# Wednesday, August 1, 2018

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

Maternal-Fetal, Pediatrics, and Fetal Clinical Chemistry

## B-310

### Fetal risk assessment in high-risk Brazilian first trimester pregnant: a retrospective observational study.

G. M. O. Soares[1], P. D. R. Cirillo[2], E. Cueva Mateo[2], F. L. O. Marinho[2]. *[1]Hermes Pardini Institute, Vespasiano, Brazil, [2]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil*

**Background**: Aneuploidies are the most common genetic abnormalities, which can be detected during prenatal care. Trisomies involving chromosomes 13 (Patau syndrome), 18 (Edward's syndrome) and 21 (Down's syndrome) are the most frequently aneuploidies identified by karyotyping or high-risk serum markers. In addition, open neural tube defects (NTDs) is also investigated in fetal risk assessment. Recently, prenatal screening for these disorders are based in tests which evaluate the concentration of serum markers and combine them with information about the pregnant health, such as age, weight, and gestational age. Together, these data estimate the fetal risk to develop the disorders above. The Multiples of the Median (MoM) values are calculated using a database resource of low-risk pregnancies with adjusted data for gestational age, insulin-dependent diabetes mellitus, multiple pregnancy, *in-vitro* fertilization, smoking status and systematic differences between laboratories and assay reagents. **Objective**: The aim of this study was to describe the high-risk gestational profile of first trimester pregnant (between 10 and 13 weeks of pregnancy) from samples collected at Hermes Pardini Institute between 2016 and 2018. **Methods:** The AutoDELFIA™ hAFP/Free hCGß (PerkinElmer, US) and AutoDELFIA® PAPP-A Kit (PerkinElmer, US) were used for detection of the total and free ß human chorionic gonadotrophin (hCG) and pregnancy associated plasma protein A (PAPP-A) levels, respectively. All the assays were performed following manufacturer's instructions. The ALPHA program (Logical Medical Systems Limited, UK) was used to calculate the risk of pregnancies with trisomies involving chromosomes 13, 18 and 21, and open neural tube defects (NTDs) in first trimester of pregnancy. In addition, this software considers the serum markers levels besides the value of nuchal translucency (NT). **Results:** One total of 10,301 patients data were analyzed between 2016 and 2018 and the median of maternal age was 32 years (51.6% of cases). Among them, 88.1% of cases were negatives for Down's syndrome and NTD, and 11.4% were positive for the disorders investigated: 9.9%, 0.8% and 0.7% showed increased risk for Down's syndrome, trisomy 18 and for others fetal risk disorders development, respectively. It were not reported risk for trisomy 13. The results observed for increased risk of the disorders at pregnant age were 2.0%, 9.7%, 49.8%, and 38.5% for 10, 11, 12, and 13 weeks, respectively. **Conclusions**: Currently, the assessment of fetal risk is indicated only for high-risk pregnancies.According to the Brazilian Ministry of Health and National Institutes of Health of US, advanced maternal age (>35 years) is one of the criteria to characterize one high-risk pregnancy.. Despite this, it was observed that the mean age of the cases evaluated was 32 years, indicating that one sample of Brazilian women had high-risk pregnancy with earlier age. Therefore, the assessment of fetal risk could be a good predictor for high-risk pregnancies, since it is less invasive than the gold standard techniques such as karyotyping, and seems to be more assertive than image tests alone.

## B-311

### The effect of the enzyme replacement therapy on the liver function tests in children with c Disease (GD)

H. A. Abdulhussain[1], H. A. Abdulamir[2], H. T. Noaman[3], H. S. Arif[4]. *[1]Ain Al-Tamer Hospital, Karbala, Iraq, [2]College of Pharmacy-Al-Nahrain University, Baghdad, Iraq, [3]College of Medicine, AL-Mustansiriyah University, Baghdad, Iraq, [4]Pediatric Department College of Medicine, Al-Nahrain University, Baghdad, Iraq*

**Background**: GD is an inherited autosomal recessive disease. It is most common in the Ashkenazi Jewish population. Many biomarkers might be involved in the etiology, pathogenesis, diagnosis and prognosis of this disease in children. Most of them are related to complications due to an involvement of many organs such as liver due to lack of the glucocerebrosidase enzyme. **Objectives:** to investigate the role of assisting liver function tests in the diagnosis and monitoring Gaucher patients receiving enzyme replacement therapy. **Methods:** A case control study was done on 67 children (male & female) age range from 1-15 years who had GD recruited from Pediatric Department and Unit of rare disease at Al Imamain Al-Kathemeaain medical city, Gastroenterology and Hepatology Teaching Hospital, Children Welfair Hospital Consultation Clinic and Central Child's Teaching Hospital. The levels of ALT, AST, ALP, total bilirubin and total protein were measured in the samples of 67 Gaucher patients who were categorized as newly diagnosed un treated patients (n=9), patients receiving ERT for 3-6 months (n=18) 6-12 months (n=20) and patients receiving ERT for more than one year (n=20) and compared with twenty newly comparable age-matched control subjects. The practical part of the study was conducted in the Department of Chemistry and Biochemistry, College of Medicine, University of Al-Nahrain from December 2016 to March 2017. The levels of these biomarkers were determined by colorimetric methods according to manufacturer instruction. **Results:** The data indicated that the mean± standard deviation (SD) levels of ALP in whole Gaucher patients (210.27 ± 61.21 U/L) were significantly higher ($p<0.05$) than that of age-matched controls (163.17 ± 49.34 U/L, respectively) while the level of total protein in patients (6.29 ± 0.73 g/dl) were significantly lower ($p<0.05$) than that of age-matched controls (6.81 ± 0.32 g/dl). On the other hand, non-significant differences were illustrated in the levels of ALT, AST and total bilirubin. These parameters were remarkably associated with the period of receiving treatment with ERT that indicated by the negative significant ($p<0.05$) correlations between the levels of AST (r=-0.476; $p<0.001$), ALT (r=-0.448; $p<0.001$), ALP (r=-0.394; $p<0.001$) and total bilirubin (r=-0.343; p=0.001) and period of receiving treatment and positive significant ($p<0.05$) correlations between the levels of total protein (r= 0.484; $p<0.001$) and the period of receiving treatment. The effect of ERT also revealed be the results obtained by ANOVA test that indicate significant ($p<0.05$) differences among the patients subgroups in the levels of ALT, AST, total bilirubin and total protein. I I **Conclusions:** Liver function tests showed to have a diagnostic value in newly diagnosed untreated patients with diversity in their response to the treatments that limit their role in the monitoring of the treatment. III

## B-312

### Reformulation of the Roche total bilirubin Gen3 reagent did not affect the relationship between BiliChek transcutaneous and Roche total serum bilirubin

J. C. Jara-Aguirre, A. M. Wockenfus, K. L. Fine, W. J. Cook, B. S. Karon. *Mayo Clinic, Rochester, MN*

**Background**: American Academy of Pediatrics guidelines recommend transcutaneous (TcB) or total serum (TSB) bilirubin measurement for many newborns. In our institution all term infants are screened with TcB prior to nursery discharge. TcB is plotted with post-natal age in hours to determine risk for severe hyperbilirubinemia using the Bhutani nomogram. Infants with high intermediate risk (HIR) or high risk (HR) TcB values have confirmatory TSB values determined, with all further treatment based upon TSB. The use of different bilirubin laboratory methods or changes in the calibration or formulation of the laboratory methods may impact the relationship between TcB and TSB, and thus the efficacy of TcB screening. In 2014 Roche Diagnostics announced a reformulation of the total bilirubin reagent that could potentially affect bilirubin results. The objective of this study was to determine whether reformulation of the Roche total serum bilirubin reagent affected the relationship between TcB and TSB. **Methods**: TcB results of all neonates in the level 1 newborn nursery with a subsequent TSB measurement within 1 hour were reviewed; during a period of six months before and after the conversion from the old Roche total bilirubin (BILTS) reagent to the new Roche Gen3 bilirubin assay. TSB was measured on a Roche Cobas c501 analyzer (Roche Diagnostics, IN). TcB measurements were performed using the BiliChek transcutaneous bilirubin monitor device (Respironics, Marietta GA), and calibrated with a disposable tip (BiliCal). Distribution of TSB results, and TcB minus TSB bias, were compared before and after the introduction of the reformulated Roche total bilirubin Gen3 assay. Median and interquartile range (IQR) TSB values, and median and IQR bias (TcB minus TSB) were calculated. A statistical difference between median values of TSB and median bias were assessed using Man-Whitney test. **Results**: A total of 301 paired (obtained within one hour of each other) TcB and TSB results were obtained, 172 before and 129 after implementation of the reformulated Roche Gen3 reagent. The distribution of TSB results, before and after the implementation, showed a similar pattern. TSB median (IQR) concentration was 7.8 (6.8-8.7) mg/dL before and 7.6 (6.7-8.4) mg/dL after implementation of the reformulated reagent (p=0.1373). Median (IQR) bias between TcB and TSB was 2.9 (2.2-3.7) mg/dL

before the reformulated reagent was implemented; and did not change at 2.9 (2.1-3.9) mg/dL after the reformulated reagent was implemented (p=0.8242). **Conclusion**: Reformulation of the Roche total bilirubin Gen3 assay did not affect the relationship between BiliChek transcutaneous and serum bilirubin; and thus no changes were needed to the neonatal TcB screening protocol as a result of the modified bilirubin reagent.

## B-313

### Association of Fibroblast Growth Factor 21 Plasma Levels with Infection in Neonates: Preliminary Results

T. Siahanidou[1], A. Margeli[2], V. Bourika[1], I. Papassotiriou[2]. *[1]Neonatal Unit, First Department of Pediatrics, Athens University Medical School, Athens, Greece, [2]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece*

**Background:** Infections remain one of the leading causes of morbidity and mortality in neonatal age and may also have severe long-term consequences. Identification of new or complementary biomarkers of neonatal infection or sepsis is of great importance. Fibroblast growth factor 21 (FGF21) is a member of the FGF superfamily, consisting of FGF19, FGF21, and FGF23. FGF21 has emerged as a key regulator in the metabolism of glucose and lipids. A possible role of FGF21 in sepsis has been suggested by the observation of increased circulating levels of this hormone during experimental sepsis in mice. Moreover, its administration has a protective effect from the toxicity of lipopolysaccharide (LPS) and sepsis. FGF21 can also reduce the severity of cerulein-induced pancreatitis in mice, further indicating that FGF21 could modulate inflammation. These findings highlight the possible role of FGF21 as a biomarker and a therapeutic tool in mice with sepsis and an inflammatory state. As the involvement of FGF21 in neonatal infection is not known yet we aimed to explore the clinical value of circulating FGF21 levels as biomarker of neonatal infection. **Methods:** Seventy-seven full-term neonates were included in the study: of them 25 with febrile bacterial infection and 52 without any infections. Along with hematologic and blood chemistry parameters, plasma levels FGF-21 were determined by means of an immunoenzymatic technique. **Results:** Plasma FGF21 levels were significantly higher in neonates with infection compared to controls ($p<0.001$). FGF21 levels on admission correlated significantly with serum CRP levels ($r_s=0.487$, $p=0.01$) and also with plasma glucose ($r_s=0.446$, $p<0.05$) and triglyceride levels ($r_s=0.419$, $p<0.05$). In multiple regression analysis, the correlation between FGF21 and CRP levels remained significant after adjustment for glucose or triglyceride levels. Receiver operating characteristic analysis of FGF21 levels resulted in significant areas under the curve (AUC) for detecting infected neonates on admission (AUC=0.965, $p<0.001$). **Conclusions:** Circulating FGF21 levels are increased at the acute phase of neonatal infection possibly reflecting and/or participating in the inflammatory process, and correlated also with metabolic parameters. Thus as sepsis is associated with insulin resistance, we can also hypothesize that the increase in plasma FGF21 observed in the neonates with infection might also be due, at least partly, to insulin resistance. Insulin resistance in sepsis is due to a decreased effect of insulin, but also reflects an imbalance between insulin and its counter-regulatory hormones such as cortisol, glucagon, growth hormone and catecholamines. FGF21 may be used as an early marker of neonatal infection, however, prior to its clinical usefulness, this protein must undergo through rigorous validation in multiple cohorts.

## B-314

### Acetylserotonin O-Methyltransferase (ASMT)/rs4446909 Polymorphism in Iraqi Autistic Children

H. A. Abdulamir[1], H. A. Abdulhussain[2], S. J. A. Al-Awadi[3], O. F. Abdul-Rasheed[4], E. A. Abdulghani[5]. *[1]College of Pharmacy-Al-Nahrain University, Baghdad, Iraq, [2]Ain Al-Tamer Hospital, Karbala, Iraq, [3]Department of Biomedical technologies, College of applied Biotechnology, Al-Nahrain University, Baghdad, Iraq, [4]Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [5]Consultant Psychiatrist, Advisor for Mental Health, Ministry of Health, Baghdad, Iraq*

**Background**: The genetic causes in addition to hormonal, neurological, and immunological basis for autism is still not fully understood, and the role of the interaction among neuro-inflammation, genetic, immunological mediators and neurotransmission impairment needs to be clearer. ASMT is an enzyme that involved in the synthesis of melatonin which is assumed to have a possible role in autism pathogenesis. **Objectives:** to explore the potential effect of the acetylserotonin O-methyltransferase (ASMT)/rs4446909 polymorphism on the risk of autism and to test the possible association between this single nucleotide polymorphism (SNP) with the severity of social and cognitive dysfunctions in male children with autism in order to assess the possibility of using this SNP in the prognosis of autism severity. **Methods:** A case control study was carried out in the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad- Iraq and Forensic DNA Research and Training Center/Al-Nahrain University\Baghdad\Iraq. The study was done on 60 male patients with autism who were recruited from Department of Pediatrics at Al-Sader Hospital, Baghdad-Iraq between November 2014 and April 2015. DNA obtained from the Erythrocytes of autistic male patients who were categorized as mild (n=20), moderate (n=20) and severe (n=20) according to diagnostic and statistical manual of mental disorders and compared with Thirty age-matched control subjects. The genetic polymorphisms ASMT/rs4446909 were detected by polymerase chain reaction-Restriction fragment length polymorphism (PCR-RFLP) method. **Results:**
It was demonstrated that there were non-significant differences in ASMT/rs4446909 polymorphism between the whole autistic group patients and control groups. On the other hand, the study of ASMT/rs4446909 polymorphism revealed that severe autistic patients who carried G/G genotype showed a significant higher autism risk when compared to individuals who carried the A/G genotype. **Conclusion:** ASMT/rs4446909 polymorphism is not found to be associated with autism in the studied children whereas an association between the severity of autism and studied genotype were illustrated. This may pinpoint the involvement of this polymorphism in the pathogenesis of autism.

## B-315

### The Effect of the Enzyme Replacement Therapy on the Kidney Function Tests & Serum Electrolyte Levels in Children With Gaucher Disease

H. A. abdulhussein[1], F. H. Al- Obaidi[2], H. S. Arif[3], H. A. Abdulamir[4]. *[1]Ain Al-Tamer Hospital, Karbala, Iraq, [2]Department of Chemistry and Biochemistry-College of Medicine-Al-Nahrain University, Baghdad, Iraq, [3]Pediatric Department - College of Medicine-Al-Nahrain University, Baghdad, Iraq, [4]College of Pharmacy-Al-Nahrain University, Baghdad, Iraq*

**Background**: Gaucher disease is an inherited autosomal recessive disease. It is most common in the Ashkenazi Jewish population. Many biomarkers might be involved in the etiology, pathogenesis, diagnosis and prognosis of Gaucher disease (GD) in children. Most of them are related to complications due to an involvement of many organs such as liver, spleen and bones by this lysosomal storage disease that caused by a lack of the enzyme glucocerebrosidase. **Objectives:** to investigate the role of kidney function test and electrolytes (urea, creatinine, sodium and potassium) level in the monitoring of the response for the treatment used for patients with Gaucher's disease in follow-up manner **Methods:** A case control study was done on 67 children (32 male & 35 female) age range from 2-14 years (mean± SD; 5.3±2.9). The levels of sodium, potassium, urea and creatinine were measured in the samples of patients who were categorized as newly diagnosed untreated patients (n=9), patients receiving ERT for 3-6 months (n=18) 6-12 months (n=20) and patients receiving ERT for more than one year (n=20) and compared with twenty age-matched control subjects (9 male & 11 female) age range from 2-14 years (mean± SD; 5.55± 3.05). **Results:** The data indicated that the level of urea in GD patients (23.39 ± 4.71 mg/dl) was significantly higher than that of age-matched controls (17.5 ± 3.05 mg/dl). Non-significant differences were illustrated in the levels of sodium, potassium and creatinine. Negative significant ($p<0.05$) correlations were obtained between the levels of urea ($r= -0.752$; $p<0.001$) and creatinine ($r= -0.536$; $p<0.001$) with the period of receiving ERT. Additionally, ANOVA test also revealed significant ($p<0.05$) differences among the patients subgroups in the levels of urea and creatinine. Results obtained from Receiver Operating Characteristic (ROC) curve revealed that urea and creatinine showed a high area under the curve (AUC), sensitivity and specificity (0.939, 77.8% and 85% for urea and 0.978, 100% and 80% for creatinine respectively) in newly diagnosed GD patients in a comparison with control. **Conclusions**: the possibility of using urea and creatinine in the diagnosis and monitoring the effect of ERT on the GD patients.

## B-316

### CALIPER Pediatric Reference Intervals for Siemens Biochemical Assays on ADVIA XPT and Dimension EXL with LM Integrated Chemistry Systems

H. Tahmasebi, V. Higgins, K. Adeli. *The Hospital for Sick Children, Toronto, ON, Canada*

**Background:** Reference intervals (RIs) are the central 95% of laboratory test results obtained from a cohort of healthy reference individuals. Accurate RIs are required for clinical interpretation of test results and diagnosis. However, several gaps exist in pediatric RIs due to the significant physiological changes that occur during pediatric development as well as difficulties involved in recruiting a large number of children and adolescents. CALIPER (Canadian Laboratory Initiative on Paediatric Reference Intervals) is a Canada-wide initiative to fill these gaps by establishing age- and sex-specific RIs on various clinical chemistry analyzers. The current study expands the CALIPER database by establishing age- and sex-specific RIs on two Siemens platforms: ADVIA Chemistry XPT and Dimension EXL with LM Integrated Chemistry Systems. **Methods:** A large cohort of healthy children and adolescents (<19 years old) who completed health questionnaire forms were recruited from GTA (Greater Toronto Area) and Hamilton regions as part of the CALIPER study and donated blood samples. Those with acute or chronic illnesses and/or recent medication use were removed from analysis. Serum samples of a total of 909 and 867 healthy participants were tested on ADVIA XPT (33 assays) and Dimension EXL (21 assays) systems, respectively. Analyte concentrations were visually inspected for age- and sex-based partitions, which were statistically confirmed using Harris and Boyd's statistics. Outliers were removed using Tukey or adjusted Tukey for parametric and nonparametric data, respectively. According to CSLI C28-A3 guidelines, age- and sex-specific 95% RIs, along with 90% confidence intervals, were calculated using either the nonparametric rank method ($n \geq 120$) or the robust method of Horn and Pesce ($40 \leq n < 120$). **Results:** Serum concentrations of several assays remained relatively constant within pediatric age range and similar between sexes, including C4, cholesterol, CRP, sodium, total iron binding capacity, and triglycerides. Other tests, such as alkaline phosphatase, enzymatic creatinine, lactate dehydrogenase, and total bilirubin, showed significant changes throughout pediatric age and differences were evident between males and females mostly after puberty. Furthermore, immunoglobulin G, total protein, and direct bilirubin and several others required age partitioning, but sex differences were not observed even after puberty. **Conclusion:** Age- and sex-specific RIs were established for a combined total of 54 assays on Siemens ADVIA XPT and Dimension EXL systems. These results will allow for a more accurate laboratory assessment of pediatric patients with the use of these two Siemens platforms in clinics and hospitals around the world. However, it is recommended that these reference values be verified, based on CLSI guidelines, using local pediatric samples and analyzers before clinical use.

## B-317

### Development of an Automated Assay for the Measurement of Free Beta Human Chorionic Gonadotropin (FBHCG) on the Siemens ADVIA Centaur XP Immunoassay System

L. Williams[1], M. Wilkie[1], D. Thomson[1], H. Candler[1], L. Smith[1], P. Kelly[1], X. Zhang[2], J. Lei[3]. *[1]Axis-Shield Diagnostics, Dundee, United Kingdom, [2]Siemens Healthcare Diagnostics, Newark, DE, [3]Siemens Healthcare Diagnostics, Tarrytown, NY*

**Background:** Human chorionic gonadotropin (HCG) is secreted by placental tissue and serves to support the corpus luteum during the early weeks of pregnancy. HCG is composed of α and β subunits while the subunits can also occur in free forms. Serum free β-HCG assessment is reported to improve detection in first- and second-trimester prenatal screening for chromosomal anomalies. The efficiency of prenatal screening in first trimester using a combination of maternal age, serum free β-HCG, serum PAPP-A, and fetal nuchal translucency measurements might be significantly improved when compared to second-trimester screening. Using this approach, various investigators have reported detection rates for Down syndrome of 85-90% at a 5% false-positive rate.[1] **Method:** A chemiluminescent immunoassay for the detection of free β-HCG has been developed. The ADVIA Centaur® Free Beta Human Chorionic Gonadotropin (FBHCG) Assay† is intended for in vitro diagnostic use in the quantitative determination of the free β subunit of HCG in serum using the ADVIA Centaur XP Immunoassay System. Free β-HCG is bound to paramagnetic microparticles coated with anti-free β-HCG antibody and is then detected by an acridinium ester (NSP-DMAE)-labeled anti-free β-HCG antibody. Following incubation, wash, and magnetic separation steps, acidic and basic reagents are added. The resulting chemiluminescence is measured. Assay performance was evaluated for precision, linearity, limit of quantification (LOQ) and method comparison to B·R·A·H·M·S Free βhCG KRYPTOR. The method comparison study was performed per CLSI EP-09-A3 using 147 patient samples. A precision study was carried out over 20 days according to CLSI EP5-A3. Linearity and LOQ studies followed CLSI EP06-A and EP17-A2, respectively. Performance of the assay was also assessed against a list of potential interfering substances and cross-reactants, following CLSI-EP07-A2. **Results:** The reportable range of the assay is up to 200 IU/L without dilution, or up to 2000 IU/L with automated 1:10 dilution. Linearity has been demonstrated up to 200 IU/L. The limit of quantitation was 0.28 IU/L. The precision study had a within-lab CV of 2.9-4.8%. The method comparison of the assay to the B·R·A·H·M·S Free βhCG KRYPTOR returned a slope of 1.03 and intercept of 0.65 IU/L by Passing-Bablok regression and a Pearson coefficient (r) of 0.99. The assay demonstrated no significant interference from hemoglobin, conjugated and unconjugated bilirubin, triglycerides, biotin, cholesterol, protein albumin, gamma globulin, rheumatoid factor, and human anti-animal antibodies. The assay demonstrated no cross-reactivity with intact HCG, follicle-stimulating hormone, luteinizing hormone, and thyroid-stimulating hormone. **Discussion and Conclusions:** The performance of the FBHCG assay on the Siemens ADVIA Centaur XP system has been assessed and the results show an accurate and precise method for the measurement of free β-HCG in human serum. **Reference:** 1. Shiefa S. et. al. Indian J Clin Biochem. 2013;28(1):3-12. †Underdevelopment.Notavailableforsale,anditsfutureavailabilitycannotbeguaranteed. 

## B-318

### Clinical case report: Patient with ring 14 chromosome with no associated deletion presenting severe clinical

C. A. Maia[1], H. L. P. B. Aragão[1], A. C. N. Peçanha[1], J. L. Silva[1], G. G. G. Fonseca[2], M. C. R. Mello[3], D. M. V. Gomes[1], S. V. L. Argolo[1], G. A. Campana[4]. *[1]DASA, Duque de Caxias, Brazil, [2]UERJ, Rio de Janeiro, Brazil, [3]USP, São Paulo, Brazil, [4]DASA, São Paulo, Brazil*

**Background:** Ring 14 chromosome syndrome has a large number of associated abnormalities. The identification of these abnormalities may be essential to provide an early diagnosis and for the genetic counseling. The case report stated the importance of early clinical suspicion for monitoring the clinical evolution of individuals with Ring 14 chromosome syndrome. Resuming the clinical discussion of patients with this rare condition. Ring 14 chromosome syndrome is a rare condition, of which exact clinical identification is still limited. The objective of this study is to provide information and data about this rare condition and to ratify the importance of conventional cytogenetics in the diagnosis and genetic counseling. **Methods:** The focus of our study is a 1-year-old male patient, submitted to conventional karyotyping with clinical indication of developmental delay and difficult-to-treat focal epilepsy, microcephaly and facial dysmorphia. With peripheral blood material, two cell cultures of lymphocytes. From the obtained material was carried out the analysis, being karyotyped 50 metaphases. It has a complete chromosomal ring with no apparent loss of chromosomal material or a small terminal deletion (telomere loss) in all cells analyzed in pure lineage, without mosaicism - 46,XY,r(14)(p13q32). The analyzed patient presented clinically the characteristics correlated to the evidenced diagnosis **Results:** In the nucleus of Cytogenetics DASA S.A in 2017, 495 karyotypes with the clinical indication delay and/or deficiency in the development were analyzed. Inside these 495 karyotypes, the age range was 0 to 9 years. The cytogenetic study of this group had 98% of normal results and in 34% there were polymorphic variants of the population in general. Only 2% had an altered karyotype. Of these 2% who presented altered karyotype all had one or more clinical characteristics added to the delay in development. This data reinforces the importance of the clinical indication in conducting the conventional karyotypic analysis. Based on the literature review, it is presumed that the genes present in the proximal 14q interval are deregulated through the process of heterochromatinization that occurs in the short arm of the chromosome. In this way, it is evidenced that clinical diagnoses, such as facial dysmorphisms observed in the presence of ring chromosomes without apparent loss of chromosome material may be related to changes in chromatin constitution, leading to a change in gene expression due to the positioning. Already the vulnerability to infections and behavioral disorders can be attributed to the 14q32 region. **Conclusion:** The clinical case report presented evidence that even a ring chromosome without associated deletion can lead to serious clinical presentations. It should be remembered that the etiological diagnosis of the deficiencies is essential for genetic counseling, and that the most commonly used CGH Array and NGS sequencing

platforms will have difficulty in identifying structural anomalies without associated losses or gains.

## B-319

### Novel Biochemical Markers Help Aid in Stratifying Patients at Risk of Preeclampsia and Adverse Events*

A. Kumar[1], B. Kalra[1], A. S. Patel[1], V. Kommareddy[1], G. Savjani[1], S. Nagalla[2]. *[1]Ansh Labs, Webster, TX, [2]DiabetOmics Inc, Beverton, OR*

**Objective:** To develop sensitive and specific objective biochemical markers to help aid in diagnosing preeclampsia.
**Relevance:** Preeclampsia is a pregnancy complication characterized by high blood pressure, presence of protein in the urine, edema, sudden weight gain, headaches, and changes in vision. Preeclampsia occurs in five to eight percent of all pregnancies. In the United States alone, Preeclampsia is responsible for about eighteen percent of all maternal deaths and fifteen percent of premature births. It is also the leading cause of premature delivery. To date, no objective biochemical marker has been found with high sensitivity and specificity to diagnose preeclampsia accurately. The current strategy to diagnose preeclampsia is through the detection of protein in the urine and onset of high blood pressure during the late second and third trimester pregnancy. However, these symptoms are also present in some normal and many other pregnancy complications such as gestation hypertension, thus increasing the number of false positives. Recent studies on maternal serum protein analysis by proteomics have shown upregulation of placental and hepatic proteins. Two of the upregulated proteins, Pappalysin (PAPP-A, a IGFBP-4 protease and PAPP-A2, a IGFBP-5 protease, produced by placenta) and glycosylated form of fibronectin (preferential binding to SNA and other lectins reflecting sialic acid and fucose carbohydrates) mostly produced by the liver were studied. **Methodology:** Specific monoclonal antibody based ELISAs for GlyFn (AL-160), Pregnancy-Associated Plasma Protein A2 (PAPP-A2, AL-109 $C_{cap}$-$C_{det}$, AL-167 $C_{cap}$-$N_{det}$), Eosinophil Major Basic Protein (proMBP) (AL-159, $proMBP_{cap}$-$proMBP_{det}$) PAPP-A-proMBP Complex (AL-112, PAPP-$A_{cap}$-$proMBP_{det}$,) and proMBP-Angiotensinogen (proMBPAGT, AL-111, $proMBP_{cap}$-$AGT_{det}$) were developed and validated. Preeclampsia status was evaluated using these biomarkers in serum samples from 545 pregnant women (PE, Control, PIH, Undiagnosed) with gestation age 20 to 35 weeks in two subsets of samples. A mathematical algorithm based on 2 decision point using PAPP-A2, GlyFn, protein urea, blood pressure have been evaluated for stratifying the patients the risk of PE and adverse events. **Validation:** ELISAs were very specific to the measured analyte and did not cross-react with other related analytes in the family. ROC analysis for each ELISA was used to calculate the area under the curve (sensitivity and specificity) of diagnosing PE vs Controls. GlyFn and PAPP-A2 ELISAs resulted in AUROC of 1.0 and 0.99 for study 1 and ROC of 0.98 and 0.99 for study 2. PAPP-A-proMBP, proMBP-proMBP and proMBP-AGT had low AUROC of 0.72, 0.64, and 0.52, respectively. Clinical cut-off was established for GlyFn and PAPP-A2 and their serum measurements showed a good concordance with the delivery status (concentrations near the cutoff delivered close to term and elevated concentrations delivered very pre-term). **Conclusions:** GlyFn and PAPP-A2 serum measurements suggest that these proteins play a critical role in preeclampsia and PAPP-A-proMBP, proMBP-proMBP and proMBP-AGT serum levels may not play a significant role in preeclampsia diagnosis. The unique combination of placental (PAPP-A2) and hepatic (GlyFn) protein biomarkers increases the sensitivity and specificity of PE diagnosis over 95%.
*Research Use Only

## B-320

### Comparison of Blood Lead Level Among School Children in Different Cities of Nepal

K. Gautam[1], S. Pradhan[1], V. Thuppil[2], D. Pyakurel[1], A. Shrestha[1]. *[1]Samyak Diagnostic Pvt. Ltd., Lalitpur, Nepal, [2]National Referral Centre for lead projects, Bangalore, India*

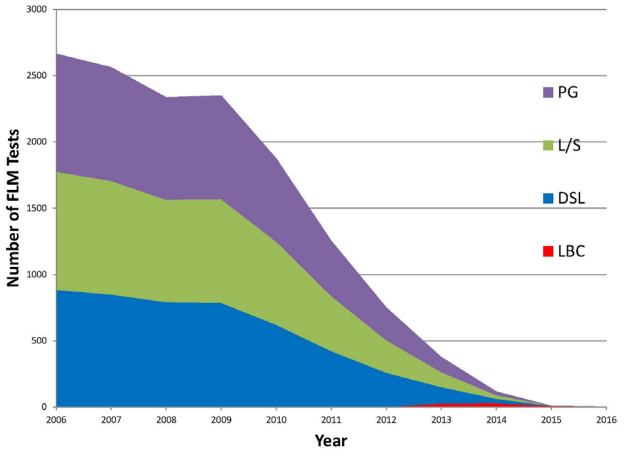
**Background:** Lead has caused serious public health problems in many parts of the world. Southeast Asia is still suffering from high disease burden from lead poisoning. Children are particularly vulnerable and even relatively low levels of exposure can cause serious health conditions. Establishing the prevalence of Blood Lead Level (BLL) shall help to screen the susceptible children and can prevent them from serious complications with early interventions. **Methods:** The cross sectional study was done on 100 school going students, 50 from industrial city Birgunj and 50 from capital city Kathmandu. Questionnaire was used to collect data. Capillary blood samples were drawn to measure Blood Lead Level. Lead Care II was used to measure Blood Lead Level. Blood Lead Level >5 µg/dl was considered as elevated BLL. SPSS ver. 22 was used to analyze the data. **Results:** The mean BLL in Birgunj came out to be 20.33±9.36 µg/dl. Mean BLL in male was 21.08±8.87µg/dl whereas that for female was 19.46±10.92 µg/dl. All the children in the study from Birgunj have elevated BLL and 84% of them have BLL >10 µg/dl. The mean BLL in children from Kathmandu was 7.01 ± 4.08 µg/dl. Mean BLL in male was 8.08 ± 4.20 µg/dl whereas that for female was 6.35 ± 3.93 µg/dl. About 62% of the children in the study from Kathmandu have elevated BLL and 12% of them have BLL >10 µg/dl. The difference in mean BLL of the children from Kathmandu and Birgunj came out to be statistically significant. ($P < 0.05$) **Conclusion:** The prevalence of BLL in children from industrial city Birgunj is alarmingly high compared to children from Kathmandu. Children exposed with chipped paints, lead acid batteries have comparatively high level of Blood Lead Level. Further study in large population is required to address the current situation regarding the lead exposure to children.

## B-321

### Rapid decline of fetal lung maturity testing at the University of Minnesota

L. M. Johnson, C. Johnson, A. B. Karger. *University of Minnesota, Minneapolis, MN*

**Background**: Fetal lung maturity (FLM) testing, first developed in 1971, has been utilized over the years to assess the potential risk for development of respiratory distress syndrome (RDS). The University of Minnesota Medical Center (UMMC) has served as a reference laboratory for FLM testing since 1975, performing thin layer chromatography quantitation of amniotic fluid lecithin:sphingomyelin ratio (L/S), phosphatidylglycerol (PG), and disaturated lecithin (DSL). However in recent years there has been a sharp decline in our FLM testing volumes, leading us to question whether these assays are clinically necessary. **Methods**: Our laboratory information system was queried from 2006 to 2016 for terms associated with FLM testing: desaturated lecithin (DSL), lecithin-sphingomyelin ratio (L/S), phosphatidylglycerol (PG), and lamellar body count (LBC). DSL, L/S, and PG were performed until 2015, and LBC from 2013 to 2016. The lipid assays were done by thin layer chromatography, and the LBC was validated on our hematology platform. Clinicians likely ordered DSL, L/S, and PG for a single patient, but we did not confirm this with chart reviews. **Results:** Graph of test volume plotted by year.
**Conclusions:** FLM testing has rapidly declined at UMMC, from a volume of 2,665 tests in 2006 to 2 tests in 2016. This precipitous decline is likely due to recent changes in clinical practice guidelines issued by the American College of Obstetrics and Gynecology (ACOG) and the Society for Maternal-Fetal Medicine (SMFM). Both sets of guidelines recommend against using FLM testing to guide management, citing studies which demonstrate that lung maturity does not necessarily reflect maturity of other organ systems, and that decisions to deliver should be more broadly based on multiple maternal and fetal parameters, not just fetal lung status. Given the changing clinical landscape, clinical laboratory directors should meet with obstetrics providers to determine whether FLM testing should be discontinued.



## B-322

### Transplacental Transfer of Fentanyl Administered During Labor and Delivery

R. Le[1], N. Novikov[2], S. E. F. Melanson[2], R. Gencheva[2], A. K. Petrides[2]. *[1]University of Massachusetts Medical School, Worcester, MA, [2]Brigham and Women's Hospital, Boston, MA*

**Background :**
Fentanyl is commonly given as an anesthetic during labor and delivery. However, the extent of transplacental transfer and fetal exposure is not well studied. In addition, screening for fentanyl in urine by immunoassay is increasingly common due to the opioid crisis and at our institution fentanyl has been detected in neonatal urine. In this study, we reviewed neonates that had urine fentanyl immunoassays performed and the relationship to maternal fentanyl exposure.
**Methods:**
All neonates with a urine toxicology panel performed as part of clinical care between January 2017 and December 2017 were included. The urine toxicology panel includes a qualitative screen for fentanyl performed by homogenous enzyme immunoassay (Immunalysis Corporation) with a cutoff of 4 ng/ml. The following variables were obtained: neonatal and maternal urine fentanyl results, time of urine collection(s), APGAR scores at 1 minute and 5 minutes, time and dose of fentanyl administration, time of delivery. A two-tailed, unpaired student's t-test was used to compare means such as APGAR scores and time from fentanyl administration to delivery between neonates with positive versus negative urine fentanyl screens. A Fisher's exact test was used to compare proportions.
**Results:**
Of the 92 neonatal urine fentanyl screens performed in this study, 25 (27%) were positive for urine fentanyl; 24 (96%) of which could presumably be attributed to fentanyl administration during labor and delivery. Eight of the 24 mothers had a urine fentanyl screen prior to fentanyl administration, all of which were negative, supporting the fact that neonatal results were secondary fentanyl administered during labor and delivery. In the remaining 67 neonates with a negative urine fentanyl screen, 59 (88%) of mothers were given fentanyl. There was no statistical relationship between maternal fentanyl administration and likelihood of positive neonatal fentanyl screen (p=0.44). Neonates with positive urine fentanyl had statistically lower APGAR scores at minute 1 (7 vs 8; p<0.05), but no difference in APGAR scores at minute 5 (8.6 vs 8.5; p>0.5). Neonates with positive urine fentanyl also had higher average time from administered fentanyl to sample collection compared to neonates with negative urine fentanyl (2951 vs 1537 minutes; p=0.05). If the total fentanyl dose was >350 mcg, neonates were significantly more likely to have a positive fentanyl screen (p<0.0001; true positive rate (TPR) of 82%; false positive rate (FPR) of 0%). Additionally, neonates with fentanyl exposure >800 minutes were statistically more likely to have a positive fentanyl screen (p<0.0001; TPR of 80%; FPR of 12%). However, total dose and length of exposure could not predict the result of the fentanyl screen in all neonates, as 16 were positive for urine fentanyl with a dose <350 mcg and length of exposure <800 minutes.
**Conclusions:**
While dose and length of exposure can predict urine fentanyl results in some neonates, there are a group a neonates with exposure to lower doses and/or shorter duration that were positive for urine fentanyl. More studies are needed to determine if genetic or other maternal characteristics such as weight can predict the extent of fetal exposure.

## B-323

### Serum Brain-Derived Neurotrophic Factor In Children With Coeliac Disease

D. Margoni[1], K. Michalakakou[2], E. Angeli[1], A. Haliassos[3], P. Pervanidou[1], C. Kanaka-Gantenbein[1], G. Chrousos[1], E. Roma[1], I. Papassotiriou[2]. *[1]First Department of Pediatrics, University of Athens Medical School, Athens, Greece, [2]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [3]ESEAP, Athens, Greece*

**Background:** Brain-derived Neurotrophic Factor (BDNF) is a neurotrophin that has a protective role in the nervous system and is involved in neural plasticity. It is abundant in the central nervous system, but is also expressed in the gastrointestinal tract. Recently, BDNF was linked to intestinal inflammation with expression in enteric cells and afferent neuronal pathways in animal models of intestinal inflammation and patients with inflammatory bowel disease (IBD). Although BDNF expression has been studied in several inflammatory conditions, scarce data exist concerning CD. Coeliac disease (CD), characterized by intestinal inflammation, has some co-morbidity with neurologic and mental disorders. The aim of this study was to evaluate circulating BDNF concentrations in patients with CD at diagnosis or on a Gluten Free Diet (GFD) for longer than one year and in healthy controls (HC). **Materials and Methods:** Fifty newly diagnosed patients with CD (aged 8.6±3.7 y, 64.0% females), thirty-nine patients on GFD for longer than one year (aged 10.4±3.4 y, 71.8% females) and 36 HC (aged 8.0±1.7 y, 33.3 % females) were included in the study. Along with anthropometric evaluation and standard blood chemistry, serum BDNF levels were measured by a specific immunoenzymatic assay. **Results:** Serum BDNF levels were significantly higher in newly diagnosed patients with CD than in controls (26,110 ± 8,204 *vs.* 19,630 ± 8,093 pg/ml, respectively, p<0.001). Similarly, BDNF levels were higher in patients on GFD than in controls (28,860 ± 7,992 *vs.* 19,630 ± 8,093 pg/ml, respectively, p<0.001). BDNF levels were significantly higher in patients on GFD than in those at diagnosis (26,110 ± 8,204 *vs.* 28,860 ±7,992 pg/ml, respectively, p=0.02). When patients at diagnosis (all of them had positive serology) were compared to those on GFD with negative serology, a trend for higher BDNF levels was observed for those on GFD, although the difference was not statistically significant (26,301±2,668 vs 30,012± 3,675 pg/ml respectively, p=0.09). No difference in BDNF levels was observed between patients at diagnosis and those on GFD with positive serology either. A correlation analysis within groups, showed that BDNF levels are independent of anti-tTG values (patients at diagnosis: r= 0.147, 0>0.31, patients on GFD: r=0.114, p>0.48). **Conclusions:** In conclusion, according to our findings, BDNF levels were higher in patients with CD than HC, regardless of adherence to the GFD. This finding could suggest a protective role of BDNF against chronic intestinal inflammation or chronic stress from the diet. It seems that BDNF plays an important role in the electrophysiological changes occurring in the CNS of CD patients. Nevertheless, data are still scarce concerning the role of BDNF in CD and further investigation is necessary.

## B-324

### Development of Amino Acid, L-carnitine and Total Protein Assays in Liquid and Dried Microsamples

D. Chace. *Medolac Laboratories, Swansea, MA*

**Objective:** The objective of this study is to develop a simple, high-throughput microsample screening method for total protein, selected amino acids and L-carnitine that are important in the nutrition of preterm infants. The nutritional requirements of premature infants are controlled by neonatologists who must deliver appropriate amounts of protein, fat and carbohydrate to maximize growth while minimizing toxicity. Enteral nutrition includes Human Breast Milk (HBM), bovine-based infant formulas and high protein supplements. HBM is insufficient to meet the requirements of VLBW or early gestational age infants and must be supplemented with protein. The goal of this study is to be able to correlate preterm infant's nutrition administration with their respective blood metabolite/protein concentrations to help neonatologists make informed decisions. To do so, a separate screening assay is under development. In this study, we report concentration of total protein, metabolites and carnitine in HMB and how they compare to other nutritional preterm infant sources.
**Methods and Justification:** Standard infant formula, a hydrolyzed infant formula and commercially available cow liquid whole milk, were spotted onto Grade 903 filter paper (75 uL) and dried overnight. Protein was measured using the Pierce™ BCA assay in liquid samples so that the new analysis of dried milk spots (1/16$^{th}$ and 1/8$^{th}$ inch) could be compared. Milk source, punch location, and size were evaluated for total protein measurement with bovine whole milk serving as a control. L-carnitine, acylcarnitines and amino acids were extracted from 3/16$^{th}$ dried milk spots punches and analyzed by MS/MS.
**Results and Summary:** The protein concentrations of liquid bovine whole milk specimens measured by the BCA assay (33.4 g/L) closely matched the manufacturer-stated concentration of proteins (34 g/L for whole milk). Precision of liquid analysis for whole milk samples was less than less than 4%. For dried milk spots (DMS) using 1/8$^{th}$ or 1/6$^{th}$ in punches, the precision was 10%. Protein concentration increased by 20% from center punch to edge of the spot. Amino acid and acylcarnitine concentrations extracted from DMS where very different from those found in Dried Blood Spots (DPS). Glutamic acid (Glu) was the dominant amino acid in bovine whole milk (283 µmol/L) and for HBM(1400 umol/L). The concentrations of amino acids are not "filtered" plasma. The median concentration of glutamate from preterm infants in DBS is 185 µmol/L. The analysis of protein from was satisfactory in bovine whole milk and achieved the concept of a microsample at 25 µL (liquid specimen) per sample analyzed. This is the first study to examine the analysis of DMS for total protein concentrations using the Pierce kit. For MS/MS analysis, only DMS were utilized. The analysis revealed metabolites that are important for evaluation in nutrition, especially Glutamate which has a purported role in gut metabolism and growth. This method will be useful in the development and implementation of a screening test for protein and metabolites in HBM.

## B-325

### Reducing discard blood draw volumes from subcutaneously implanted ports (PORT) in patients with End Stage Renal Disease (ESRD)

U. Garg, J. Moore, R. Trujillo, P. Monachino, A. Wiebold, A. Ferguson, G. Oroszi, V. Chadha. *Children's Mercy Hospitals and Clinics, Kansas City, MO*

**Background**: To monitor clinical status, dialysis and transplant patients with ESRD frequently require blood draws. To preserve their veins and to avoid frequent intravenous access, these patients, especially young children, require PORT placement. Between blood draws, the PORT is flushed with saline and filled with heparinized saline to prevent blood clotting. To avoid contamination from PORT fluids, a fixed amount of blood is withdrawn and discarded before the blood sample is withdrawn for laboratory analyses. Currently, the recommend discard blood volume is 5 mL which is 5 times the reservoir volume of most PORTs and attached catheters. The volume of discarded blood can be significant, particularly in young patients with ESRD who are already anemic and receive Epogen and iron therapy. This can be a leading cause of iatrogenic anemia. In the present study we evaluated the possibility of reducing the discard blood volume from 5 to 3 mL without compromising laboratory results. **Methods**: After obtaining informed consent, 12 ESRD patients who had PORT placed as part of their clinical care were included in the study. The study period was from February to October 2017. Fifty paired blood samples were drawn from these patients for basic metabolic panel (BMP consisting of sodium, potassium, chloride, bicarbonate, urea, creatinine, calcium and glucose) and complete blood count (CBC consisting of hemoglobin, WBC and platelets) for clinical indications only. The study design included blood wastage of 3 mL and collection of additional 2 mL blood for a total volume of 5 mL. This was followed by collection of additional blood as needed for regular laboratory analyses. Along with regular samples analysis (control), 2 mL aliquots (experimental) were also tested at the same time on the same analyzers. Results for BMP and CBC from control and experimental samples were compared using Bland-Altman analysis. Coefficient of correlation ($R^2$) by regression analysis were also determined. **Results**: On Bland-Altman analysis, the differences between all except 4 control and experimental paired values were within the preset acceptable variability limits. The $R^2$ for all analytes ranged between 0.90 for calcium to 0.99 for creatinine, urea and hemoglobin ($p<0.0001$). **Conclusion**: For the tested analytes, the discard blood volume can be reduced from 5 mL to 3 mL. This 40% decrease in the amount of wasted blood can have significant impact on reducing iatrogenic anemia. We plan to extend the study to other analytes.

## B-326

### Development of an Automated Immunoassay for the Measurement of Pregnancy-associated Plasma Protein A (PAPP-A) on the Siemens ADVIA Centaur XP Immunoassay System

S. Poynton[1], L. Smith[1], C. Anderson[1], P. Kelly[1], X. Zhang[2], J. Lei[3]. *[1]Axis-Shield Diagnostics, Dundee, United Kingdom, [2]Siemens Healthcare Diagnostics, Newark, DE, [3]Siemens Healthcare Diagnostics, Tarrytown, NY*

**Background:** Pregnancy-associated plasma protein A (PAPP-A) is a placenta-derived glycoprotein. During pregnancy, it is produced by the trophoblast. PAPP-A levels in maternal serum rise with gestational age. The functional significance of PAPP-A is unclear. Some studies suggest that reduced PAPP-A concentrations are associated with chromosomal abnormalities in the fetus.[1] Maternal serum PAPP-A assessment between 11 and 14 weeks of pregnancy is reported to have significant utility in screening for Down syndrome and other chromosomal anomalies. A combination of maternal age-related risk, free β-HCG, and fetal nuchal translucency measurements may substantially increase the efficiency of prenatal screening compared to second-trimester screening. Using this approach, various investigators have reported detection rates for Down syndrome of 85–90% at a 5% false-positive rate.[2] **Method:** A chemiluminescent immunoassay for the detection of PAPP-A has been developed. The ADVIA Centaur® PAPP-A Assay† is intended for in vitro diagnostic use in the quantitative measurement of PAPP-A in human serum using the ADVIA Centaur XP Immunoassay System. PAPP-A is bound to microparticles coated with anti-PAPP-A antibody and is then detected by an acridinium ester (NSP-DMAE)-labeled anti-PAPP-A antibody. Following incubation, wash, and magnetic separation steps, acid and base reagents are added. The resulting chemiluminescence is measured. Assay method comparison to B·R·A·H·M·S PAPP-A KRYPTOR was performed per CLSI EP-09-A3 using 101 patient samples. A precision study was executed over 20 days according to CLSI EP5-A3. Linearity and functional sensitivity studies followed CLSI EP06-A and EP17-A, respectively. Per CLSI EP07-A2, the assay was tested for interference from hemoglobin, bilirubin (conjugated and unconjugated), triglyceride, biotin, cholesterol, immunoglobulin G, protein albumin, rheumatoid factor, and human anti-animal antibodies. The assay was also tested for cross-reactivity with alpha-2-macroglobulin, angiotensinogen, angiotensin 1 and 2, sex-hormone binding globulin, human chorionic gonadotrophin, alpha-fetoprotein, and prolactin per CLSI EP07-A2. **Results:** The reportable range of the assay is up to 10 IU/L without dilution, or up to 100 IU/L with automated 1:10 dilution. Linearity has been demonstrated up to 10 IU/L. Functional sensitivity was observed at 0.01 IU/L. In the precision study, the assay demonstrated within-lab CV of 2.9–4.9%. The method comparison of the assay to the B·R·A·H·M·S PAPP-A KRYPTOR returned a slope of 1.07 and an intercept of 0.05 IU/L by Passing-Bablok regression, and a Pearson coefficient (r) of 0.99. The assay demonstrated no interference and no cross-reactivity with the tested cross reactants. **Discussion and Conclusions:** The feasibility of the automated PAPP-A assay on the Siemens ADVIA Centaur XP System has been assessed and the results show an accurate and precise method for the measurement of PAPP-A in human serum. **Reference:** 1.

Fialova L, et. al. Bratisl Lek Listy. 2002;103(6):194–205. 2. Shiefa S. et. al. Indian J Clin Biochem. 2013;28(1):3–12.

†Under development. Not available for sale, and its future availability cannot be guaranteed.



## B-327

### CALIPER continuous reference curves for biochemical markers: Advantages over traditional partitioned reference intervals

S. Asgari[1], V. M. Higgins[2], C. McCudden[3], K. Adeli[2]. *[1]SickKids Hospital, Toronto, ON, Canada, [2]SickKids Hospital, University of Toronto, Toronto, ON, Canada, [3]The Ottawa Hospital, University of Ottawa, Ottawa, ON, Canada*

**Background**: Despite the critical importance of reference intervals for accurate interpretation of laboratory test results, they have traditionally been severely lacking in the pediatric population. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has made significant strides to close this gap by establishing a pediatric reference interval database based on data from thousands of healthy children and adolescents (www.caliperproject.ca). CALIPER reference intervals have traditionally been partitioned by age, using the Harris & Boyd method to determine statistically significant age partitions. However, analyte concentration does not change abruptly with age, but rather changes dynamically. In this study, we establish continuous reference intervals for biochemical markers using the CALIPER database to provide a more accurate estimate of age-related changes in biomarker concentration. **Methods:** Data from CALIPER subjects aged 1-<19 years were used to establish continuous reference intervals for eight analytes, including alanine aminotransferase, albumin, alkaline phosphatase, total bilirubin, calcium, creatinine, phosphate, and uric acid. Data from subjects <1 year of age were excluded. Continuous reference intervals (i.e. 2.5$^{th}$ and 97.5$^{th}$ quantiles) were established using non-parametric quantile regression via a univariate B-spline with a penalty to impose monotonicity and quantile non-crossing constraints using R software. This method is robust to various departures from assumptions, including normality, symmetry, linearity, and variance homogeneity, as well as outliers. **Results:** Reference curves were established for several biochemical markers, showing the dynamic age-related trends in analyte concentration. A table of reference values for each 6-month age bin was also established. Calcium and alanine aminotransferase concentration remained relatively stable throughout the age range, showing little dependence on age. Total bilirubin, creatinine, and uric acid continuously increased with age. Alkaline phosphatase showed a non-linear relationship with age, increasing until puberty, and subsequently decreasing into adulthood. Although less pronounced, phosphate exhibited a similar age-related dynamic to alkaline phosphatase. **Conclusion:** Continuous reference intervals better reflect the dynamic age-related trend in analyte concentration. However, the feasibility of implementing continuous reference intervals into clinical practice remains an issue, particularly considering the limitations of current laboratory information systems. We provide tables of 6-month age bins to increase their feasibility, although this inherently reduces the accuracy of continuous reference intervals.