Wednesday, August 2, 2017

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

Pediatric/Fetal Clinical Chemistry

B-247

Heads up! Fetal scalp lactate misclassification error rates based on simulation modelling for the Lactate Pro meter

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Background: : Fetal scalp lactate is a surrogate measure of fetal metabolic acidosis. The most common cause of fetal metabolic acidosis is intrapartum hypoxia, which can lead to poor neonatal outcomes including low Apgar score and hypoxic ischemic encephalopathy (HIE). Point of care testing (POCT) is used in some centers to measure fetal scalp lactate as an adjunct to electronic fetal monitoring (EFM). The goal of implementing this technique in modern obstetric practice is two-fold: to identify fetuses with abnormal EFM experiencing significant hypoxic distress that require immediate delivery; and to identify stable fetuses who do not require intervention, despite abnormal EFM. Kruger et al. established 4.8 mmol/L as a threshold for the Lactate Pro meter for predicting poor outcome. Wiberg and Källénin used the Lactate Pro meter in their 2017 study to establish a normal reference range for fetal scalp lactate. The aim of this study is to establish the rates of misclassification of fetuses at 4.8 mmol/L using the Lactate Pro meter in populations with both normal and abnormal EFM results.

Methods: Descriptive statistics from the Wiberg and Källénin data for fetuses with abnormal EFM and or Apgar scores < 9, and those with normal EFM and Apgar scores ≥ 9 were used. The distributions of lactate were skewed so data was logarithmically transformed and then simulated datasets of 1000 were created for each group. A Monte Carlo simulation model was performed to assess the levels of misclassification at the 4.8mmol/L cut off with various levels of bias (+/- 1mmol/L) and imprecision (CV 0-20%) for both groups.

Results: The misclassification rates were affected far more by bias than imprecision in both groups. Furthermore, imprecision had a smaller impact on misclassification rates when associated with positive bias than with negative bias. In the abnormal EFM group, simulation with biases of 0 to +1mmol/L resulted in false positive misclassification rates of 10-70%, while negative biases up to 1mmol/L lead to false negative misclassification rates from 5-20%. In the normal group, false positive misclassification rates were also 10-70% for positive biases of up to 1mmol/L, while the false negative misclassification rates ranged from 1-5% with biases of 0 to -1mmol/L.

Conclusion: Simulation models predicted that up to 70% of lactate readings below 4.8mmol/L would be misclassified as above the threshold with biases up to 1mmol/L, which would result in a large proportion of fetuses receiving unnecessary intervention. Though lower than the rates for positive bias, with negative biases of up to 1mmol/L, 5-20% of fetuses with scalp lactates of greater than 4.8mmol/L would be misclassified as less than 4.8mmol/L, and as such would not receive needed intervention. This study calls into question the ability of POCT with the Lactate Pro meter for fetal scalp lactate to provide meaningful information to guide obstetric management.

B-248

Performance Evaluation of the ADVIA Chemistry Total Bilirubin_2 (TBIL_2) Assay in Neonatal Samples

P. Datta, S. Patel, J. Snyder, J. Dai. *Siemens Healthcare Diagnostics, Newark, DE*

Background: The ADVIA® Chemistry Total Bilirubin_2 (TBIL_2) Reagents, available on ADVIA Chemistry Systems from Siemens Healthcare, measures total bilirubin in human serum and plasma (lithium heparin) samples. Bilirubin is the end-decomposition product of the heme part of red blood cells. It is extracted and biotransformed in liver and excreted in bile and urine. Bilirubin when exposed to light is transformed into several isomers, the double bonds isomerizing in their usual Z-Z configuration to E-E, E-Z, and Z-E configurations. Total bilirubin measurements are used in the diagnosis and treatment of hemolytic, biliary, and liver disorders, including hepatitis and cirrhosis. The assay was originally validated for adult bilirubin samples only and now has been tested with neonatal samples.*

Methods: The ADVIA Chemistry TBIL_2 Reagents are based on a chemical oxidation method using vanadate as an oxidizing agent. The bilirubin is oxidized by vanadate at about pH 2.9 to produce biliverdin. In the presence of the detergent and the vanadate, both conjugated (direct) and unconjugated bilirubin are oxidized:

$$Bilirubin + Surfactant + VO_3^- \rightarrow Biliverdin$$
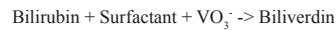
This oxidation reaction causes a decrease in the optical density of the yellow color, which is specific to bilirubin. The decrease in optical density at 451/545 nm is proportional to the total bilirubin concentration in the sample. The Siemens Chemistry Calibrator is used to calibrate the assay. CLSI protocols are used to measure the correlation of the assay with the Roche total bilirubin (BILT3) assay on neonatal samples; limits of blank (LoB), detection (LoD), and quantitation (LoQ); interference of hemoglobin A1 and F, indican, and CYANOKIT; and analytical measuring range. The published total bilirubin assay range was confirmed according to CLSI protocol across neonatal (0-5 days) to other ages (up to >90 years).

Results: LoB, LoD, and LoQ of the assay were 0.0, 0.1, and 0.1 mg/dL, respectively. Analytical range was minimum up to 35 mg/dL. When tested with 119 neonatal samples (serum: 13 hours to 39 days) and 5 neonatal samples spiked with bilirubin up to 31.6 mg/dL concentration, the ADVIA Chemistry TBIL_2 assay compared as follows with the Roche BILT3 assay: ADVIA = 1.06(Roche) - 0.24 mg/dL, r = 0.99 (range 0.7-31.6 mg/dL). Interference in the assay (up to) was <10% for hemoglobin A and F (1000 mg/dL), indican (10 mg/dL), and CYANOKIT (40 µg/mL). The literature reference range was confirmed[1]. Earlier the assay was shown to have imprecision <4.7% at bilirubin concentrations of 1-15 mg/dL across all ADVIA Chemistry platforms and <10% interference to ascorbic acid (up to 50 mg/dL) and lipemia (up to 750 mg/dL of triglycerides concentrate).

Conclusion: The data obtained in this study show that the ADVIA Chemistry Total Bilirubin_2 assay can be used for quantitative determination of total bilirubin in human serum and plasma on the ADVIA Chemistry Systems.

References:

1. Wu AHB. Tietz clinical guide to laboratory tests. 4th ed. St. Louis (MO): Saunders Elsevier; c2006. p. 316.

* For Investigational Use Only. The performance characteristics of this device have not been established. Pending 510(k) clearance. Not available for sale for this indication for use.

B-249

Study on Strategic Approach of Prenatal Testing for Trisomy 21 in General Pregnancy Population

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Background: : Recently, non-invasive prenatal testing (NIPT) by analysis of cell-free DNA (cfDNA) in maternal blood has shown promise for highly accurate detection of common fetal autosomal trisomies. Its clinical studies have primarily included pregnant women identified by prior screening to be at high risk for aneuploidies. It has been uncertain if the results of NIPT in such high-risk pregnancies are applicable to the general pregnancy population. It is known that guideline for application of NIPT is developing. This study is to help establishing guideline for prenatal screening through assessment of the performances of various combination method with NIPT and integrated test.**Methods:** We collected 57,639 integrated test data performed by Green Cross laboratories(Yongin, South Korea). Full integrated test was performed during 1st (PAPP-A, free hCG) and 2nd (AFP, hCG, uE3, inhibin A) trimester. 6 scenarios were supposed. S1 scenario is to perform integrated test (cut off 1:270 for Down syndrome) without NIPT. S2 scenarios are to perform NIPT to high risk pregnancies of integrated test with various integrated cut offs, 1:270 for S2-1, 1:500 for S2-2, 1:1,000 for S2-3, 1:2,000 for S2-4. S3 scenario is to perform NIPT without integrated test. Screening performance such as sensitivity, specificity, positive predictive value, negative predictive value, number of pregnancy needed invasive testing like amniocentesis was calculated to each scenario. Also the comparison of cost between scenarios was performed.**Results:** Among 57,639 data, there were 3.1% pregnancies with below 1:270 cut off, 5.0% for below 1:500 cut off, 8.3% for below 1:1,000 cut off and 13.1% for below 1:2,000 cut off. S1 showed lower screening performance and higher frequency of invasive test. S2-1 showed the possibility of false negative like S1, lower frequency of invasive testing and medical costs compared with S1. S2-2 showed the possibility of false negative, the similar medical costs as S1. S2-3 and S2-4 showed no false negativity and higher medical cost. S3 showed the lowest cost effectiveness.**Conclusion:** We thought that the introduction of NIPT could help improving the screening performance for Down syndrome. Also, considering the cost

effectiveness, the practice of integrated test as first line test should be kept. At future, the exploration of the acceptability NIPT to service users and the comparison between NIPT and invasive test should be studied for identify the main barriers and facilitators for implementing NIPT within general pregnancy population

B-251

Comparison of Two Automated Immunoassays for Progesterone to Mass Spectrometry to Aid in the In Vitro Fertilization Setting

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Background: Progesterone levels vary during the menstrual cycle, pregnancy and embryogenesis. Progesterone levels are measured to monitor ovarian functions, during infertility treatment, pregnancy or abnormal uterine bleeding. The gold standard method for progesterone measurement is tandem mass spectrometry. However, in the In Vitro Fertilization (IVF) setting, where low progesterone levels are monitored, due to the need for fast turnaround time, decreased cost and sample preparation requirements, automated immunoassays are most often implemented. The main aim of our study was to determine the precision and accuracy of progesterone levels measured on the ADVIA Centaur® CP analyzer and Architect i1000SR (Abbott) analyzer relative to the reference method (LC-MS/MS). Our study will significantly optimize the utility of progesterone assay in patients undergoing IVF. The main aim of our study was to compare the analytical performance of two automated immunoassays for progesterone to the reference method, Liquid chromatography-tandem mass spectrometry (LC-MS/MS). Our study will significantly optimize the utility of progesterone assay in patients undergoing IVF.

Method: De-identified serum samples were collected from 23 adult female patients undergoing IVF at the Texas Children's Pavilion for Women. Analytical performance parameters of precision, linearity, accuracy, and correlation were measured on the ADVIA Centaur® CP analyzer and Architect i1000SR (Abbott) analyzer. Results from these immunoassays were compared with an LC-MS/MS method on AB SCIEX 5500 tandem mass spectrometer.

Results: Intra and inter-assay precision for the Architect was <10 %, for Centaur it was <8%, and for LC-MS/MS it was <9 %. Linearity for the Architect was 0.11 to 40.53 ng/ml, for Centaur it was 0.21-60 ng/ml and linearity for LC-MS/MS was 0.17-25.6 ng/ml. For all serum progesterone samples (ranging from 0.7 to 63.9 ng/ml), Deming regression equation for Centaur CP relative to LC-MS/MS was y=0.89x+0.64, r=0.99, (n=23), Centaur CP showed a negative bias of -4.3%. However, for low progesterone samples (ranging from 0.7 to 2.2 ng/ml), Deming regression equation for the Centaur CP relative to LC-MS/MS was y=0.75x+0.57, r=0.90, (n=15), Centaur CP showed a positive bias of 21.9%. On the other hand, for all serum progesterone samples (ranging from 0.4 to 66.1 ng/ml), Deming regression equation for the Abbott Architect relative to LC-MS/MS was y=0.89x+0.04, r=0.99(n=23), the Architect showed a negative bias of -11.1%. For low progesterone samples (ranging from 0.4 to 2.0 ng/ml), Deming regression equation of the Architect relative to LC- MS/MS was y=0.87x+0.26, r=0.92 (n=15), the Architect showed a positive bias of only 6.9%.

Conclusion: Optimization for the progesterone assay can be achieved by comparison across platforms, using LC-MS/MS method. Architect progesterone assay showed better performance and closer correlation than the Centaur assay to the gold standard mass spectrometry, particularly for the testing of low levels of progesterone. Clinically it is very important to report the progesterone levels accurately immediately without delay to initiate IVF treatment. Thus, we aim to use the Architect progesterone assay to test low levels of progesterone and aid in clinical challenges met during IVF treatment.

B-252

Acute Infection Etiology of Febrile Children

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Background: A novel assay (ImmunoXpert™) that integrates measurements of three blood-borne host-response proteins (TRAIL, IP-10, and CRP) was recently developed to assist in differentiation between bacterial and viral disease. Here we compare the assay performance with standard laboratory parameters that are routinely used in clinical practice to facilitate diagnosis of infection etiology in febrile children.

Methods: We studied serum remnants collected from children aged 3 months to 18 years with suspicion of acute infection presenting at the ED or admitted. Reference standard diagnosis was based on predetermined criteria plus adjudication by an expert panel blinded to assay results. Assay performers were blinded to reference standard. Assay cut-offs were defined before un-blinding.

Results: Of 529 potentially eligible patients, 100 did not fulfill infectious inclusion criteria and 68 had insufficient serum. The resulting cohort comprised 361 patients, with 239 viral, 68 bacterial, and 54 indeterminate reference standard diagnoses. The assay distinguished between bacterial and viral infected patients with 93.8% sensitivity (95% CI: 87.8%-99.8%) and 89.8% specificity (85.6%-94.0%); 11.7% had an equivocal assay outcome. Overall the assay outperformed other laboratory parameters, including: (i) white blood count (WBC; cut-off 15,000cells/µl, sensitivity 72.7% (61.7%-83.8%), P<0.002; specificity 83.2% (78.3%-88.1%), P<0.05); (ii) CRP (cutoff 40 mg/L, sensitivity 88.2% (80.4%-96.1%), P<0.37, specificity 73.2% (67.6%-78.9%), P<0.001); (iii) Procalcitonin (PCT; cutoff 0.5 ng/ml, sensitivity 63.1% (51.0%-75.1%), P<0.001), specificity 82.3% (77.1%-87.5%), P<0.03); (iv) absolute neutrophil count (ANC; cut-off 10,000 cells /µl, sensitivity 68.2% (56.6%-79.7%), P<0.001; specificity 92.9% (89.6%-96.3%), P<0.30).

Conclusion: The host response-based assay was more accurate than routine laboratory parameters and biomarkers (WBC, ANC, CRP, PCT) in distinguishing bacterial from viral etiologies in febrile children. It has the potential to help clinicians avoid missing bacterial infections or overusing antibiotics.

Figure 1: Diagnostic performance of host-signature assay compared to routine laboratory parameters and other biomarkers
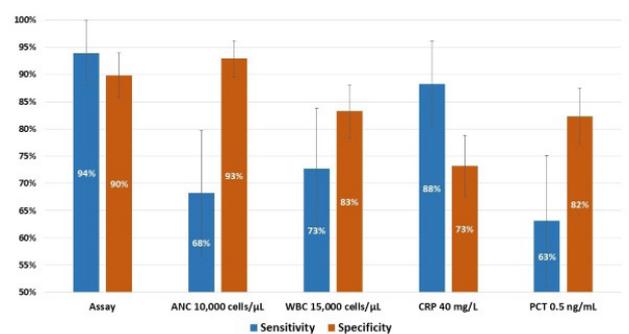
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Comparison of LeadCare Ultra® to ICP-MS as an initial screen for blood lead levels

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Objective: In 2012, the CDC issued new guidelines for assessing children's blood lead levels (BLLs) based on the National Health and Nutrition Examination Survey (NHANES). The study defined a BLL reference cut-off of 5 µg/dL based on the 97.5[th] percentile. Prior to these guidelines, children had a BLL "of concern" at or above 10 µg/dL. Given the change of cut-off, it is critical that laboratory methods are accurate

at the lower BLL cut-off of 5 μg/dL. Well-established methods for measuring BLLs include Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) because they are highly accurate and precise methods. However, the instruments are very costly and require expertise to operate and maintain. The LeadCare Ultra® (Magellan Diagnostics) is an FDA-approved instrument that uses anodic stripping voltammetry (ASV) to measure BLLs. The LeadCare Ultra is both cheaper and easier to run and maintain than GFAAS or ICP-MS, and thus is an attractive alternative for clinical labs that are currently sending out lead level testing to reference laboratories. However, there have been no prior studies assessing the performance of the LeadCare Ultra at the BLL cut-off of 5 μg/dL. Therefore, our objective was to perform a method comparison study of the LeadCare Ultra with ICP-MS to determine whether the LeadCare Ultra provides acceptable performance at a BLL cut-off of 5 μg/dL and thus could be used to effectively screen children for elevated BLLs.

Methods: 50 μL of heparinized capillary whole blood was transferred into a vial with 250 μL of 0.1M HCl to lyse cells and release lead into the solution for analysis on the LeadCare Ultra. For the method comparison, a blood sample was sent to Medtox (St. Paul, MN) to be analyzed by ICP-MS. Method verification studies included accuracy, precision, reference interval, and reportable range.

Results: To assess accuracy, we analyzed 75 samples and statistically compared results of the paired BLLs. We used CLIA proficiency testing criteria of ± 10% or ± 4 μg/dL for allowable total analytical error. Although all measurements passed these criteria, we found that the LeadCare Ultra has a large positive bias of 1.68 μg/dL in the BLL range of ≤ 4 μg/dL (n=38), low negative bias of 0.05 μg/dL in the BLL range of 5-10 μg/dL (n=23), and a negative bias of 0.81 in the BLL range ≥ 10 μg/dL (n=14). The bias across the entire BLL range from 1-45 μg/dL was 0.72 μg/dL (n=75). Deming regression had a slope of 0.91 (95%CI: 0.87-0.94) and intercept of 1.30 (95%CI: 0.94-1.66). Both within-day and between-day precision was less than 10%.

Conclusion: The LeadCare Ultra was compared to ICP-MS and found to be acceptable as an initial screen (BLL cut-off of 5 μg/dL) due to the low bias in the BLL range of 5-10 μg/dL. The LeadCare Ultra does have a large positive bias in the BLL range of ≤ 4 μg/dL (1.68 μg/dL), and therefore should not be relied upon to accurately quantify lead levels below the cut-off of 5 μg/dL.

B-255

Prevalence of sampling errors in umbilical cord blood samples and the effect on determination of reference intervals

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Background: The measurement of umbilical cord blood gas (UCBG) analytes is a standard procedure intended to document fetal metabolic status at birth. Unfortunately, UCBG analysis is complicated by the anatomy of the cord which consists of a central vein (UV) entwined by two arteries (UA). Due to this architecture, errors from double sampling of the vein or arteries ('same' sampling) or inadvertent swapping of the samples ('swap' sampling) often occur. Such errors have been previously reported to occur in 10-30% of UCBG samples but have not been adequately considered in the establishment of reference intervals (RIs). **Objectives:** To establish a quality indicator for detecting sampling errors in a cohort of deliveries performed at Mount Sinai Hospital (Toronto, Canada) and to determine the effect of such sampling errors on establishing RIs for UCBG analytes. **Methods:** A total of 1301 spontaneous vaginal births were identified from May to October 2015 with at least one associated UCBG sample. Of these, 863 were selected for further analysis using the following inclusion criteria: (1) singleton pregnancy; (2) 5th to 95th centile for birth weight (2640 to 4110 grams); (3) APGAR score >7 at 1 minute; and (4) no resuscitation We developed a scoring system based on differences between paired samples as a sampling error quality indicator, observed about 35% "same" and 5% "swap" errors, and applied the 2.5th and 97.5th centiles as RIs based on the remaining 442 pairs. **Results:**

Table 1 - Reference Intervals of Umbilical Cord Blood Gas Analytes							
Analyte	Difference Calcula-tion	Difference Cut-Offs		UA Reference Interval		UV Reference Interval	
		"Same" Within	"Swap" Less Than	Uncor-rected	Cor-rected	Uncor-rected	Cor-rected
pH	UV-UA	± 0.02	- 0.02	7.09 to 7.35	7.10 to 7.30	7.19 to 7.39	7.21 to 7.39
PCO_2 (mmHg)	UA-UV	± 3	- 3	35 to 75	44 to 77	33 to 56	33 to 53
PO_2 (mmHg)	UV-UA	± 5	- 5	10 to 41	9 to 28	16 to 42	19 to 45
sO_2 (%)	UV-UA	Not used	Not used	14 to 85	11 to 62	29 to 88	41 to 89
BD_{ecf} (mmol/L)	UA-UV	Not used	Not used	-0.3 to +11	-1.4 to +9.8	+0.4 to +10.2	+0.3 to +10

Conclusions: Correction for "same" or "swap" sampling made a conspicuous difference for UA results, which most directly reflect fetal status at birth. Our finding that UV RIs were only slightly affected by sampling errors suggests that most errors involved collecting UV instead of UA. Nevertheless, UV results are important for confirming UA validity. Detection of common sampling mistakes is crucial for establishing appropriate UCBG RIs.

B-256

Rapid Detection of Early Stage *Mycoplasma pneumonie* Infection Using Single-Walled Carbon Nanotube/Colloidal Gold-Based Immunochromatographic Strips

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Background: Current clinical diagnosis of *Mycoplasma pneumonia* infection typically fall under two major categories: immunological assays (ELISA, immunoprecipitation, etc.) to detect IgG and/or IgM, and PCR-based assays to test for the nucleic acids of the pathogens. However, a positive result from immunological test usually indicated the existing infection condition of the patient while the PCR test normally requires days of turnaround time. The objective of the current research was to develop quicker and easier way to detect Mycoplasma pneumonia from patient throat swaps.

Methods: To achieve a better detection efficiency, the single-walled carbon nanotubes (SWCNT) were coupled with the colloidal gold-monoclonal antibody immunochromatographic strips (CGIC). Two hundred microliters of anti-*M. pneumoniae* (P1) mouse monoclonal antibody (IgG) at 0.5 mg/ml was added into 10 ml colloidal gold solution (pH 8.5) with shaking for 30 minutes. One thousand microliters of 5% BSA was supplemented with shaking for another 30 min. Ten mg of SWCNT (DKnano, China) was dispersed in 100 ml miliQ pure water supplemented with one hundred microliters of Tween 20 by ultrasonicating for 5 min. Five μl of SWCNT solution was mixed with the above colloidal gold/IgG solution with shaking for 30 min. The mixed solution was centrifuged at 9500 rpm for 30 min at 4°C. The pellet was resuspended in 5000 μl of the suspension buffer (0.01mol/L pH 8.5 Tris-HCl, 25% sucrose, 2.5% BSA, 1.5% Tween-20, 0.25% PEG-20000, 0.20% sodium caseinate). Repeating the centrifugation at 9500 rpm for another 30 min at 4°C, and resuspending the pellet in 240μL of suspension buffer. The final SWCNT-colloidal gold-labeled antibody conjugate was jetted onto the glass fiber and dried at 37°C for 4hr. The specific detection antibody (Test line, T) at 1.5 mg/ml and the goat-anti-mouse control antibody (Control line, C) were transferred onto the nitrocellulose membrane and dried at 37°C for 2 hrs. The finalized detection strip was consisted of sample pad, conjugate pad, immobilized nitrocellulose membrane, and absorbent pad. Throat swap was used to collect the samples from the patients and the samples were diluted into 500μl of 1xPBS. Eighty microliters was used for the SWCNT/CGIC strip test for 15 minutes.

Results: A sequential cohort of 137 patients aged 3-14 years with fever/cough/fatigue were recruited with custodian-signed informed consent during Dec 1, 2016 to Jan 31, 2017 at the pediatric respiratory department of 3rd Affiliated Hospital of Jilin University. Among them, 97 were diagnosed as positive for *Mycoplasma pneumoniae* based on CRP, WBC, NEUT, LYM, body temperature, pulmonary iconography, immunological passive particle agglutination (PPA, Serodia-Myco II, Fujirebio, Japan), and RT-PCR (Daan gene Co. Ltd. China), and other clinical symptoms. SWCNT/CGIC strips achieved a sensitivity of 72.2% (70/97) and a specificity of 100.0% (40/40). The corresponding positive and negative predictive values were 100.0% (70/70) and 59.7% (40/67), respectively.

Conclusion: The SWCNT/CGIC strips demonstrated its effectiveness as an easy and quick detection for *Mycoplasma pneumoniae* infection.

B-257

A Creative Approach to Establishing Pediatric Reference Intervals for Ionized Calcium

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Background: Reference intervals (RIs) represent the range of laboratory test results derived from a group of healthy people. It is important for laboratories to provide accurate RIs, however challenges exist. In practice, pediatric RIs are difficult to establish or verify. Establishing RIs for analytes such as serum ionized calcium (iCal) is especially challenging due to analyte instability and because analyzer manufacturers typically provide only adult RIs for whole blood specimens. The objectives of this study were to (i) establish pediatric and adult iCal RIs in serum using screened, retrospective patient data (ii) verify established adult RIs using healthy donors and (iii) assess bias and RI transferability between serum and whole blood iCal values.

Methods: This study was exempt by the Mayo Clinic Institutional Review Board. Serum iCal results from 7/1/2008-7/1/2016 were included (263,724 results from 42,109 outpatients) using GEM3000 Premier analyzers (Instrumentation Laboratories, Bedford, MA). ICD-codes were used to exclude patients with heart failure, bone/renal disease, hypertension, and malignancy. Only the first result per patient was included, yielding n=506 pediatric and n=944 adult values. Age and sex partitioning was assessed. Central 95%RIs and 95% confidence intervals (CIs) were calculated using quantile regression. Age breaks were applied when the calculated range differed by >0.15mg/dL. Healthy adult donors were used to verify adult RIs and assess bias between serum and electrolyte-balanced lithium-heparin whole blood.

Results: No significant difference between males and females was observed. The table summarizes the established RIs and CIs. Healthy adult donors verified the RIs in serum and whole blood. Serum demonstrated a 2%(0.1mg/dL) bias compared to whole blood.

Table 1. Reference Intervals for Serum iCal

Age	N	Central 95% RI	95% CI on lower bound	95% CI on upper bound	Verified with Healthy Donors?
14 days-<1 yr	36	(5.21, 6.01)	(4.88, 5.54)	(5.94, 6.56)	n/a
1-<2 yrs	35	(5.04, 5.84)	(4.82, 5.26)	(5.74, 6.11)	n/a
2-<3 yrs	42	(4.87, 5.67)	(4.46, 5.28)	(5.39, 5.83)	n/a
3-<24 yrs	573	(4.83, 5.52)	(4.80, 4.85)	(5.46, 5.58)	Yes (n=8, age >17)
24-<98 yrs	767	(4.57, 5.43)	(4.49, 4.64)	(5.36, 5.49)	Yes (n=20)

Conclusion: Pediatric and adult RIs for iCal were established using screened, retrospective patient data and verified in serum and whole blood. Despite a 0.1mg/dL positive bias in serum, the established RIs are transferable to either matrix.

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Transference and Verification of CALIPER Pediatric Reference Intervals to Ortho VITROS 5600 Chemistry Assays

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Background: Reference intervals (RIs), defined as the central 95% of laboratory test results obtained from a healthy reference population, are essential to accurately interpret pediatric test results. Although the concept and utility of pediatric RIs is straightforward, developing them can be quite complex, particularly in the pediatric population, As a result, pediatric RIs have been severely lacking, with several laboratories using adult RIs to inappropriately fill the gap. CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) developed an age- and sex-specific pediatric RI database based on thousands of healthy children and adolescents aged 0-<19 years of age. Originally established for Abbott assays, CALIPER has performed a series of transference studies to make CALIPER RIs applicable to additional analytical platforms. This study transfers reference intervals for 34 biochemical assays to the Ortho VITROS 5600 Chemistry System.

Methods: In accordance with CLSI guidelines, a method comparison was performed between Abbott and Ortho assays by measuring 200 patient serum samples. If the comparison data yielded an $r^2 \geq 0.95$, simple linear regression using the least squares approach was used to estimate the line of best fit. For data yielding $r^2 < 0.95$, Deming regression is used. If data yields $r^2 < 0.70$, the reference intervals were not transferred due to poor correlation. The appropriateness of the linear model was assessed using Q-Q, standardized residual, and Bland-Altman plots. If the linear model was deemed appropriate, the equation of the line of best fit was calculated and used to transfer RIs and corresponding confidence intervals from Abbott to Ortho assays. Transferred RIs were verified using 86 healthy pediatric serum samples from the CALIPER cohort. RIs were considered verified if >90% of reference samples fell within the transferred confidence limits.

Results: Reference limits for most biochemical assays successfully transferred from Abbott to Ortho assays, with the exception of calcium and CO_2, which failed to transfer due to poor correlation between Abbott and Ortho assays, with r^2 values of 0.611 and 0.305, respectively. Of the 32 successfully transferred analytes, 27 successfully verified with >90% of reference samples falling within transferred confidence limits. For five of the assays, transferred reference intervals could not be verified using healthy children samples, including total bilirubin, magnesium, LDH, ASO, and C4.

Conclusion: This transference study broadens the utility of the CALIPER pediatric RI database to laboratories using the Ortho VITROS 5600 analyzer. These evidence-based RIs will improve the accuracy of pediatric test result interpretation in healthcare institutions using this clinical chemistry analyzer. Prior to implementing these RIs, all laboratories should verify them for use on their individual analytical platform and local population as recommended by CLSI.

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Principle Component and Correlation Analysis of Biochemical and Endocrine Markers in the CALIPER Cohort of Healthy Children and Adolescents

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Background: Reference intervals provide a baseline for which laboratory test results are compared in clinical practice. Reference values of several biomarkers are known to be affected by age and sex. However, an important additional covariate which is often overlooked is the concentration of other biomarkers. Biomarker concentrations are often not independent of each other, but may correlate due to various physiological processes. Identifying significantly correlated biomarkers in healthy individuals can be valuable when interpreting patients' laboratory test results. Here, we report a comprehensive biomarker correlation study in the healthy CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) cohort using principle component and heat map analyses.

Methods: CALIPER collected blood samples from healthy pediatric subjects (0-<19 years). Here, we used data from children aged 1-<19 years, as those <1 year only had a small number of blood tests examined, limiting the ability to examine correlations. In total, we examined 35 routine chemistry markers and 20 fertility and endocrine markers measured using Abbott Architect assays. Outliers were detected and removed using a principle component analysis (PCA) score plot and Mahalanobis distance. PCA was performed by finding an alternate set of linearly uncorrelated variables called principal components. Loading plots were created, displaying main patterns of biomarker concentration and dependencies between them. Spearman's rank correlation coefficients were computed and displayed with a heatmap. Analysis was performed on all subjects, including children (<10 years), and adolescent males and females (≥10 years).

Results: A high level of agreement was observed between Spearman's heatmaps and PCA loading plots. Eighteen of 595 chemistry marker correlations examined had moderately strong Spearman's coefficients ($r \geq 0.50$) across all subjects, with fewer and unique correlations found when children and adolescents were analyzed separately. Seventeen of 190 fertility and endocrine marker correlations examined had moderately strong Spearman's correlations across all subjects. Again, fewer and unique correlations were found when children and adolescents were analyzed separately. Comparing loading plots and heatmaps between the age and sex separated groups, interesting correlation patterns were apparent. Thyroid hormones were a good example of this, where FT3 is positively correlated (heatmaps) and clustered in (loading plots) TT4 and FT4 in all subjects, children, and adolescent males. However, in adolescent females this is not apparent. Additionally, a high dependency between most fertility and endocrine markers with age is present in adolescent females, but is much less apparent in children and adolescent males.

Conclusion: This study provides, for the first time, a robust PCA and Spearman's correlation analysis for 35 chemistry and 20 fertility and endocrine markers in a healthy pediatric population. Identifying biomarker correlations will enable clinical laboratorians and physicians to consider the potential influence of other biomarkers on their biomarker of interest when establishing reference intervals, ordering laboratory tests, and interpreting laboratory test results.

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Urinary metabolome in autistic children and in their unaffected siblings: preliminary data on the role of oxidative stress and gut dysbiosis

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Background: Autism Spectrum Disorder or Disease (ASD) is a nonspecific term encompassing a wide range of complex neurodevelopmental disorders, characterized by repetitive and distinctive patterns of behavior and difficulties with social communication and interaction. The development and expression of ASD depend on multiple interactions between genetic, epigenetic, and environmental factors. Behind the plethora of potential risk factors, recent evidences suggest that most of environmental factors may arise from alterations in the gut microbial ecosystem. Indeed, perturbation of the gut microbiota can influence brain development and affect social, emotional, and anxiety-like behaviors by immune-mediated, neural, or humoral mechanisms. Current knowledge points out the role of metabolites derived from the gut microbiota in autism: they can modulate the behavioral phenotype of the host, greatly influencing host metabolic pathways and the immune system and determining the individual susceptibility to disease. The aim of this study was to identify the urine metabolic fingerprint in 21 autistic patients aged 4-16 years (18 males) and to compare it with that obtained in 21 healthy siblings aged 4-17 years (10 males). In addition, we investigated the gut microbiota composition in ASD patients. Methods: Samples analysis was done by Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy. For each sample, 630 µL were added to 70 µL of a 1.5 M phosphate buffer solution pH 7.4 with a final concentration of 0,5 mM TSP (trimethylsilyl propanoic acid). Each sample was stirred for 60 seconds and transferred into a 5 mm NMR tube for analysis. Spectra were acquired at 499,839 MHz using a Varian Unity Inova 500 MHz spectrometer (Bruker s.r.l., Milan, Italy). Gut microbiota in ASD patients was characterized by targeted sequencing of bacterial 16S rRNA genes in stool samples. Multivariate statistical analysis was performed by using the software SIMCA-P+13.0 (Umetrics, Umeå, Sweden). The final dataset was scaled using unit variance (UV) scaling to minimize the effect of spectral noise or high variability of the variables. Partial least Square Discriminant Analysis (PLS-DA) identified the set of important variables on the projection (VIPs); they were considered the most important metabolites responsible for differences in urine metabolome between groups. Results: Compared with controls, the urine metabolome in ASD children showed increased levels of hippurate, glycine, creatine, tryptophan and D-threitol as well as decreased levels of glutamate, creatinine, lactate, valine, betaine and taurine. These results suggest that children with ASD may show an imbalance in amino acid metabolism: the increase in tryptophan urine levels indicates alterations of the serotonergic pathway as well as the increase of hippurate, a mammalian-microbial co-metabolite, is closely related with gut dysbiosis. Concomitantly, the decrease in glutamate urine level suggests impairment in excitatory neurotransmission. Changes in urine levels of creatine and taurine are associated with an increasing oxidative stress, which in turn has been previously found in several studies on ASD. Gut dysbiosis in autistic children was characterized by a significant increase in *Clostridium spp*. Conclusion: In conclusion, oxidative stress and gut dysbiosis are involved in metabolic perturbations associated with ASD.

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Neonatal Bilirubin Testing in a Community Laboratory: An Opportunity for Physician Education

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Background: Significant hyperbilirubinemia must be diagnosed in newborn infants and neonates without delay to prevent the potential development of neurological impairment. If hyperbilirubinemia is suspected in a patient receiving care outside of a hospital setting, community laboratories in Ontario, Canada can provide urgent testing for neonatal bilirubin if requested by the ordering physician. **Objective:** Quantify the turnaround time (specimen collection to result reporting) for urgent and routine neonatal bilirubin testing in our community laboratory. Identify the prevalence of severe and critical hyperbilirubinemia results within our neonatal patient population, as defined by the Canadian Paediatric Society (CPS) and Ontario Association of Medical Laboratories (OAML). **Methods:** The turnaround time (TAT) and patient results from all neonatal bilirubin testing between Jan 2016 and Dec 2016 (*N*=1132) were retrospectively reviewed. Urgent testing was requested for 30.4% (*N*=264) of specimens. The average and median TAT time for each testing priority was tabulated as well as the frequency for which it exceeded 24 hr. Neonatal bilirubin (total serum bilirubin) results were evaluated against: our laboratories age-specific reference intervals (<24 hr, 34-103 µmol/L; 1-2 d, 103-120 µmol/L; 3-5 d, 68-205 µmol/L; 6 d-1 m, <70 µmol/L); and the age-specific concentrations for identifying severe (0-28 d, >340 µmol/L) and critical (0-28 d, >425 µmol/L) hyperbilirubinemia from the CPS and critical hyperbilirubinemia from the OAML (24-48 hr, >260 µmol/L; 49-72 hr, >310 µmol/L; >72 hr, >340 µmol/L). **Results:** Specimens requested for urgent neonatal bilirubin testing had a relatively shorter TAT (mean±SD, 8.3±4.5 hr; median, 8.0 hr) than those processed routinely (mean±SD, 12.3±14.0 hr; median, 10.0 hr). TAT exceeded 24 hr for 3.0% of urgent neonatal bilirubin tests and 9.7% of routine neonatal bilirubin tests, respectively. 90.0% and 81.3% of 1-2 day old neonates respectively receiving urgent and routine neonatal bilirubin testing had results above the upper limit of their reference interval. Regardless of age, the majority (>53%) of neonates receiving urgent testing had total serum bilirubin concentrations above the upper limit of their respective reference interval. The incidence of severe or critical hyperbilirubinemia, as defined by the CPS and OAML, within the examined population was relatively rare. For all urgently (*N*=264) tested neonates, one >72 hr old patient had a bilirubin concentration >340 µmol/L. The CPS defined concentration for critical hyperbilirubinemia (>425 µmol/L) was only exceeded by one neonate who received routine testing. Neonates receiving routine testing had a bilirubin concentration exceeding the OAML critical level hyberbilirubinemia for *N*=1 24-48 hr old (>260 µmol/L) and *N*=5 >72 hr old (>340 µmol/L) patients. **Conclusion:** Severe or critical hyperbilirubinemia is uncommon within the examined population. Urgent neonatal bilirubin testing provided by our community laboratory has a relatively shorter TAT than routine testing. However, it likely does not serve physician needs when the detected level of bilirubin within the neonate necessitates immediate therapeutic intervention. It is recommended that testing for suspected neonatal hyperbilirubinemia should be performed in an acute care setting, not a community laboratory. This study can be used by other community or reference laboratories to educate physicians on the associated risk of this diagnostic practice.

B-263

Biochemical sample testing in new MiniCollect® Blood Collection Tubes

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Background: Where small sample volumes are critical, especially for infants, elderly or obese patients, the new MiniCollect tube allows highest flexibility and accuracy by collecting blood in unprecedented simplicity. The MiniCollect Serum Separator and Lithium Heparin Separator tubes are intended to collect, transport, separate and process capillary blood for testing serum and plasma, respectively in the clinical laboratory.

Methods: Studies were done at Steyr Hospital (Austria) and Laboratory Rainbach (Austria) using MiniCollect tubes with old design vs. new design. Altogether, 80 hospitalized and 70 healthy subjects were recruited. Informed consent was given by all donors and the studies were approved by EC Upper Austria. Directly after blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. After centrifugation for 10 min at 3000g, 28 common biochemical analytes (venous) and 10 analytes (capillary) were tested using an AU680 and DxI800 (Beckman Coulter, precision within-run < 3%, total < 3%). Comparison testing to Microtainer (BD) was included. Analysis was done with the instrument's accompanying reagents. Statistical evaluation was done by STATISTICA 13.

Results: Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of tubes with old and new design for performance testing did not reveal any clinically nor statistically significant deviations (p<0.05). The values in both serum tubes of venous collection resulted in an initial highest deviation of 3.2%, and in plasma tubes of 4.4%. Comparable highest deviations for initial values in relation to

48h values were obtained for serum (5.3%) and plasma (6.4%). Capillary collection led to a highest deviation for LDH of 6.9% in serum, and in plasma tubes of 8.7%.

Conclusion: From a clinical perspective, the MiniCollect Serum Separator and Plasma Separator tubes with the new design are substantially equivalent to the tubes with the old design. The newly designed tubes provide an essentially enhanced blood collection device for skin-puncture testing. As the fundamental advantage is the guarantee of the sample integrity for high quality results in case of critical sample collections and transport of the tubes, the supporting information and data obtained from adult populations are more than adequate to establish safety and effectiveness for the patient indication.

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Evaluation of glucose concentration as a reliable indicator of blood gas sample contamination with total parenteral nutrition, lipid emulsion, and concentrated dextrose solutions

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Background: Blood gas samples from the neonatal intensive care unit (NICU) can be contaminated with intravenous solutions high in lipid and glucose such as total parenteral nutrition (TPN), lipid emulsion, or 50% dextrose in water (D50). We currently use glucose concentration >250 mg/dL as a sign of potential contamination. We performed *in vitro* spiking of blood gas samples with either a 1:1 mixture of TPN and lipid emulsion (mimicking administration in the NICU), or D50, to determine whether glucose concentration was a reliable indicator of blood gas sample contamination with these solutions; and determine whether a glucose threshold could be established to predict whether contamination produced blood gas and electrolyte results with greater than allowable error.

Methods: Residual lithium heparin arterial blood gas samples were pooled, transferred into tubes and spiked with a mixture (1:1) of TPN solution and 20% lipid emulsion (Intralipid, Germany), to final concentrations of 0, 1, 3, 5, 10 and 15%. Similarly D50 was added to pooled samples to final concentrations of 0, 1, 1.5, 2 and 2.5%. Samples were transferred to blood gas syringes and analyzed for pH, pO2, pCO2, hemoglobin, sodium, potassium, and ionized calcium on a Radiometer ABL 90 Analyzer (Radiometer, Denmark). After centrifugation plasma glucose was measured using a Roche Cobas c501 (Roche Diagnostics, IN). We determined whether a glucose threshold could be established to detect contamination with TPN/lipid emulsion or D50, and to predict when blood gas or electrolyte values exceeded laboratory-defined allowable error for each analyte.

Results: Addition of 3% or more of the TPN/lipid emulsion solution resulted in glucose concentrations >300 mg/dL, while addition of even 0.5% D50 resulted in glucose >400 mg/dL. Any amount of added TPN/lipid emulsion or D50 caused both pH and pO2 to exceed allowable error (±0.02 for pH and ±7.5 mm Hg for pO2). Addition of TPN/lipid emulsion up to 15% of sample volume had minimal impact on ionized calcium. For all other parameters addition of TPN/lipid emulsion resulted in greater than allowable error only when glucose concentrations >500 mg/dL, which occurred with 5% sample dilution. D50 contamination up to 1.5% (producing glucose results >900 mg/dL) did not cause greater than allowable error for any parameters other than pH, pO2 and pCO2.

Conclusion: Glucose concentration can be used as an indicator of significant sample contamination with either TPN/lipid emulsion or D50 solution. Sample contamination with TPN/lipid emulsion or D50 produces unreliable blood gas (pH, pCO2 and pO2) results. Contamination with concentrated D50 solution does not impact hemoglobin or electrolyte parameters even when the measured glucose concentration exceeds 900 mg/dL. In contrast with TPN/lipid emulsion contamination, greater than allowable error occurs in many measured blood gas parameters when glucose exceeds 500 mg/dL. The findings suggest that NICU samples with glucose >500 mg/dL should be rejected as potentially contaminated to prevent inaccurate results from being reported.

B-265

Verification of CALIPER pediatric Reference Intervals using a large community based pediatric population

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Background: The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) program used CLSI guidelines to establish pediatric reference intervals and transfer RIs to other platforms. The applicability of these RIs to other pediatric cohorts has not yet been assessed. The aim of this study was to verify CALIPER-established pediatric reference intervals (RIs) for calcium, iron, ALT, AST, total bilirubin, and alkaline phosphatase (ALKP).

Methods: Test results from a community laboratory obtained from September 2015-August 2016 were extracted from the information system for children <19 years of age. Individuals with abnormal results for clinically-related analytes were excluded. The remaining dataset was age- and sex-stratified according to CALIPER-recommended partitions, pared of outliers via log-transformation and Tukey's test, and used to verify the CALIPER RIs. If CALIPER RI did not pass with a 10% allowance, RIs were adjusted based on population data and verified with a second dataset obtained from September 2016-January 2017.

Results: CALIPER RIs for all age and gender partitions for calcium (n=140) and iron (n=3727) were verified. All partitions for total bilirubin (n=16,802) and ALT (n=6421), two partitions for AST (n=6504), and two partitions for ALKP (n=3609) failed verification, requiring the establishment of population-based RIs. In these cases, the population-based RI more closely matched the limits of the 95% confidence interval (CIs) for each CALIPER RI.

Conclusions: Our data demonstrate the applicability of CALIPER RIs to a filtered, community-based cohort and highlights the importance of verifying proposed RIs for specific populations. CALIPER RIs were adopted for some analytes and partitions, but for others, population-based RIs were required. This was particularly the case for analytes with non-Gaussian distributions, where a larger sample size is required to obtain narrow 95% CIs about the RI limits. With the exception of total bilirubin, the adopted RI was equivalent to the CALIPER RI when the CI was taken into consideration.

Verified age- and sex-specific pediatric reference intervals for biochemical markers						
Analyte	RI Origin	Age	Female Lower Limit (CI)	Female Upper Limit (CI)	Male Lower Limit (CI)	Male Upper Limit (CI)
Total Bilirubin	CALIPER	0 - 14 days	<	250 (249-252)	<	250 (249-252)
(mol/L)	CALIPER	15 days - <1 yr	<	10 (8- 11)	<	10 (8- 11)
	Population	1 - < 9 yrs	2 (2- 2)	11 (11- 12)	2 (2- 2)	11 (11- 12)
	Population	9 - <12 yrs	2 (2- 2)	12 (12- 13)	2 (2- 2)	12 (12- 13)
	Population	12 - <15 yrs	2 (2- 2)	22 (21- 24)	2 (2- 2)	22 (21- 24)
	Population	15 - < 19 yrs	3 (2- 3)	22 (22- 23)	3 (2- 3)	22 (22- 23)
ALT	CALIPER	0 - <1 yrs	<	25 (17-33)	<	25 (17-33)
(U/L)	Population	1 - <13 yrs	9 (8- 9)	30 (29- 30)	9 (8- 9)	30 (29- 30)
	Population	13 - <19 yrs	7 (7- 8)	28 (28- 29)	9 (9- 9)	38 (37 - 38)
AST	CALIPER	0 - 14 days	<	155 (140-169)	<	155 (140-169)
(U/L)	CALIPER	15 days - <1 yr	<	63 (48 - 78)	<	63 (48 - 78)
	CALIPER	1 - <7 yrs	<	41 (26- 55)	<	41 (26- 55)
	Population	7 - <12 yrs	20 (20- 20)	38 (38- 41)	20 (20- 20)	38 (38- 41)
	Population (Female) CALIPER (Male)	12 - <19 yrs	14 (13- 14)	28 (28- 29)	<	32 (17- 47)

Evaluation of the BD Vacutainer® UltraTouch Push™ Button Blood Collection Set in a tertiary pediatric hospital

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Introduction: Blood collection in pediatrics can be stressful for the child and challenging for the phlebotomist. Smaller gauge needles are preferred as they lessen patient discomfort and improve difficult venous access during venipuncture. However, smaller gauged needles are associated with increased incidence of sample hemolysis, which can interfere with many laboratory tests and carries the risk of a re-draw. BD introduced the BD Vacutainer® UltraTouch™ Push Button Collection Set (BD UltraTouch™) with ultra-thin wall technology which provides a needle with a larger inner diameter without increasing the outer diameter (gauge size). The aim of this study was to evaluate the performance of the BD UltraTouch™ in a tertiary pediatric hospital and examine the effect of a smaller gauge needle on sample hemolysis rates.

Methods: Blood was collected from patients, aged 7-18 years old, in the phlebotomy clinic at The Hospital for Sick Children, Toronto, Canada, during an evaluation period of four days. We compared hemolytic index (HI) and potassium results in patients with blood drawn using the BD UltraTouch™ versus the current Terumo Surshield Safety Winged Infusion Set (Terumo Surshield™). Patients were age–matched when possible during the evaluation period. 61 specimens collected with the BD UltraTouch™ and 41 specimens collected with Terumo Surshield™ were included in the study. The acceptance criterion for potassium was ±4% between the needle sets. Results from the evaluation period were validated on a larger cohort of patients following the implementation of a hospital wide switch in needle sets. We examined the HI and the rate of critical potassium results due to sample hemolysis using one month of data when phlebotomy was performed using the Terumo Surshield™ and following the implementation of the BD UltraTouch™ for phlebotomy.

Results: Analysis of the evaluation period showed 87% (53) of patients collected with BD UltraTouch™ had a hemolytic index (HI) <15; 13% (8 patients) had HI of 15-50, indicating mild hemolysis. Using the Terumo Surshield™, 71% (30) of patients had HI <15; 21% (9) had HI 15-50; and 7% (3) had HI >51. There was no significant difference in HI between needle sets. Furthermore, no significant differences in hemolysis rates between the BD UltraTouch™ 21G, 23G and 25G needles were observed. Potassium results showed acceptable differences between samples collected with the two needle sets. Validation using a larger cohort of patients showed no significant difference in the rates of gross hemolysis (HI >300) between the needle sets; 1.2% (n=7645) when using the BD UltraTouch™ vs. 1.3% (n=8852) using the Terumo Surshield™. In addition, there was no significant difference in the rate of potassium critical results due to sample hemolysis between BD UltraTouch™ or Terumo Surshield™ sets, as both needle sets reported approximately 80% samples with critical potassium were due to hemolysis.

Conclusions: These data show there were no significant differences in sample hemolysis or the number of critical potassium results due to hemolysis when using the new BD UltraTouch™ vs. the Terumo Surshield™. This allows for the use of smaller gauge needles without increasing the risk of sample hemolysis.

Evaluating pediatric eGFR approximation with growth-curve derived height and creatinine reference intervals

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Background: Although less prevalent than in adults, Chronic Kidney Disease (CKD) among children has steadily increased over time. Late recognition, precluding early intervention to improve growth and development, remains a concern. The aims of this study were to test, in principle, an approximated calculation of pediatric estimated glomerular filtration rate (eGFR) when empirical height data is not available, and to determine whether children identified by the lower approximated eGFR were also flagged by an elevated creatinine. Comparisons of pediatric creatinine reference ranges generated by the Caliper study versus site specific reference ranges employed at local pediatric and adult health centers were also carried out.

Methods: Retrospectively, one year of creatinine results for children and adolescents aged 2-18 years were collected from the local pediatric and adult/community health centers. Data were sorted to select for outpatient, non-nephrology, non-emergency department results. Approximate eGFR was calculated using the updated Bedside Schwartz equation by applying age- and gender-specific height data from WHO growth charts at the third, fiftieth, and ninety-seventh percentiles.

Results: When an approximation of eGFR was calculated using growth curve heights, the majority of children identified with values under 75 mL/min/1.73m² were age 13 and older, representing half of identified children at the pediatric center and 98% at the adult/community center. The majority of these values also fell between 60 and 75 mL/min/1.73m². Using the 3rd percentile from the growth curves, the percentage of children with an approximated eGFR under 75 mL/min/1.73m² was 2.9% at the pediatric center (P)and 15% at the adult center (A). At the 50th and 97th percentiles these percentages were 1.26% (P) and 5.3% (A), and 0.65% (P) and 1.4% (A), respectively. Of children identified (approx. eGFR <75 mL/min/1.73m²) using the 3rd percentile of height, all under age 11 years had an elevated creatinine flag by both locally established (IWK) ranges and by Caliper-transferred reference ranges. Between ages 11 to <15 years, creatinine did not flag by IWK ranges, but did flag by Caliper ranges. At 15-years and older, IWK reference ranges did not flag a creatinine elevation, while Caliper ranges flagged creatinine elevations for females, but poorly for males. This trend was also apparent, in a lesser degree, in the subset of children identified by using the 50th percentile of height. Chart review of selected cases indicates there may be value added in providing additional information to physicians, with flagging creatinine values or approximation of eGFR, particularly for older children and adolescents.

Conclusions: Approximating eGFR using WHO growth charts for age- and gender-specific heights to apply the Bedside Schwartz equation predictably identified an increasing proportion of children with lowered eGFR as lower height percentiles were applied. Interestingly, even at the 3rd percentile, all children identified under age 11 also had a creatinine reference range flag. Caliper reference ranges flagged more children than the IWK reference ranges, particularly for older children and females. Application of the calculation likely generates false positives, depending on the height percentile used, but may identify subclinical cases that would benefit from closer scrutiny.

Defining the reference range of prealbumin in healthy children and adolescents

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Background: The definition of reference ranges in laboratory is a great challenge, especially in pediatric population. In Brazil, laboratories release their test result with RR based on population from other countries. Prealbumin is a protein synthesized in the liver and it´s important for nutritional evaluation pediatrics because it transports thyroxine and carries retinol-binding protein. To define the reference range (RR) of prealbumin in healthy children and adolescents aged 1-12 years 11 months and 29 days from Cuiabá, capital of Mato Grosso.

Methods: This is a descriptive study, conducted in 1,866 healthy children and adolescents from kindergartens and schools of the capital city, obtained by random sampling. A questionnaire assessing the individual background and their relatives besides demographic and anthropometric data had been also carried out. Were defined as inclusion criteria of the study, children and adolescents in the group 1-12 years 11 months and 29 days without any underlying disease or diagnosed clinical complaints at the time of collection. In addition, participants should not take any regular medication. The samples were collected during fasting period and were determined by nephelometry (BN II Siemens). Regarding statistic methodology, we did analyse the homogeneity of variances by Bartlett´s test for each parameter by age and subsequently by ANOVA or Kruskal-Wallis test to check the differences between age groups. The test "pos hoc" Bonferroni was applied when it was found the difference to regroup similar age groups, thus constituting a new age bracket. After this procedure the Bartllet test was applied, as it result, we did conduct ANOVA or Kruskal-Wallis to check if the groups remained. Then proceeded to the exclusion of extreme values (outliers) taken as those values above or below the mean ± 3 standard deviations. After excluding outliers, obtained the RI as the mean ± 2 standard deviations of the remaining values. The significance level was 5%. The project was approved by the Research Ethics Committees of the institutions involved. **Results:** The subjects were grouped into four different age groups: aged 1 year; 2-7 years; 8-10 years and 11-12 years. The RRs found for each age group were 0.09 g/L to 0.22 g/L for the group aged 1 year; 0.11 g/L to 0.26 g/L for the group aged 2-7 years; 0.13 g/L to 0.28 g/L for the group aged 8-10 years and 0.13 g/L to 0.31 g/L for the group aged 11-12 years.

A few studies have defined the RR of prealbumin levels in children and adolescents worldwide. The IFCC studied newborns and elderly people aged up to 60 and grouped them into six age groups, showing that prealbumin increased progressively with age. In the age group 1-2 years, the values (0,11 to 0,26 g/L) were very similar to those found in this study (0,09 to 0,22 g/L) for the 1 year-old age group.

Conclusion: The RR of prealbumin levels observed in children and adolescents from Cuiabá may be used as a reference for interpreting results of blood tests in Brazilian children and adolescents. Further studies from other Brazilian states should be done.

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Establishment of pediatric reference intervals for alkaline phosphatase in Korean children based on retrospective analysis

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Background: Hypophosphatasia is an inborn error of metabolism characterized biochemically by deficient activity of the tissue-nonspecific isoenzyme of alkaline phosphatase (ALP). ALP activity is one of sensitive markers of hypophosphatasia. However, it is particularly important and difficult to establish appropriate reference intervals for ALP due to development and growth of children. No more than three subgroups according to the sex were divided on reported studies of pediatric reference intervals in Korea. We aimed to establish more age- and sex- specific partitioned reference intervals for ALP in Korean children by using reported studies.

Methods: Blood test results were collected for 4,290 children aged 0-18 year from Korean National Health & Nutrition Examination Survey data which established from a healthy community children, and reported Severance Hospital data and multicenter study data of which children underwent minor surgeries in seven university hospitals. Medical records were reviewed to exclude patients with underlying diseases or any illnesses other than the diagnosis for the minor surgery. The reference interval settings complied with the Clinical & Laboratory Standards Institute (CLSI) guidelines. After eliminating outliers, we used the 2.5 percentile as the lower limit and the 97.5 percentile as the upper limit in the same subgroup as CALIPER. According to CLSI EP9-A2 guideline, reference intervals were transferred to assays performed on five different clinical chemistry platforms including Roche cobas 8000 and modular DP, Abbott C16000, Toshiba 200FR NEO, and Beckman Coulter AU5800.

Results: Currently, provided reference intervals for ALP using Roche cobas 8000 were as follows: female, 151-465 IU/L (15 day-1 year), 153-388 IU/L (1-10 year), 134-428 IU/L (10-13 year), 64-258 IU/L (13-15 year), 54-158 IU/L (15-17 year), 38-96 IU/L (17-19 year); male, 153-511 IU/L (15 day-1 year), 145-364 IU/L (1-10 year), 151-435 IU/L (10-13 year), 102-420 IU/L (13-15 year), 77-305 IU/L (15-17 year), 52-192 IU/L (17-19 year). Compared with the results of the CALIPER study, the reference intervals for ALP were similar. Among five different hospital analyzer platforms, the results of each platform except Beckman Coulter AU5800 were not significantly different.

Conclusions: This is the first study to present detailed Korean pediatric reference intervals for ALP using three databases. It should assist laboratorians and pediatricians in interpreting test results more accurately and thereby to improved diagnosis of hypophosphatasia.

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reference intervals for serum and urinary creatinine urinary albumin and albumin creatinine ratio in healthy children and adolescents in cuiaba mato grosso brazil

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Background: : Establishment of Reference Intervals (RI) is an arduous task for clinical laboratories however it´s fundamental importance to medical decision making. A few studies on pediatric populations have been carried out to determine creatinine dosage by colorimetric assay and cinetic analysis, as well as to quantify urinary albumin by turbidimetry. The objective of this study was define the reference ranges (RR) for serum and urinary creatinine, urinary albumin and albumin/creatinine ratio in the urine of healthy children and adolescents from Cuiabá, Mato Grosso, Brazil. **Methods:** This cross-sectional study was approved by the Ethics Committee of the University of São Paulo and Julio Muller University Hospital of Federal University of Mato Grosso. In a total of 1,886 healthy children and adolescents from daycare centers and municipal schools in the city of Cuiabá were evaluated . A questionnaire assessing the individual background and their relatives besides demographic and anthropometric data had been also carried out. Were defined as inclusion criteria of the study, children and adolescents in the group 1-12 years 11 months and 29 days without any underlying disease or diagnosed clinical complaints at the time of blood and urine collection. In addition, participants should not take any regular medication. The samples were collected during fasting period and were determined using cobas® 6000 (analyser series - Roche Diagnostics). Regarding statistic methodology, we did analyse the homogeneity of variances by Bartlett´s test for each parameter by age and subsequently by ANOVA or Kruskal-Wallis test to check the differences between age groups. The test "pos hoc" Bonferroni was applied when it was found the difference to regroup similar age groups, thus constituting a new age bracket. After this procedure the Bartllet test was applied, as it result, we did conduct ANOVA or Kruskal-Wallis to check if the groups remained. Then proceeded to the exclusion of extreme values (outliers) taken as those values above or below the mean $\pm$ 3 standard deviations. After excluding outliers, obtained the RI as the mean $\pm$ 2 standard deviations of the remaining values. **Results:** The serum creatinine was studied in 8 aged group: 1-2 years; 3 years; 4 years; 5 years; 6-7 years, 8 years; 9-11 years and 12 years and the Reference Range (mg/dL) for all these age groups were, respectively: 0,20-0,40; 0,22-0,46; 0,27-0,47; 0,29-0,51; 0,32-0,57; 0,33-0,62; 0,35-0,69 e 0,38-0,72. The Reference Range of urinary creatinine (mg/dL) was proposed into three different age groups: 1-3 years (up to 125); 4-11 years (up to 182) and 12 years (up to 261). The studied showed that urinary albumin level was up to 2,12 mg/dL and albumin/creatinine ratio in an isolated sample was up to 0,03 mg/dL in both cases, the subjects were group into a single age group. **Conclusion:** Defining RR for these analytes in Brazilian population may allow us to better interpret test results in Brazil. Other similar studies in different regions may corroborate these results.

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Performance Evaluation of the ADVIA Centaur PlGF and sFlt-1 Assays

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Background: Preeclampsia (PE), a disease found in pregnant women, is characterized by hypertension and proteinuria. Rapid, accurate diagnosis of PE is essential for best clinical practice and maternal/fetal care. PlGF and sFlt-1, both produced by the placenta, regulate fetal angiogenesis. The sFlt-1/PlGF ratio becomes elevated in PE; therefore, this ratio is valuable in helping diagnose PE. Siemens Healthineers (Tarrytown, NY, U.S.) has developed ADVIA Centaur® PE assays* for detecting PlGF and sFlt-1 in serum and plasma to be used in tandem for a clinically relevant ratio of diagnostic value. This study evaluated performance of the ADVIA Centaur PlGF and sFlt-1 assays.

Methods: The assays were evaluated on ADVIA Centaur Immunoassay Systems for precision, method comparison, linearity, limit of detection (LoD), limit of quantitation (LoQ), calibration interval, onboard stability (OBS), hook effect, and endogenous interferences. Clinical agreement with Roche ELECSYS PE assays for >150 clinically relevant samples was also evaluated. Also, sensitivity and specificity of the PE cutoff

ratios were evaluated at ≤34 and >34 weeks gestation from normal and physician-diagnosed PE patients.

Results: The PlGF and sFlt-1 assays have respective ranges of 10-10,000 and 30-85,000 pg/mL, with acceptable sensitivity and specificity within early and late gestational windows. The precision results were <8% CV, and both were linear within each assay's range, with no observed hook effect. The LoD and LoQ were <10 pg/mL (PlGF) and <30 pg/mL (sFlt-1). The calibration interval and OBS for the ADVIA Centaur PlGF and sFlt-1 assays were >14 and >28 days, respectively. <10% endogenous interference was observed for all tested interferents for both assays. In addition, the ADVIA Centaur and ELECSYS PE assays showed strong positive (98.2%) and negative (98.8%) agreement by clinical outcome.

Conclusions: These studies indicate that the ADVIA Centaur PE assays are precise and sensitive for measuring PlGF and sFlt-1 across a wide range of concentrations.

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