UMets; both at > 3x ULN. An additional 16 patients had adrenal or extra-adrenal lesions, but none were diagnosed with pheochromocytoma despite elevations of UCats or UMets that did not exceed 1.5x ULN. Another 45 patients had slightly elevated UCats that were medication related, stress related or both.

Conclusions: This study showed that either UCats or UMets suffice for cost-effective pheochromocytoma screening. However, to achieve 100% sensitivity, testing for both may be required where clinical suspicion is reasonably high. To avoid false positive UCats in patients who cannot discontinue certain medications, elevations ≥ 2.5x ULN should be sought for diagnosis.

P138

Optimization of serum indices' thresholds for 19 analytes on the Alinity chemistry analyser

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Objectives: The Alinity clinical chemistry analyser (Abbott® Laboratories) has been introduced as a next-generation *in vitro* diagnostics system. However, hemolysis, icterus, and lipemia (HIL) interference at thresholds initially defined by the manufacturer may hamper the accuracy of the results. We verified the performance of 19 assays in the presence of varying degrees of HIL and evaluated the results for acceptability.

Design and Methods: Total of 130 samples were prepared to evaluate 19 routine chemistry analytes in quintuplicates. Measurement were performed at baseline and with each known spiked concentration of hemolysate, intralipid and conjugated bilirubin. Serum indices were measured spectrophotometrically by the Alinity system's built in HIL methods using four wavelength pairs. For each analyte, average result recovery, relative to baseline measurements were determined with each interference concentration for evaluation of acceptability against the optimal allowable error, defined as 1.5x precision goal, set by the Institute for Quality Management in Healthcare.

Results: Most analytes met our criteria at manufacturer-defined thresholds of interferences. However, lower thresholds of hemolysis are required for ALT and creatinine (Jaffe method). When icteric above 68.4 mmol/L and 171 mmol/L, phosphate and creatinine had results beyond allowable error. At triglyceride concentration above 2.3 mmol/L, interference exceeded allowable error for total protein.

Conclusions: Verification of serum indices thresholds has enabled optimization and modification to accommodate acceptability criteria different from the manufacturer's. While we observed a general agreement between the manufacturer's claims and our results, lower thresholds of HIL indices may be required for ALT, creatinine, phosphate and total protein.

P139

An in vitro comparison of immunoassays for the monitoring of infliximab biosimilar drugs serum levels

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Objectives: To determine if immunoassays designed to measure serum infliximab (Remicade®, Janssen) levels can be used to measure serum levels of the infliximab biosimilars Renflexis® (Merck) and Inflectra® (Pfizer). To demonstrate common antigenic epitopes between biosimilars and infliximab.

Design and Methods: The ability of 4 different infliximab ELISA assays to measure the biosimilars was evaluated by assaying drug free serum spiked with drug at eight concentrations of 1.0 – 10.0 μg/ml. All samples were run in triplicate on each plate at 2 sites and the mean value assessed against the expected concentration using Deming regression analysis. Three levels of each drug were added to patient samples known to contain anti-infliximab antibodies (ATI) and recoveries calculated.

Results: Linearity was demonstrated across the assay range for all drugs/methods. Slopes vary by < 10% which is clinically insignificant. Recovery of drug from ATI serum produced equivalent results at comparable levels [SDs 2- 14%.]

Conclusions: These biosimilars can be monitored using ELISA assays designed to measure infliximab.

P140

Nova StatStrip glucose meters: Performance tracking over 3 years

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Objectives: Management of point-of-care (POC) programs involves reviewing performance of multiple devices remote from the central laboratory. While improvements in middleware facilitate transmission of quality control (QC) results and report generation, long-term assessment of instrument performance remains challenging. We present our solution for tracking Nova StatStrip glucose meters performance over three years.

Design and Methods: Our POC middleware provides monthly reports of the mean and percent coefficient-of-variation (%CV) for each of two QC levels and for each glucose meter. QC means are subtracted from the common QC target mean and plotted as bias by month (goal: approaching zero). QC %CV are subtracted from the target maximum %CV and difference plotted by month (if approaching zero or less than zero, performance is poor). New lot numbers, maintenance, other meter issues are noted.

Results: We present here three years of performance data tracked in

this manner for 46 unique glucose meters in use in our Health Centre. Considering all meters, some trends may be explained by seasonal variation (i.e. humidity/temperature), timing of distributing new lots, and education programs. Considering individual meters, we observed trends in training of new staff and targeted education appropriately. We also identified meters that, although not failing performance goals, were repeatedly poor performers and were suspended from use for further maintenance and troubleshooting.

Conclusions: Our verified Nova StatStrip glucose meters exhibited stable performance. The implemented tracking permitted early detection and intervention for meters in need of attention, as well as users in need of targeted education.

P141

Defining hemolysis interference thresholds for serum ionized calcium measurement at Alberta Public Laboratories in Calgary, Alberta

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Objectives: Ionized calcium (iCa) is the active form of blood calcium and is measured using blood gas analyzers. Hemolysis, which can occur during phlebotomy, dilutes and sequesters iCa. While blood gas analyzers do not assess hemolysis, laboratories could use a different analyzer to assess hemolysis and identify results for negative interference. We aimed to define and implement a hemolysis threshold for flagging iCa results.

Design and Methods: Ten serum pools were serially diluted with hemolysate, and tested for iCa, pCO_2, and pH using GEM 4000 analyzers. Hemolysis was assessed using the Roche hemolysis index (H-index) on a Cobas 6000 c501 analyzer. Average thresholds for an analytical interference (Total Allowable Error; 3SD of CAP peer group = -0.03 mmol/L), reference change value (-5.3%), and clinical interference (-0.1 mmol/L by clinician consultation) were calculated from the relationship between H-index and iCa for each serial dilution.

Results: The mean (SD) concentration of iCa in serum pools was 1.26 (0.07) mmol/L, which was reduced to 1.09 (0.06) mmol/L when the H-index was 2000 ($P < 0.05$). For an analytical interference (-0.03 mmol/L), the average predicted H-index was ~238 (52). For a reference change (-5.3%) the average predicted H-index was 599 (82). For a clinical interference (0.1 mmol/L), the average predicted H-index was 927 (160). Atmospheric exposure slightly increased pH and pCO2 ($p < 0.05$) over the course of the experiment, however iCa did not change ($p \geq 0.05$).

Conclusions Only an H-index of ~ 900 (gross hemolysis) led to a clinically significant interference in serum iCa measurement. This threshold was implemented by our laboratories.

P142

IgE monoclonal gammopathy uncovered by an integrated high sensitivity and interference free approach

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Objectives: To report and illustrate how IgE monoclonal gammopathy (MG), an extremely rare disease, challenges techniques and professional expertise.

Design and Methods: In a referring hospital, a 68 years old male diagnosed in 2015 having free kappa light chain disease was monitored with serum free light chain (FLCs; index varying from 1.69 to 3.88). Bone marrow FISH analysis (October 2018) showed 21% plasma cells and 17p13.1 deletion. On January 2019, a blind serum protein electrophoresis (SPE) request was sent to CHUM. CHUM analysis was done according to our MG algorithm based on high sensitivity techniques, reflex testing, antigen excess awareness along with step-by-step integrated professional interpretation.

Results: Monoclonal peak of 25.6 g/L measured in SPE; reflex immunofixation (IF): suggestive of free kappa light chain MG with final IF interpretation postponed, relying on reflex testing: FLCs (kappa = 15.0, lambda = 5.54 mg/L) [IgD] negative (< 19 kU/L; RF < 100) and [IgE] (23.6 kU/L; RV < 100). Nephelometric IgE result was the first reflex result released. Upon professional integration of all data IgE antigen excess was highly suspected because our IgD in-house modified radial immunodiffusion assay avoids antigen excess. IgE was then assayed with serial dilution ranging from 1/10 to 1/100 000. Linear dilution was observed for 5 dilutions ranging from 1/500 to 1/100000 giving a mean [IgE] of 6 640 000 kU/L ($SD = 0{,}78 \times 10^6$). IgE antigen excess tolerance (Siemens reagent; BN ProSpec nephelometer) was then established to 1×10^5 kU/L.

Conclusion: Integrated professional interpretation of all required biological data is critical as well as use of confident methods avoiding antigen excess.

P143

Wine colored samples? Development of laboratory reporting guidelines for hydroxocobalamin (OHCob) interference in patients pulled from house fires

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Objectives: OHCob is used for the treatment of cyanide poisoning secondary to smoke inhalation from house fires. OHCob turns bodily fluids wine-red, which can interfere with spectrophotometric assays. The objective was to investigate impact of OHCob on chemistry, coagulation, and urinalysis assays.

Designs and Methods: Normal and abnormal discard plasma/urine samples were spiked with a high dose (1.5 mg/mL) of OHCob or equivalent diluent volume (control). Samples (n = 5) were run on > 40 assays using Beckman Coulter DxC600/Access2, STA-Compact STAGO, and Siemens Clinitek Atlas analyzers. If interference was > 10%, dose-response treatments were performed on subset of assays. Serial samples obtained from a patient administered OHCob in the emergency department (ED) were analyzed for changes to colour and chemistry measurements.

Results: Spiking studies revealed positive interference (range 26-1298%) to total bilirubin, lactate, magnesium, uric acid, creatinine-enzymatic, prothrombin time, partial prothrombin time and d-dimer. There was negative interference (range 12-63%) to alanine aminotransferase, aspartate aminotransferase, creatinine-Jaffe and creatine kinase. Urinalysis dipsticks were falsely increased (grades up to 3+) on glucose, ketones, blood, nitrates and leukocytes. Subsequent dose-response treatments showed statistically significant ($p < 0.05$) increase/decrease interferences. Interference in samples from a patient administered a single dose of OHCob was not detected by hemolysis index (HI), but showed gradual recovery on select chemistry assays as red colouration faded over time.

Conclusion: OHCob impacted 16 analytes with varying degrees of interference, which will aid in developing sample handling and reporting procedures. Lack of HI flagging underscores importance of communication with ED to identify these samples.

P144

Establishment of reference Interval of anion gap from hospital population

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Objectives: Across three main hospital sites, CVH, MH and QHC, anion gap (AG) is reported without a reference interval (RI). The aim of this study was to establish a common AG RI across three sites using available patient data.

Design and Methods: Test results from three sites for December 2018 were extracted from the laboratory information system for sodium (Na), chloride (Cl), total CO_2 (TCO_2), albumin and AG. Individuals with reported values for Na, Cl, TCO_2, or albumin outside the RI were excluded. Outliers were removed from the remaining dataset using Tukey's test. An AG RI for each site and a common RI was established

using the non-parametric method. The common AG RI was verified with a second independent data set of healthy volunteers.

Results: The AG RI for CVH (n = 1163) and MH (n = 151) was 5 – 13. The AG RI for QHC (n = 266) was 5 – 11, which had a lower upper limit compared to other sites, likely due to differences in patient population as QHC is an ambulatory site. When all three sites (n = 1580) were analyzed together, the common AG RI of 5-13 was established. This RI was verified with 27 healthy volunteers. All 27 individuals reported AG values within the common RI.

Conclusions: The AG RI for two sites, 5-13, was similar, but the third site had a lower upper limit, likely due to differences in the patient population. Analysis across all sites showed a common AG RI of 5-13 and was implemented institution-wide.

P145

Impact of prevalence of hypocalcemia on the accuracy of albumin-adjusted calcium

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Dynacare; Dynacare; Dynacare; Dynacare; Dynacare; Dynacare

Objectives: Many laboratories provide albumin-adjusted calcium (Adj-Ca) in lieu of ionized calcium (iCa^{2+}). However, the reliability of Adj-Ca has been questioned. To assess the accuracy in an outpatient setting, we compared the ability of total serum calcium (TCa) or Adj-Ca against pH-adjusted serum iCa^{2+} to classify calcium status.

Design and methods: Reference intervals for iCa^{2+} and TCa were verified. Results for 12 months were extracted. Adj-Ca was calculated by inhouse regression of albumin (bromocresol green) against TCa. Agreement between iCa^{2+} and TCa or Adj-Ca was assessed by Cohen's kappa. A subset analysis was performed on samples with albumin values < 35 g/L.

Results: Adult reference intervals for iCa^{2+} and TCa were verified at 1.15-1.35 mmol/L and 2.15-2.60 mmol/L, respectively. The Adj-Ca equation, Adj-Ca = TCa + {0.0169x[46.142-Alb]}, was derived using 93,760 paired results. Clinical agreement versus iCa^{2+} was assessed using 6579 paired results, 4.3% with albumin concentrations < 35 g/L. Of these, 4.9% were hypocalcemic, 81.2% were eucalcemic, and 13.9% were hypercalcemic, as determined by iCa^{2+}. Versus TCa, Adj-Ca had higher agreement with iCa^{2+} in both the larger patient cohort and the hypoalbuminemic subset (Cohen's kappa of 62.6% and 32.3% for TCa versus 76.2% and 65.0% for Adj-Ca). However, Adj-Ca overestimated the calcium status in 47% of hypocalcemic patients, while TCa underestimated the calcium status in 43% of hypercalcemic patients.

Conclusions: The accuracy of Adj-Ca is dependent on the prevalence of hypocalcemia. Adj-Ca is suitable to correct for erroneous eucalcemia in hypercalcemic patients but will classify ~50% of hypocalcemic patients as eucalcemic.

P147

Investigating non-standard body fluid testing patterns at DynaLIFE Medical Labs

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DynaLIFE Medical Labs and University of Alberta; DynaLIFE MEDICAL LABS; DynaLIFE Medical Labs; DynaLIFE MEDICAL LABS; DynaLIFEdx and the University of Alberta

Objectives: Tests ordered on non-standard body fluids are a common challenge for clinical laboratories. The sheer number of possible combinations diminishes the feasibility of conventional validation approaches, yet analytical performance validation is a regulatory requirement of both CAP and ISO15189. In this study, our objectives were to characterize body fluid testing patterns and design a tractable validation plan for the body fluid tests performed at DynaLIFE.

Design and Methods: De-identified data for 17 orderable tests on body fluids were obtained for January 1, 2016 to December 31, 2018. Testing was performed on the Siemens Advia, and data analyzed using Microsoft Excel.

Results: Of the 20,693 body fluid test results, 90% were accounted for by just 5 analytes: total protein, 25%; glucose, 22%; lactate dehydrogenase, 19%; albumin, 15%; and creatinine, 9%. The most commonly encountered body fluids were pleural, 42%; peritoneal, 26%; unidentifiable, 15%; synovial, 7%; and Jackson Pratt (JP) drain, 6%. An abbreviated validation plan was designed, including 2-level precision (5 replicates x 5 runs; one level at the LoQ), 5-level linearity, matrix effects via spiking, and saline dilution recovery. The breakdown of body fluid types per test was used to prioritize the fluid(s) used for each validation. For example, albumin in pleural and peritoneal fluid, creatinine in JP drain fluid, rheumatoid factor in synovial fluid and potassium in pericardial fluid.

Conclusions: Retrospective evaluation of body fluid testing enabled prioritization of validation experiments to balance limited resources and accreditation requirements. Strategies to enforce unambiguous fluid source descriptions are necessary to ensure quality results.

P148

Does mixing improve the comparability between hemoglobin measured on the GEM Premier 4000 blood gas analyzer and the Sysmex XN hematology analyzer?

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Objectives: To evaluate various mixing strategies to reduce the difference in hemoglobin results between the Sysmex XN hematology (SH) analyzer and the GEM Premier 4000 blood gas (GBG) analyzer.

Design and Methods: Hemoglobin results were obtained from patients who had a test result from both SH and GBG where blood gas samples were either mixed by hand (n = 21) or mixed using a rotational mixer (n = 63). Eight volunteers provided venous blood samples, one EDTA whole blood vacutainer and two balanced heparin syringes, to determine the optimum rotational speed to minimize the differences between SH and GBG results. Group 1 (n = 4) compared 10 rpm to 20 rpm and Group 2 (n = 4) compared 20 rpm to 40 rpm. Data were analyzed using difference plots comparing the difference between SH and GBG hemoglobin results to their average.

Results: Applying a standard manual mixing technique by hand (n = 21) or by using a rotational mixer (n = 63) gave a large spread of biases between the SH and GBG (95% limits of agreement, LOA: -3.5 to 11.4 and -8.9 to 8.5, respectively). These values exceeded the performance goals set by IQMH for hemoglobin on hematology analyzers (± 4 g/L at < 100 g/L and ± 5% at ≥ 100 g/L). No rotational speed was shown to minimize the bias between SH and GBG hemoglobin measurements. The difference in GBG values in Group 1 was similar to Group 2 and did not exceed ± 2.4%.

Conclusion: Standardized hand-mixing and rotator mixing of syringe collected venous blood did not reduce the difference between hemoglobin measurements on the SH and GBG.

P149

The impact of a CPOE best practice alert at reducing repetitive Hemoglobin A1c testing

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Objectives: Hemoglobin A1c (HbA1c) is one of the primary tests for diagnosis and monitoring of diabetes and reflects the average blood glucose control over the last few months. The 2018 Canadian Diabetes Guidelines recommend that HbA1c be assessed every 3 months when glycemic targets are not met, or therapy is being adjusted. Computerized physician order entry (CPOE) and has led to inappropriate repeat testing and overutilization of HbA1c. The objective of this study was to assess the effectiveness of a CPOE Best Practice Alert (BPA) or "pop-up" intervention aimed at reducing duplicate HbA1c testing within 90 days.

Design and Methods: The intervention was a CPOE BPA pop-up to alert the ordering physician of a duplicated HbA1c order within 90

days. The BPA pop-up alert informed the ordering provider of the previous order date, HbA1c result and offered the opportunity to cancel the order. The effectiveness of BPA pop-up was assessed by the cancelation rate and the reduction of duplicated HbA1c tests.

Results: The HbA1c BPA pop-up alert was triggered over 350 times in the first month following the intervention. Repeat HbA1c orders triggering the BPA pop-up alert were canceled 61% of the time.

Conclusions: Implementation of a BPA pop-up alert dramatically decreased repeat testing of HbA1c. Expansion of the rules to include a pop-up for add-on testing within the Core Laboratory and changing the rule to cancel the test by default following the BPA pop-up are expected to lead to further reductions in testing.

Keywords: HbA1c, Utilization, CPOE pop-up, duplicate testing

P150

Validation of software and a high-performance liquid chromatography method for blood spot hemoglobin to screen newborns for sickle cell disease

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Objectives: On April 1, 2019, the newborn screening panel was expanded in Alberta to screen infants for sickle cell disease. The objective of this study is to validate Bio-Rad Variant software and a high performance liquid chromatography (HPLC) method for bloodspot hemoglobin analysis.

Design and Methods: Precision was accessed with QC material in duplicate over 10 days for retention time (RT) and % peak area for fetal hemoglobin (HbF), adult hemoglobin (HbA) and variant hemoglobins (HbS, HbC, HbE, and HbD). One hundred and nineteen newborn bloodspots and 5 proficiency testing samples were exchanged with BC to compare screen result and HPLC hemoglobin pattern. Seventeen newborn bloodspots were sent for DNA analysis. The limit of detection (LOD), carryover, hemoglobin stability and interferences were verified.

Results: The RT and % peak area CV were $<5\%$ and $<13\%$, respectively for all hemoglobins. There was 100% concordance with BC for 70 normal and 54 abnormal samples. All 17 newborn bloodspots sent for DNA analysis agreed with the hemoglobin pattern. Two chromatograms gave discrepant hemoglobin pattern interpretations by biochemists. Carryover was not detected. The LOD was peak area 1%. Transfusion and bilirubin concentrations $>200\mu mol/L$ interfered. Degraded hemoglobin bloodspots were not software flagged broad peak but gave high FAST peak areas and low HbF, HbA and total areas.

Conclusions: The Bio-Rad newborn screen bloodspot hemoglobin HPLC method is precise and accurate. Lab developed criteria have been established to flag chromatograms suspicious for degraded hemoglobin or transfusion for all biochemists to interpret. Transfused samples will go directly for DNA analysis.

P151

Developing common reference intervals in Alberta: Taming the Wild West Phase 2

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Objectives: Healthcare providers utilize reference intervals (RI) to support appropriate interpretation of laboratory test results. Standardizing RI within/across analytical platforms improves patient care by unifying result interpretation and standards of practice. Previously, common RI were developed for twelve frequently ordered clinical chemistry tests in Alberta (previously published). This second phase aimed to develop common RI for five additional chemistry tests.

Design and Methods: Common RI for ferritin, urea, magnesium, ALT and GGT were determined through a posteriori indirect sampling approach. RI were primarily derived from de-identified results obtained from community patients, but mixed populations (inpatients/community) were also assessed to ensure appropriate sample sizes. Bhattacharya analysis identified statistically appropriate RI; partitions were reviewed for clinical significance and final RI was defined by group consensus.

Results: An environmental survey of current RI in the province for the five analytes demonstrated significant variation across the province and included several historical/incomplete RI (instruments: Roche Diagnostics, Beckman Coulter, Ortho Clinical Diagnostics, Siemens Healthineers). After data filtering and appropriate partitioning, Bhattacharya analysis included large data sets ($N=1{,}000$-$300{,}000$) and analytical/clinical review was completed (e.g. Table 1).

Conclusions: Large data sets, a posteriori indirect sampling, Bhattacharya analysis, and clinical assessment are important components for developing common RI.

P152

Prospective evaluation of the Siemen's thyroid-stimulating immunoglobulin (TSI) assay for diagnosis and prognosis of Graves' disease

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Objectives: Graves' disease (GD) is an autoimmune condition caused by autoantibodies directed towards thyroid stimulating hormone receptor (TSHR), resulting in hyperthyroidism. Testing for autoantibodies in GD is challenging because assays must discriminate between TSHR autoantibodies that may stimulate, or block, thyroid hormone production. The study aim was to evaluate the latest TSHR autoantibody assay, the TSI assay from Siemens, compared to currently available TRab assay from Roche.

Design and Methods: One hundred patients will be enrolled in this study. Study patients are seen at a University of Calgary endocrine clinic for thyroid assessment, suspected GD patients are approached to participate. Consenting patients receive standard care including TRab measurement and must provide additional blood for TSI measurement. TRAb assay was measured on cobas 8000 platform (Roche Diagnostics). TSI samples were stored frozen, and batch tested on Immulite platform (Siemens Medical Solutions). Graves' diagnosis was adjudicated by endocrinologists that were blinded to TSI result.

Results: Within-run and between-run precision of the TSI assay spanning low-to-high QC levels was 4.5-5.2% and 8.6-6.7%, respectively. The TSI and TRab assay results ($N=40$) were correlated ($R^2=0.7$; $P<0.01$) with 88% concordance. Using manufacturer cut-offs, the respective sensitivity, specificity, positive predictive value, and negative predictive value for TSI was 90, 75, 89, 75%, and for the TRab assay was 93, 91, 100, 100%.

Conclusion: Preliminary data suggest TSI assay is a good method for GD detection that correlates well with TRab. Further patient enrollment and the prognostic properties of these assays are ongoing.

P153

Evaluation of a mass spectrometry-based assay for quantitative measurement of homovanillic acid, vanillylmandelic acid, and 5-hydroxyindoleacetic acid

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Objectives: Urinary measurement of homovanillic acid (HVA), vanillylmandelic acid (VMA), and 5-hydroxyindoleacetic acid (5-HIAA) are important in the diagnostic work-up for neuroblastic and carcinoid tumors. This study's objective was to evaluate a liquid chromatography tandem mass spectrometry (LC-MS/MS) method to replace the current methods for measurement of HVA, VMA, and 5-HIAA.

Design and Methods: The candidate method uses a C-18 column

equipped on a Shmadzu UFLC paired to an AB Sciex QTRAP 5500 triple quadrupole MS. Between-day precision at two levels, limit of quantitation, and linearity was assessed for all three analytes of the assay. Method comparison was assessed against either high performance liquid chromatography-electrochemical detection method (HPLC-ECD) for 5-HIAA or LC-MS/MS methods for HVA and VMA.

Results: Between-day precision of the LC-MS/MS method showed %CV of less than 7.8% across all levels and analytes. Limit of quantitation studies (%CV $<20\%$) was confirmed at 1.0 μmol/L for HVA and VMA and at 0.4 μmol/L for 5-HIAA. Linearity for the candidate method was acceptable for all analytes for up to a concentration of 500 μmol/L. HVA and VMA method comparison to another established LC-MS/MS method showed similar performance. Method comparison of the candidate method for 5-HIAA measurement to the previously used HPLC-ECD method showed similar performance.

Conclusions: Evaluation of the candidate LC-MS/MS assay for multiplexed measurement of HVA, VMA, and 5-HIAA showed comparable performance to the previous and other LC-MS/MS. With acceptable analytical performance and the utility to simultaneously measure the three analytes simultaneously, the method was accepted for clinical use.

P154

Serum protein electrophoresis by capillary electrophoresis can miss IgA monoclonal immunoglobulins hidden in the beta regions

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Objectives: Well documented limitations of serum protein electrophoresis (SPEP) testing are not commonly appreciated by all general practitioners, who often order SPEP to screen for plasma cell dyscrasias in the community population. Here we evaluated the ability of capillary electrophoresis (CE) to identify analytically significant monoclonal immunoglobulins using data from a community laboratory.

Design and Methods: A total of 4,687 CE results were retrospectively compared, and 401 CE results prospectively compared, to confirmatory serum immunofixation electrophoresis (SIFE) results, and the sensitivity, specificity, positive and negative predictive values calculated. Chi-square statistics were used to compare groups of data.

Results: CE successfully detected 93.4% of positive SIFE cases (n = 1,236). There were 81 false negative cases, of which the majority had analytically significant monoclonal immunoglubulins hidden in the beta regions. A significant proportion of beta-migrating cases were IgA immunoglobulins (n = 42) (chi-squared [127, $p<0.05$]). Interestingly, well over half of these IgA cases (n = 23) migrated in the gamma region by SIFE. This discrepancy is due to the unique migration of some IgA immunoglobulins by CE. Consequently, this is a population of immunoglobulins that are uniquely undetectable by CE. Prospective data demonstrated 91.4% CE sensitivity, supporting the fact that potentially 9% of monoclonal immunoglobulins are not detectable by CE.

Conclusions: These data support the need to inform physicians of SPEP limitations by including a comment on patient reports stating that negative SPEP results do not rule out monoclonal immunoglobulins, and the use of more analytically sensitive tests may be required if clinically indicated.

P155

Comparability of urinary albumin and creatinine measurements: a proficiency testing provider perspective

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Objectives: Accurate measurement of urinary albumin (UA) excretion is crucial for assessment of kidney damage. The Institute for Quality Management in Healthcare (IQMH) provides ISO 17043:2010 accredited Proficiency Testing (PT) programs for UA, urine creatinine (UC) and albumin/creatinine ratio (ACR). Nine years of data was evaluated to determine average bias and imprecision and compared to performance goals.

Design and Methods: Included were 23 PT samples of human urine distributed between May 2010 and January 2019. Participants' results were assessed against the all methods' mean. Robust statistics based on ISO 13528 were used to calculate peer group means and standard deviations to eliminate outlier effects.

Results: UA included 99 peer groups; average absolute method bias: 5.1% (0.0%–24.3%), average CV: 5.5% (1.2%–19.9%). For UC, 125 peer groups; average absolute method bias: 3.8% (0%–12.1%); CV: 3.5% (0.0%–10.7%). For ACR, average all methods' CV: 12.0% (7.2%–24.4%). Method bias estimates for UA and UC were within the desirable goals for 93% and 85% of samples, respectively. For UA, 65% of the peer group CVs were within the desirable CV goals; 100% for UC, and 70% of all methods' CVs for ACR. See table for peer group results.

Conclusion: Overall biases for UA and UC met the desirable goals, although some methods were suboptimal. Efforts to standardize UA should address this shortcoming and reduce the overall variability of results leading to better detection of kidney disease.

Keywords: urine albumin, urine creatinine, standardization, kidney disease, proficiency testing

P156

Community physicians in Ontario continue to order of serum 25-hydroxy-vitamin D testing inappropriately

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Dynacare; Dynacare; Dynacare; Dynacare; Dynacare

Objective: On December 1, 2010 a new Ontario Health Insurance Plan (OHIP) laboratory requisition was issued requiring clinicians to classify the patient as insured or uninsured for the ordered serum 25-hydroxy-vitamin D (vitamin D) testing. Our objective was to assess the impact of the OHIP requisition change on the frequency of insured vitamin D testing performed by our community reference laboratory and to characterize the vitamin D status of our patient population.

Design and Methods: All vitamin D test orders from July 2005 to July 2018 were extracted from our laboratory information system. Quantitative trends in monthly test volumes and patient vitamin D status were analyzed.

Results: $N = 1{,}540{,}141$ insured vitamin D tests were performed. Monthly volume increased exponentially from $N = 884$ tests in July 2005 to a peak of $N = 30{,}533$ tests in March 2010. Volume decreased 89% the month after the new laboratory requisition was launched but average monthly increases of 3.1% per month were subsequently observed. $N = 13{,}796$ insured tests were performed in July 2018. Vitamin D sufficiency (75 to 250 nmol/L) trended upward from 36% in July 2005 to 53% in July 2018. The average monthly prevalence of vitamin D deficiency (<25 nmol/L) was $<1.9\%$.

Conclusion: Mandating ordering physicians to classify vitamin D insurance status on the laboratory requisition did initially reduce insured test volume; however, subsequent ordering has consistently increased month-to-month. This increase is not justified by vitamin D patient status. Renewed test utilization strategies are urgently needed to mitigate the undue financial burden of inappropriate vitamin D test ordering.

P157

Understanding the performance characteristics of urine dipstick "blood" and microscopic "RBC" testing

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Objectives: Test inquiries concerning urine blood (dipstick) and microscopic RBC testing are numerous, probably due to the need for follow-up referrals and investigations that are expensive, time consuming and worrying for both physicians and patients. To ensure appropriate understanding and expectations for these tests, as well as consistent performance over time, we assessed their monthly concordance rates.

Design and Methods: Urine samples with both blood (dipstick) and microscopic results were included in this 9-month study (21,852/

month) (Beckman Coulter Iris iChem Velocity, Iris iQ200 Sprint).

Results: Approximately 70% of urines were negative for blood by dipstick, and 80% were negative for RBCs by microscopy (≤2/hpf). Using microscopy as the gold standard, dipstick analysis was 98% specific (0.7% CV) and 82% sensitive (monthly range 71%-100%; 8.7% CV), with a 1.8% FP-rate (monthly range 0.1%-2.6%; 18% CV, excluding 1 month) and 18% FN-rate (11% CV, excluding 2 months). The NPV of the dipstick for blood was 97%, while the PPV was 89%. Urines with blood results of 0.3mg/L were excluded from the above calculations, as the negative:positive ratio was 56%:44% for microscopy. This comprised approximately 20% of the dipstick results. Approximately 10% of results were positive (>2.0mg/L).

Conclusions: Blood dipstick screening for urine blood is sensitive and specific for negative results, and results >2.0mg/L. However, results of 0.3mg/L have a poor predictive value and should be considered borderline and repeated with a new sample. Monitoring dipstick sensitivity, FP-rate and FN-rate compared to microscopy may be a useful QI indicator for laboratories.

P158

Development of mismatch-assisted non-covalent DNA catalytic reaction

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Objective: Non-covalent DNA catalytic reactions offers amplified isothermal detection of nucleic acids without the use of enzymes. Enzyme-free signal amplification shows promise for point of care testing and in situ intracellular detection. This study aims to develop a new non-covalent DNA catalytic reaction showing improved amplification efficiency and kinetics, and simple substrate preparation.

Design and Methods: The catalytic reaction utilizes a duplex DNA substrate comprised of a probe strand and an output strand which contains a single-base mismatch. The single-base mismatch destabilizes interaction between the probe and output DNA strands while accentuating the interaction between the target and the probe to expedite the toehold-mediated strand displacement reaction between the substrate and target. Cyclic signal amplification and target DNA recovery is achieved using a mismatch-free fuel DNA, which displaces the target DNA from the probe DNA through toehold-exchange reaction (Figure 1).

Results: The mismatch-assisted catalytic reaction achieved 40-fold signal amplification within one hour with a limit of detection of 1.9 nM, which represents a 11-fold increase in amplification efficiency compared to reactions using substrates without mismatch.

Conclusions: The mismatch assisted non-covalent nucleic acid catalytic reaction shows promise for detection of nucleic acids in test tubes and cancer cells.

P159

Novel alpha-1-antitrypsin allelNull(Canada): two missense mutations (p.Glu366Lys and p.Ile100Asn) in cis

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Objectives: a_1-antitrypsin deficiency (AATD) predisposes patients to pulmonary disease due to inadequate protection against neutrophil elastase released during an inflammatory response. Homozygosity or compound heterozygosity for the Z A1AT variant (c.1096G>A (p.Glu366Lys)) is the most common cause. Null variants are rarer and manifest as complete absence of plasma A1AT. Our objective was to explain the results of AATD investigations performed on an 8 yo patient from Edmonton. His A1AT phenotype was determined to be M (wild type)/Null by isoelectric focusing (IEF) but M/Z by targeted genotyping. Gene sequencing revealed two heterozygous variants: Z and p.Ile100Asn (c.299T>A). The Ile100Asn substitution is predicted to disrupt an a-helix resulting in intracellular degradation prior to hepatocyte secretion.

Design and Methods: Family testing was conducted to verify potential inheritance of an A1AT allele carrying the two mutations *in cis*, as this arrangement of the mutations would explain "Z" detection by genotyping but not by IEF.

Results: A novel variant Null(Canada) (c.[299T>A;1096G>A] (p.[(Ileu100Asn;Glu366Lys)])) was confirmed. A sibling was identified as having AATD on the basis of compound heterozygosity for two alleles: Null(Canada) and the common Z allele. A separate pedigree from Newfoundland was subsequently recognized as carrying Null(Canada).

Conclusions: With one exception, all reported A1AT variants are characterized by a single mutation. *In cis* mutations such as Null (Canada) may be more common than previously recognized as historical testing methods would not have detected them. Combined approaches that include gene sequencing and segregation studies allow recognition of rare A1AT variants, including *in cis* mutations.