PEDIATRIC AND MATERNAL-FETAL DIVISION NEWS
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AACC Better health through laboratory medicine.

## FROM THE MIND OF THE CHAIR

Hi again everyone! Thanks for viewing the July 2018 edition on the PMF newsletter! This issue is jam packed with lots information about the upcoming 70th AACC Annual Scientific Meeting & Clinical Laboratory Expo in Chicago. In particular, we highlight many of the pediatric and maternal fetal medicine related sessions that are available for meeting attendees! I am looking forward to seeing many at one or more to the PMF sponsored events! Please plan to attend our annual **Joint division mixer on Monday night, July 30 at 7:30**! Here you can catch up with old friends from the PMF, Clinical Translational Sciences, and Informatics Divisions, and honor our award winners! As in past years, we have two abstract awards winners, **Houman Tahmaseb** (Student/Young Investigator) and **Ioannis Papassotiriou** (Best Abstract). The 2018 award winner for **Outstanding Contributions to Pediatric and Maternal Fetal Laboratory Medicine** is **Dr. David Grenache**. Please join me in congratulating each of our award winners on their outstanding contributions to our field!

Along with the joint division mixer, there will be a networking event on Sunday evening at the opening mixer! New this year, the **Division networking event at the opening mixer** will give interested attendees a chance to mingle with Division board members, play games, and even join new divisions! Please stop by the PMF table to network, play PMF related games and even register for the grand prize, dinner with the PMF board!

There are also several scientific sessions sponsored by the PMF division at the upcoming meeting. This year we are also hosting a special session on Tuesday, July 31 at 1:00 entitled "**Current and Future Activities of NHANES and Collaborative Opportunities".** This session will provide interested attendees with an update on the AACC sponsored project on pediatric reference intervals, the work of the NHANES organization, and opportunities for our two groups to collaborate.

Finally, do not miss the PMF poster walk lead by Dr. Amy Pyle-Eiola on Wednesday, August 1 from 12:30 – 1:30 PM!

Also in this issue, we continue our ABC's of Pediatric Laboratory Medicine series with our B installment on Biotin interferences. Our excerpts from the literature focuses on a blood test for traumatic brain injury (TBI) and practical challenges and recommendations for verifying reference intervals. I hope you find the content of this newsletter both interesting and helpful! Thank you for your ongoing support of the efforts of the PMF Division! See you in Chicago!

Alison Woodworth

Chair, AACC PMF Division

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# THE ABC'S OF PEDIATRIC LABORATORY MEDICINE:

## "B" is for Biotin as a Potential Immunoassay Interference

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Biotin (Vitamin B7) is a water-soluble B-complex vitamin necessary for human growth and health. It is an essential cofactor for several carboxylase enzyme complexes that includes 5 biotin-dependent carboxylases: acetyl-CoA carboxylase (ACC) 1, ACC2, methylcrotonyl-CoA carboxylase (MCC), pyruvate carboxylase, and propionyl-CoA carboxylase (PCC), all of which are involved in carbohydrate, amino acid, and lipid metabolism (1).

Biotin deficiency is rare; conditions requiring increased biotin intake are: i) inherited metabolic diseases (eg. Multiples carboxylase deficiencies such as infantile holocarboxylase synthetase deficiency and later onset biotinidase deficiency) (2), ii) disorders of mitochondrial energy metabolism, iii) seborrheic dermatitis in infants and iv) post-gastrectomy patients.

Because lack of biotin is rare, there is no Recommended Dietary Allowance (RDA) or Recommended Nutrient Intakes (RNI) for it. Normal daily recommended intakes for biotin are generally defined as follows (3):

- Infants and children
  - Birth to 3 years of age: 10 to 20 micrograms (mcg).
  - 4 to 6 years of age: 25 mcg.
  - 7 to 10 years of age: 30 mcg.
- Adolescents and adults
  - 30 mcg.

In recent years, biotin supplementation has progressively increased, due to medically prescribed therapies as well as over-the-counter supplements for hair, skin, and nail. The later can be doses as high as 10 mg/day of biotin (4). Recently, high doses of biotin were found to be a therapeutic option in the treatment of: i) progressive multiple sclerosis (5) and ii) biotin-thiamine responsive basal ganglia disease. This last one is an orphan neuro-metabolic disease caused by mutation in the SLC19A2 gene coding for a thiamine transporter (6).

Normal daily recommended intake for biotin derived naturally from foods, is not expected to interfere with immunoassays based on biotin and/or streptavidin-biotin system. Most of the biotin interferences have been described in patients that were using very high doses of biotin. The most commonly involved clinical chemistry systems are hormone immunoassays, based on streptavidin-biotin and/or biotin detection system, including Roche Elecsys and Beckman Coulter Access/DXI analyzers (7,8,9). Also, the multitest assay platform systems based on streptavidin-biotin and/or biotin detection system (Immulite 2000, Vitros, Access/DXI, Elecsys), have not been exempt from biotin interferences for non-hormone immunoassays (folate, IgE, anti-TPO, anti-TSHR, tumor marker, cardiac markers) (10). Morevover, some platforms with serologic testing capabilities for HBsAg, anti-HBc IgM, HBeAg, anti-HBe antibodies, anti-HAV and anti-HAV IgM, have been described to have biotin interferences as well (10,11,12). These findings imply a corresponding risk of assay misinterpretation in cases of anemia, allergy, autoimmunity, malignancies, cardiac injury and infectious disease.

Some pediatric patients that use Biotin as treatment for inherited metabolic diseases are not exempt of the Biotin interferences. A report on six children, 3 with disease of biotin–

thiamine–responsive basal ganglia disease and 3 infantile and neonatal mitochondrial diseases, receiving high-dose biotin (2-15 mg/kg/day) had been described. Unexpected laboratory test results suggestive of Graves' disease in all the patients during routine examination showed excessively elevated levels of free thyroxine (T4) (competitive immunoassay) and low levels of TSH (non-competitive immunoassay), and elevated levels of anti–thyrotropin receptor antibodies (competitive immunoassay). Antithyroid medication was initiated in at least three children (only 1 of 3 children had symptoms attributable to hyperthyroidism); after an investigation of these unusual laboratory test results and discontinuation of the biotin treatment (24 to 48h for TSH and T4 and 7 days for anti–thyrotropin receptor antibodies, the effect of biotin interference, that produced the false thyroid test results was normalized (13).

The mechanism of biotin interferences in different assays system for non-competitive and competitive immunoassays has been described for most hormone immunoassays (4,9,10). The proposed mechanism of biotin interferences in a non-competitive immunoassay for detection of Hepatitis B antigen (HBe Ag) using a VITROS platform is described in Figure 1 (12). In this case, the serum sample is incubated with a mixture of a biotinylated monoclonal anti-HBe Ag antibody, and an HRP-labeled monoclonal anti-HBe Ag antibody. The immune complexes formed between the two antibodies and the serum HBe Ag are captured to the solid phase via streptavidin coated well. The chemiluminescence produced after signal reagent enhancer is directly proportional to the HBe Ag in the serum sample (Figure 1A). Description of how a very high dose of biotin in the serum will result in reduced binding of the immune complexes to the solid phase, and hence a falsely negative HBe Ag result is described as well (Figure 1B).

In the case of a competitive immunoassay for antibody of Hepatitis B antigen detection (anti-HBe)(Figure 2) is described next. The serum sample is incubated with an anti-HBe-specififc antibody, linked to HRP labeled. Subsequently, biotinylated anti-HBe-specififc antibody is added to the mixture. The biotinylated anti-HBe-antibody complex then binds to streptavidin-coated well on the solid phase. After removal of the liquid phase, the emitted chemiluminescence is inversely proportional to anti-HBe in the serum sample (Figure 2A). Description of how a very high dose of biotin in the serum saturates the streptavidin binding sites, thereby resulting in little or no labeled anti-HBe-antibody complex binding to the solid phase, and hence a falsely positive anti-HBe (Figure 2B). In this example, the VITROS HBeAg and anti-HBe immunoassays are susceptible to biotin interference, leading to a clinically significant change in qualitative interpretive results of originally borderline HBeAg-reactive to negative result and an originally anti-HBe-negative to reactive result (11,12).

On November 28, 2017, the US Food and Drug Administration alerted health care professionals and patients regarding an interaction with high dose biotin administration and some laboratory testing, resulting in incorrect results. The safety communication noted that biotin in patient samples may cause falsely high or low results, depending on the test. The announcement also noted that several dietary supplements on the market contain biotin levels up to 650 times the recommended daily intake of the vitaminmay cause interference with laboratory tests. The safety announcement also noted that patients may not divulge taking supplements during a medication history and that some health care professionals may be unaware of this potential interaction.

Different laboratory approaches have been described to investigate and eliminate the biotin interferences, when discordant results are present and biotin interference is suspected. Biotin depletion using streptavidin agarose (Thermo Fisher Scientific Inc) or streptavidin-coated microparticle (Roche Elecsys reagent pack), have been used to incubate with the serum sample to remove the Biotin interference and measure the analyte in the supernatant. Adopting these methods as troubleshooting tools enables laboratories to confirm biotin

interference in the appropriate clinical setting; remove the interference and report accurate and reliable results (8,14).

Detection of biotin interference is difficult to identify, as usually this interference mimics a biochemical patterns of laboratory result, as it happens in the case of hyperthyroidism. Adopting some specific pre pre-analytical activities such as: i) reviewing medication history, ii) asking the patient if they were receiving any vitamins or supplements, before scheduling an appointment or iii) letting them know through the preparation instructionsheet that critical laboratory test that could be affected by biotin with a comment such as: *"If you are taking any vitamin or supplement that contains biotin you need to suspend at least 48h before your appointment. In the case you have any questions call the laboratory".*

In, summary the increased use of biotin-containing supplements may affect patient laboratory results. Biotin Interference may cause false-negative (under-estimation) or false-positive (over-estimation) results in non-competitive and competitive and immunoassays, respectively. Clinical laboratories should recognize that the patient's use of biotin-containing supplements may adversely affect certain laboratory test results. Open communication and collaboration between laboratory directors, medical technologists, and clinicians plays a key role in detecting biotin interferences and prevent misdiagnosis.

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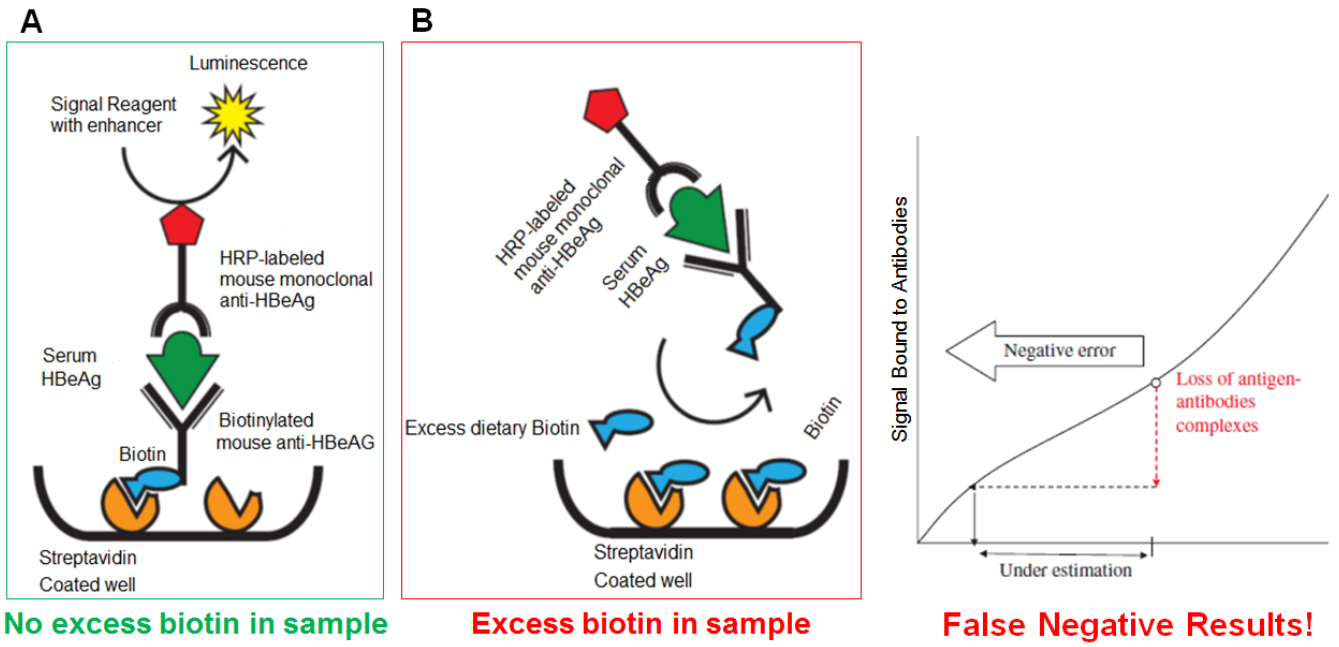


**Figure 1: Mechanism of biotin interferences in a non-competitive immunoassay for detection of Hepatitis e antigen (HBe Ag) using a VITROS platform.** *Adapted and modified from Thyroid 2016 ,26 (6), 860-863* (9).

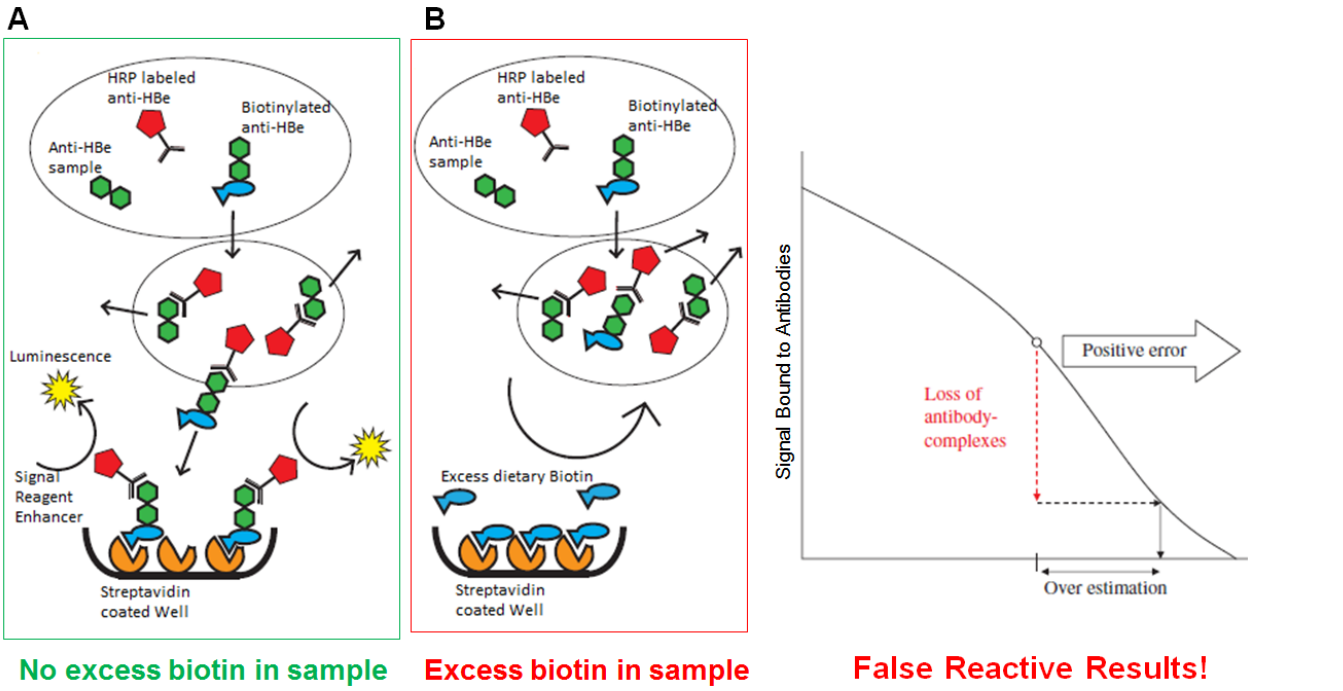


**Figure 2: Mechanism of biotin interferences in a competitive immunoassay for detection of anti-Hepatitis e antibody (anti-HBe) using a VITROS platform.** *Adapted and modified from Thyroid 2016 ,26 (6), 860-863* (9).

## Excerpts from the Literature

**Kelly Doyle**, PhD, DABCC, FAAC, Clinical Chemist, Intermountain Healthcare, Salt Lake City, UT, USA

**What to know about the recently FDA approved mTBI blood test**

Our hospital lab was contacted by physicians, administrators, and parents of children engaged in high school sports who were interested in the recent announcement by the FDA in February 2018 approving the marketing of a two-marker blood test for mild traumatic brain injury (mTBI) by Banyan Biomarkers (1). You may be in a similar situation in your laboratory or may be approached in the future about bringing this test in house for your pediatric patients. Below are a few highlights of the approved test called Brain Trauma Indicator.

The test measures concentrations of two proteins, Ubiquitin C-terminal Hydrolase-L1 (UCH-L1) and Glial Fibrillary Acidic Protein (GFAP) that are released during the injury event in the brain. Time-course studies demonstrate GFAP increases 14-16 hours post injury and decreases to baseline within 72 hours with or without neurosurgical intervention. UCH-L1 concentrations are greatest at time of injury and continue to decrease to baseline with 24-36 hours post injury (2).

Initially developed for military use and evaluated under the Breakthrough Devices Program of the FDA, the assay is not yet ready for clinical use although the company has partnered with bioMerieux for product development.

The test is rousing for many reasons. For instance, a non-imaging-based blood test for mTBI supports clinical and governmental initiatives to reduce patient radiation exposure—of significant interest in pediatrics where radiation absorption in kids can be significantly higher than adults. Also, as stated by FDA Commissioner Scott Gottlieb, M.D. who remarked in the FDA's announcement that "A blood-testing option for the evaluation of mTBI/concussion not only provides health care professionals with a new tool, but also sets the stage for a more modernized standard of care for testing of suspected cases. In addition, availability of a blood test for mTBI/concussion will likely reduce the CT scans performed on patients with concussion each year, potentially saving our health care system the cost of often unnecessary neuroimaging tests."

Test approval appears to be largely based on a multi-center clinical trial of 1,947 blood samples from patients >18 years old who were suspected of mTBI or concussion (3). Test performance was compared to CT scan results and the FDA indicated that the test predicted the presence of intracranial lesions on a CT scan 95.5 percent of the time, and those without intracranial lesions on a CT scan 99.6 percent of the time.

The clinical community will want to keep in mind that this testing is not solely for concussion assessments but may aid in assessing bleeding in the brain from a variety of TBIs.

Potential Limitations

Unfortunately, FDA approval is specific for adult use as blood samples evaluated in the clinical trial were from individuals >18 years old. Even so, while the clinical trial data looks promising, recent publications suggest UCH-L1 and GFAP may not be specific to TBI. For instance, Posti et al. (4) show that patients with orthopedic trauma and negative CT findings for TBI can have elevated concentrations of these proteins

in their blood as both are expressed outside of the CNS.

Additionally, the FDA approval letter suggests that the turnaround time is 3 - 4 hours which may limit clinical usefulness as one weighs the costs of imaging to those associated with occupying an ER room while waiting to make the admit/discharge decision.

Ultimately, one's perspective of the test may be that its intention is less about providing a definitive diagnose for concussion but to rule out probable detection of blood by CT scan and thereby mitigate the need for costly imaging.


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(4) Posti P, Hossain I, Takala R, et al. Glial Fibrillary Acidic Protein and Ubiquitin C-Terminal Hydrolase-L1 Are Not Specific Biomarkers for Mild CT-Negative Traumatic Brain Injury. J Neurotrauma 2017; 34:1427–1438.



**Brenda Suh-Lailam**, PhD, DABCC, FAAC, Assistant Director, Clinical Chemistry and Mass Spectrometry, Ann & Robert H. Lurie Children's Hospital of Chicago. Assistant Professor of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA


**Verification of reference intervals in routine clinical laboratories: practical challenges and recommendations (BS-L)**

Ozarda Y, Higgins V and Adeli K. Clin Chem Lab Med 2018; aop

Reference intervals (RIs) play an important role in the interpretation of laboratory test results. To obtain RIs, laboratories can adopt medical decision limits defined by expert consensus for specific analytes, or establish RIs from a reference population of healthy individuals. When RIs are established, the central 95% of results obtained constitute the RI for that analyte. Most laboratories find it challenging to establish RIs as a minimum of 120 samples are required per partition. Even more so in pediatric settings where it is a challenge to recruit healthy children and obtain sufficient sample volume, to determine RIs for the multiple age and gender partitions that exist. Hence most laboratories follow the CLSI guidelines that allow for RIs from other sources e.g. manufacturers' package inserts, publications, text books, and multicenter studies, to be verified or transferred, for use in their laboratories.

The goal of the Ozarda et al. publication was to review current challenges to RI verification and transference, and provide recommendations that will facilitate this process in clinical laboratories. When transferring and verifying RIs, a method comparison using values that span the width of the RIs should be performed if similar methods and reference populations

have been used. In the absence of a bias, it is recommended to verify the transferred RIs using a minimum of 20 samples from healthy individuals in the local population. The authors identify the CALIPER project as a source of RIs that laboratories can verify for use in their laboratories as these contain age- and sex-specific pediatric RIs. This publication is a good educational read for any laboratorian struggling with verifying pediatric RIs for their laboratories, as the authors not only review the approach recommended by the CLSI EP28-A3c guidelines, but also additional approaches such as indirect data mining methods which are not as commonly used. These alternate approaches to verifying RIs can be useful in situations where obtaining 20 samples from healthy children to verify a specific RI is challenging.

## 2018 AACC Annual Scientific Meeting and Clinical Lab Expo: PMF Sessions of Interest and Meeting Highlights

**JULY 29-AUGUST 2, 2018 IN CHICAGO, ILLINOIS**

### Sunday, July 29th

Opening Plenary:

Brian Druker, MD

Imatinib as a Paradigm of Targeted Cancer Therapies.11001.

### Monday, July 30th

Plenary Session:

Kenneth Setchell, PhD

Genetic Defects in Bile Acid Synthesis Causing Liver Disease-Diagnosis and Treatment—Translational Medicine from Mass Spectrometry Discovery to the Bedside..12001.

Brown Bag Sessions:

Update on Gestational Diabetes Mellitus: Current National and International Diagnostic Criteria. 42122 or 52222

Scientific Sessions:

**Update on Thyroid Disease in Pregnancy**
Developed in cooperation with Pediatric and MaternalFetal Division. 32102

Pregnancy has profound effects on the thyroid gland and its function. As a result, assessment of thyroid status during pregnancy can be complicated, confusing and even controversial. This session will present information on the diagnosis and management of thyroid disease during pregnancy and postpartum, including important new published guidelines.

Cervical Cancer Screening as an Example of a Global Health Strategy in Resource-Limited Countries. 32431

### Tuesday, July 31st

Plenary Session:

Denise Galloway, PhD

HPV-Associated Cancers and the HPV Vaccine.13001.

Brown Bag Sessions:

Measurement of Steroid Hormones by Mass Spectrometry. 43115 or 53215

Inborn Errors of Metabolism: From Newborn Screening to Diagnosis. 43125 & 53225.

Minimum Retesting Intervals: Importance, Determination, Advantages, Challenges of Enforcement. 43127 or 53227

Scientific Sessions:

Clinical Assay Issues: What Endocrinologists Will Ask You. 33107

Special Presentation:

PMF Meeting: Current and Future Activities of NHANES and Collaborative Opportunity. 1:00-2:30 PM. Hyatt Regency McCormick Place, Regency Ballroom C (Level 2)

## Wednesday, August 1st

Plenary Session:

James Collins, PhD

Nucleic Acid Detection Using CRISPR-Dx. 14001

Brown Bag Session:

Embracing Pathology's Stepchild: A Practical Guide to Clinical Chemistry Education. 44106 or 54206

Scientific Sessions:

Accurate Measurement of Thyroid Hormones in Disease and Pregnancy. 34107

Poster Walk:

PMF Division poster walk with Dr. Amy Pyle-Eiola. 12:30-1:30 PM

**New Approaches for Drug Screening in Pediatrics.** Developed in cooperation with Pediatric and MaternalFetal Division. 34106

Immunoassay drug screens are commonly used for their convenience. However, these report qualitative results based on quantitative cutoffs designed for workplace testing, and their use in a medical setting may be inappropriate. Detection of drug exposure, even at low concentrations, is critical in pediatrics to guide treatment. This session discusses novel approaches and experiences of two labs in addressing these issues.

**Endocrine Disrupting Chemicals in Children and Environmental Health—Emerging Opportunities for the Clinical Laboratory** Developed in cooperation with Mass Spectrometry and Separation Sciences Division, Pediatric and Maternal-Fetal Division. 34212

Exposure to endocrine disrupting chemicals (EDCs) affect human health and development. To reliably assess the impact of these chemicals on diseases, analytical measurements need to meet the same standards as those used in clinical laboratories. This creates unique opportunities for clinical laboratories to help improve EDC measurements and human health.

## Thursday, August 2nd

Scientific Sessions:

**Jumping the Pediatric Reference Interval Hurdles** Developed in cooperation with Pediatric and MaternalFetal Division. 35109

Ideal methods for establishing reference intervals are generally not feasible for pediatric populations, forcing labs to turn to alternative approaches. This session will review CLSI guidelines for generating reference intervals and discuss real-world applications to pediatrics.

## Please Join Us!

**Event: Opening Mixer. Sunday 7/29 6:45-8:00 PM**

**Event: Joint Section Mixer (with the Informatics and Clinical Translational Science Divisions). Monday 7/30 7:30-9:00 PM. Marriott Marquis Chicago, Astronomy (Level 2)**

# PMF Division Awardees

Please help us congratulate the winners of this year's PMF Division Awards. The awards will be presented during the Pediatric and Maternal-Fetal, Industry, Informatics, Clinical Translational Science, and Industry Divisions Joint Mixer.

## Best Abstract by a Student or Young Investigator:

- **Houman Tahmaseb,** The Hospital for Sick Children in Toronto, Toronto, Ontario, Canada

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

## Best Abstract:

- **Ioannis Papassotiriou**, The "Aghia Sophia" Children's Hospital in Athens, Greece
- Title: Association of Fibroblast Growth Factor 21 Plasma Levels with Infection in Neonates: Preliminary Results

## Outstanding Contributions to Pediatric Maternal-Fetal Laboratory Medicine:

**David G. Grenache, PhD,** Chief Scientific Officer, TriCore Reference Laboratories, Albuquerque, New Mexico, USA

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