

## The Monitor

Volume 31, Issue 1 Winter 2012

### From the Mind of the Chair

Dear colleagues and friends in the Pediatric and Maternal Fetal Division,

I have been the chair of the division since January 2010, and this will be my last editorial comments as chair. It has been a real pleasure to meet most division members during the annual mixer at the AACC annual meetings, during scientific programs, as board members, or even through electronic presentations over the years. Your energy and comments were always appreciated. The division board used your comments to expand our roles and meet your needs.

During my tenure as chair, we were active to support our division objectives.

- A. **Education** – there were many symposia and webinars at the AACC and at the International Congress of Pediatric Laboratory Medicine (ICPLM) meetings, developed in collaboration with our division, to enhance the knowledge of the clinical laboratory and health care professional.
- B. **Communication, collaboration and consultation** – through our listserv, our newsletter and the questionnaire to membership, our division is able to provide a forum for the dissemination of information about analytes, methods of analysis, reference ranges for pediatric and maternal-fetal patients. The listserv also provides assistance to clinical laboratory and health care professionals with immediate problems in the field of Pediatric and Maternal-Fetal Clinical Chemistry. The mixer during the AACC annual meeting is another tribune to enable collaboration within our division and with the divisions of Molecular Pathology, Clinical Translational Science and Industry.
- C. **Recognition** – in 2011 the Pediatric and Maternal-Fetal Division Award for Outstanding Contributions to Pediatric and Maternal-Fetal Clinical Chemistry to recognize excellence was given to Dr Vijaylaxmi Grey. Two poster awards and two honorable mentions were given for the best poster and the best student and young faculty poster.

I encourage you to participate in our upcoming questionnaire since it provides a forum for direct communication with the board. Our pediatric and maternal fetal listserv is also active and features topics that are unique and complex. I thank all the persons who contribute with their advice.

Please take some time to browse our division webpage. We have made significant changes, and added new features for dissemination. We are now embarking on the next phase of the collaboration between AACC and the National Children's Study. Our division will proceed with measurement of more than 300 blood spots from newborns for the analysis of amino acids and steroids. This initiative is happening in the realm of our long-term commitment to the establishment and continuous review of pediatric reference intervals. Let us know if you are interested in taking part in this initiative!

As you see with the current edition of our newsletter, we moved away from the traditional format and into the future. Please do not hesitate to contact us with special topics you would like to read in the newsletter.

I would like to introduce Dr Sharon Geaghan who will take the leadership role of the division chair in January 2012. You will greatly appreciate her dedication and sense of humor over the next two years! I want to thank the board members for their support, and Penny Jones and her staff at the AACC Head Office for her helpful advice during the past two years.  
In closing, I would like to provide my best wishes for health, happiness and success throughout 2012.

Best regards,

Nathalie Lepage, PhD

Past Chair, Pediatric and Maternal-Fetal Division

## Reference Interval Corner

### Serum Alpha-Fetoprotein Levels in Healthy Infants and Children

By Sharon M. Geaghan, MD

A longtime gap in the pediatric reference interval literature has existed for serum Alpha-fetoprotein (AFP) values. AFP is a glycoprotein first synthesized in the yolk sac, and later by the fetal liver and gastrointestinal tract. Though AFP constitutes the major serum protein in the fetus, its function is unknown. AFP circulates in extraordinarily high levels in fetal serum, passing into the fetal urine which becomes the major component of amniotic fluid. The concentration of AFP in fetal serum and in amniotic fluid peaks at 13 weeks and decreases thereafter<sup>1</sup>. After 12 weeks gestational age, this protein is found in increasing concentrations in maternal serum<sup>2</sup>, as it is diffuses across fetal membranes and is also transported by diffusion into the maternal placental circulation<sup>3</sup>. Measurement of maternal serum AFP in nanograms per milliliter is expressed as a multiple of the mean (MoM) of an unaffected population, to normalize AFP distribution of values and make results across different populations and laboratories comparable. These maternal measurements are the basis of prenatal screening programs for a wide variety of fetal conditions associated with low or high maternal serum AFP levels, and when elevated, are mostly false positives. This application of maternal AFP assessment is outside the scope of this brief.

Up until now, the literature has been missing a top quality study of healthy infants and children where AFP has been tested on current platforms and that includes all the early years of life, until AFP is demonstrated to reach adult levels. In particular, the 2-3 years of age range has yet to be studied, and Blohm's group has highlighted that AFP has not reached adult levels at 2 years of age<sup>4</sup>.

This is a critically important analyte to have solid pediatric reference intervals for, due to the association of AFP elevation with a variety of tumors in pediatric patients in this age range. The elevation can help in a differential diagnosis, help planning for tissue sampling or definitive cancer surgeries, and is used for prognosis, gauging responsiveness to treatments and as a monitoring tool for relapse/recurrence surveillance.

The publication "Pediatric Reference Intervals for Serum Alpha-Fetoprotein"<sup>5</sup>, authored by William Roberts, included 466 males and 447 females from 6 months to 6 years of age, comprised of pediatric patients undergoing elective surgeries, and evaluated by a physician's assistant for eligibility and for exclusion criteria. The samples were run on two automated chemistry analyzers: the Roche Diagnostics Modular Analytics E170 and the Beckman Coulter Access 2. When sample size allowed (N>120), nonparametric reference intervals were created and when samples were insufficient in number, (n<120), transformed parametric intervals were created using EP Evaluator Release 8 software (Data Innovations). Data was analyzed independently by analyzer and by age and gender. Partitioning was based on statistically significant gender and age group differences.

The conclusions were that AFP decreases with advancing age; reaches adult levels at 3 years of age; and that gender differences are significant, with females having higher upper limits of reference intervals as compared to males. Results from the two analyzers compared well, although it is not recommended that results be interchanged. The project is a product of the pediatric reference interval initiative sponsored by Child Health Improvement through Laboratory Diagnostics (CHILDx) Project at ARUP Laboratories.

An excellent review of the differential diagnosis and utility of AFP levels in pediatric oncology and medicine from the German cooperative groups is available <sup>6</sup>. For the curious, there is a hereditary persistence of alpha-fetoprotein, described in 19 families to date. This is a benign autosomal dominant disorder which can be confirmed first by measuring family members AFP levels, and if indicated, investigating point mutations of the AFP gene promoter <sup>7</sup>.

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## The ABC's of Pediatric Laboratory Medicine: O is for Opioid

By Sarah M. Brown, PhD, Assistant Medical Director of Core Laboratories, St. Louis Children's Hospital

Opioids are synthetic and semi-synthetic derivatives of the natural alkaloid constituents of the opium poppy. Opioids are very effective analgesics and are widely used for the treatment of acute and chronic pain. Opioids frequently used in the in-patient population include parenteral formulations of morphine, hydromorphone, and fentanyl. These are often administered intravenously via patient controlled analgesic (PCA) devices. Opioids commonly used in the outpatient setting are oral formulations of codeine, hydrocodone (Vicodin) and oxycodone (Oxycontin). Methadone, used for opioid withdrawal therapy both in inpatient and outpatient settings, is being increasingly used for pain as well. Opioids have high potential for abuse. A 2011 review from the National Institutes on Drug Abuse (NIDA) reported that among adolescents, prescription drugs are the third most commonly abused substance, and of prescription drugs, opioid analgesics are the most commonly abused. Sustained – release formulations, used commonly in outpatient pain management, are especially associated with a high abuse potential. The NIDA –funded 2010 *Monitoring the Future* study showed that 3% of 8<sup>th</sup> graders, 8% of 10<sup>th</sup> graders, and 8.0% of 12<sup>th</sup> graders had abused Vicodin and 2% of 8<sup>th</sup> graders, 5% of 10<sup>th</sup> graders, and 5% of 12<sup>th</sup> graders had abused Oxycontin for nonmedical purposes at least once in the year prior to being surveyed. This article will review the pharmacology and metabolism of opioids, and the role of the clinical laboratory in opioid testing.

### ***Opioid Pharmacology***

The pharmacology of opioids is mediated by opioid receptors. Opioid receptors are G-protein coupled receptors located in the cell membranes of many tissues throughout the body, including cells of the central nervous system and the gut. Activation of the opioid receptors results in 1) block of neurotransmitter release by inhibition of calcium ion influx into the pre-synaptic terminal, and 2) opening of post-synaptic potassium channels, leading to decreased action potential generation. Opioidergic neurotransmission influences many CNS functions; the effect depends in part on the location of the receptors. For example, activation of receptors in the respiratory center will lead to decreased respiratory rate, in the myenteric plexus to decreased gut motility, and in the dorsal root ganglia to analgesia.

There are four classes of opioid receptors: mu, kappa, delta, and nociceptin receptors. The most well studied opioid receptor is the mu receptor. Activation of the mu receptor results in analgesia, sedation, itching, nausea, euphoria, decreased respiration, pupil constriction, and decreased gut motility. Activation of the kappa receptor results in dysphoria, and antinociception (analgesia) in animal models. The delta receptor is less well studied but has been shown to elicit analgesia, although to a lower extent than with activation of the mu receptor. Results of studies of whether or not activation of the delta receptor results in decreased respiration are contradictory. The nociceptin receptor is a newly-described receptor that has approximately 60% sequence homology with the classical opioid receptors.

The results of nociceptin receptor activation are still not well understood, but believed to be involved in instinct and emotion by antagonism of dopamine signaling pathways. Opioids can bind to one or more receptors, but generally have high affinity for only one receptor type.

In addition to the four receptor classes, there are four classes of endogenous opioid receptor ligands: the endorphins, enkephalins, dynorphins, and nociceptin. The enkephalins are pentapeptides with high affinity for the delta opioid receptors, and the endorphins and dynorphins are more complex peptides that have highest affinities for the mu and kappa receptors, respectively. Nociceptin is a 17 amino acid peptide with affinity for only the nociceptin receptor. All four classes of endogenous opioid have similar heterocyclic ring constituents that are very similar to the structure of the exogenous opioids (see figure).

Opioid receptor ligands can be agonists, antagonists, or partial agonist/antagonists. As the name implies, full agonists activate the receptor, resulting in the complete physiological response. Antagonists reverse or block the effect of agonists. Partial agonists are generally agonists at one receptor, and antagonists at another, such that the full physiological response is often not observed. Generally, there is a ceiling effect seen with partial agonists/antagonists – a dose beyond which there is not an increase in the desired response. Partial agonists are believed to have a lower incidence of development of abuse than full agonists. Morphine is the prototypical mu full agonist. Naloxone (Narcan) is the prototypical antagonist, and reverses the effects of opioid agonists. Naloxone has highest affinity for the mu receptor, but at higher doses will antagonize the other receptor types as well. Because of this, naloxone is used as the antidote to opioid overdose. Other antagonists are used extensively in research to help distinguish which receptor type is activated by investigational compounds. Some partial agonist/antagonists are used therapeutically. Nalbuphine (Nubaine) is used at some institutions to treat sickle cell pain that is refractory to other opioids. Buprenorphine is a mu receptor partial agonist used primarily to treat opioid abuse.

Chronic use of opioids can lead to hypersensitivity to pain (hyperalgesia) and tolerance. The mechanism of tolerance is downregulation of opioid receptor gene expression after chronic exposure to opioids, resulting in decreased receptor density at the cell surface and a need for increase opioid dose to achieve the desired effect. While the mechanism of opioid induced hyperalgesia is not yet understood, the outcome is a requirement for an increased dose to achieve the desired effect. Increased dose increases receptor exposure, which downregulates gene expression, and thus a vicious cycle is begun.

### ***Opioid Metabolism***

Opioids are extensively metabolized. First pass metabolism, mediated by hepatic mixed-function oxidases, typically involves dealkylation or deacetylation. An example of dealkylation is codeine (methyldmorphine) → morphine. An example of deacetylation is heroin (diacetylmorphine) → 6-monoacetylmorphine. CYP3A4 and CYP2D6 are the enzymes most often involved in first pass metabolism of opioids. Patients with liver dysfunction may not respond ideally to opioid prodrugs, such as codeine, that require liver function for biotransformation to the active form. Second pass metabolism typically involves conjugation to a water-soluble moiety such as glucuronic acid, rendering the

metabolite more readily excreted in the urine. The uridine glucuronyl transferases (UGTs) are involved in the second pass metabolism of opioids. Some opioids have active metabolites. These include first pass metabolites, such as hydromorphone, and second pass metabolites, such as morphine-6-glucuronide. Care must be taken in choosing an appropriate opioid analgesic for patients with impaired renal function, as active metabolites may accumulate in patients with decreased renal clearance. An opioid without active metabolites may be more appropriate to decrease the risk of potential adverse events in these patients.

### ***Opioids and the Clinical Laboratory***

The clinical laboratory tests that pertain to opioid analgesic therapy or abuse include immunoassay and mass spectrometry for identification. Some molecular testing for mutations in metabolic enzymes is also available. Identification is important in both the emergency setting and the pain management setting. Immunoassays are widely used for screening in the clinical lab. The disadvantages to immunoassay are the degree of cross-reactivity, the cut-off limit, limited number of compounds detectable per screen, and the need for a confirmation step. Mass spectrometry, coupled to gas or liquid chromatography, is more specific and sensitive. The drawbacks to mass spectrometry are cost and technical difficulty in operating/maintaining. When a patient presents with symptoms of opioid overdose, it may be necessary to test for salicylates and/or acetaminophen as well. This is because there are several co-formulations of opioid plus salicylate and/or acetaminophen, such as Vicodin (hydrocodone + acetaminophen), used widely in outpatient pain management.

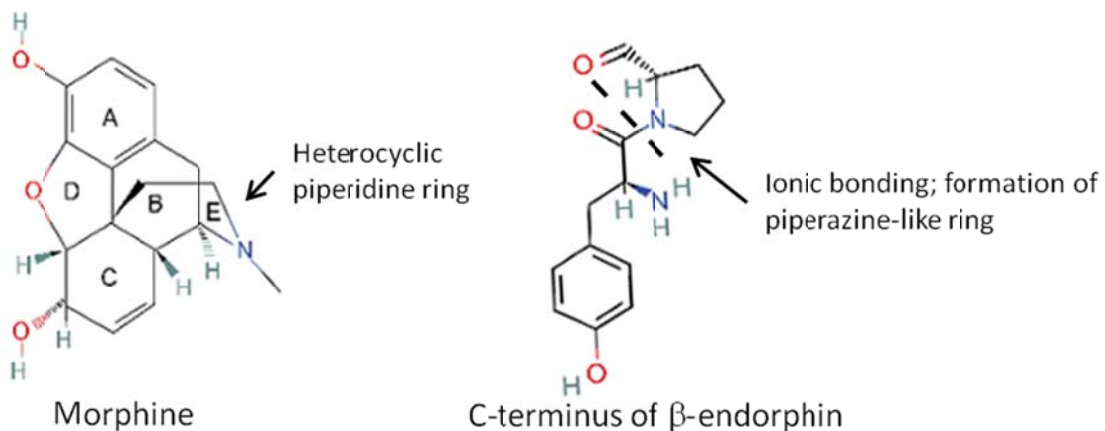
A challenge for clinical laboratories is supporting pain management therapies by providing adequate testing for compliance. Monitoring compliance is important because outpatient opioid pain therapy is a source of abused drugs. A 2006 National Survey on Drug Abuse and Health revealed that 56% of individuals reporting misuse of pain relievers in the year prior to the survey obtained the medication from friends or family, and 81% of those reported the friend or relative obtained the medication from just one doctor. Therefore relying on behavioral cues such as “doctor shopping” is insufficient to predict non-compliance. Of particular concern in this setting is the interpretation of a negative result by immunoassay. This could indicate that the patient is non-compliant, or it could simply be a false negative. Based on forensic (i.e. workplace testing) or overdose models, most immunoassay cutoffs are too high to detect opioids when a therapeutic dose is maintained. In 2009, Mikel et al showed that 12-40% of patients on opioid therapy were missed by nominal immunoassay cutoffs. Also, immunoassays are not equally sensitive to the entire opioid drug class. Use of more specific and specific technology such as LC-MS/MS is more appropriate for this clinical application.

With the recent interest in pharmacogenomics and personalized medicine, the roles of genetic variants in the genes coding for enzymes in the opioid absorption, distribution, metabolism and excretion pathways are being investigated. Polymorphisms affecting expression or function of the gene product have been found in both *CYP2D6* and *CYP3A4*. Currently, determination of only *CYP2D6* status is clinically available. It is estimated that 5-10% of Caucasians,  $\leq 1\%$  of Asians, and 0-35% of African-Americans have allelic variants associated with decreased clearance. It is estimated that 1-7% of Caucasians and 9-30% of African-Americans have allelic variants associated with rapid metabolism and

clearance. Phenotypically, patients can be classified as “poor metabolizers”, “intermediate metabolizers”, “extensive metabolizers”, and “ultrapid metabolizers”. Extensive metabolizers are those that have normal gene expression/gene product function and have the normal, expected response to standard doses. “Poor metabolizers” are those with inactive or dysfunctional enzymes. A prodrug requiring biotransformation for pharmacological activity, such as codeine, may be ineffective in poor metabolizers. Ultrapid metabolizers have more functional enzyme due to gene duplication. Due to more rapid metabolism, these patients may require a larger dose than is normally effective. Molecular testing for determining CYP3A4 status is available clinically, however at present the only opioid for which genotyping is clinically significant is codeine.

In summary, opioids are effective analgesics that are commonly used and abused in both adult and pediatric populations. Knowledge of the pharmacology and metabolism of these compounds is important for understanding their physical effects and the complications that can arise with opioid analgesia therapy. A large percentage of the abused opioids are obtained by friends or family members, who obtain the drugs from physicians. Monitoring pain management patients for compliance may be a way to deter opioid abuse. The clinical laboratorian should be aware of the limitations of the different analytical techniques used for identification of opioids, and be able to assist the physician through test result interpretation and education.

**Figure 1. Structural similarities between exogenous and endogenous opioids.**





**Table 1. Commonly used opioids, major metabolites, and CYPs involved in metabolism.**

Opioid	Major Metabolite	CYP3A4/CYP2D6	Active Metabolite?
<b>Morphine</b>	Morphine Glucuronide	-	Yes
<b>Codeine</b>	Morphine	2D6	Yes
<b>Hydrocodone</b>	Hydromorphone	2D6	Yes
<b>Hydromorphone</b>	Hydromorphone Glucuronide	-	Yes
<b>Oxycodone</b>	Oxymorphone	2D6	Yes
<b>Oxymorphone</b>	Hydroxy-oxymorphone	-	No
<b>Fentanyl</b>	Norfentanyl	3A4	No
<b>Methadone</b>	EDDP*	3A4	No

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## **“Ich bin ein Berliner” : Scientists Appreciate the International Congress of Pediatric Laboratory Medicine (ICPLM) Held in Berlin, Germany, May 13-15, 2011**

The International Congress of Pediatric Laboratory Medicine (ICPLM) took place in Berlin, Germany on May 13 through 15, 2011, and drew more than 330 registered participants. The Congress was arranged to just precede the opening of the IFCC Congress, at the same venue. Four Plenary Lectures and ten Symposia offered more than 30 speakers, and presentations were well acclaimed. Our own PMF AACC Board members in attendance as speakers and/or organizers included: Michael Bennett (past member), Vijay Grey (past member), Patti Jones (past member), Nathalie Lepage (Past Chair), and Sharon Geaghan (Chair). Additionally, more than 90 scientific posters were exhibited across the spectrum of pediatric laboratory medicine.

Under the dynamic leadership of Chair Klaus Kohse (Germany) and Vice Chair Vijay Grey (Canada), the ICPLM raised more than 60,000 € in sponsorship monies and more than 50,000 € from registration fees, making this a most financially successful Congress.

In addition to a scientifically rewarding experience, the participants celebrated the finale with a dinner cruise on the picturesque River Spree, and were delighted by one of most extraordinary, historically important\* and architecturally exciting cities in the world.

If you weren't fortunate enough to attend, the short papers proffered are to be found in a special edition of *Clinical Biochemistry* (44: 7, May 2011, pp 445-565) devoted to the XII<sup>th</sup> International Congress of Paediatric Laboratory Medicine, thanks to the work of Guest Editors, Vijaylaxmi Grey and Klaus P. Kohse.

\*History buffs: For a historical treat, see “Ich bin ein Berliner”

[http://en.wikipedia.org/wiki/Ich\\_bin\\_ein\\_Berliner](http://en.wikipedia.org/wiki/Ich_bin_ein_Berliner), a celebrated June 26, 1963 speech expressing U.S. support for West Berlin during the Cold War, and one of the most acclaimed oratories of President John F Kennedy. The words were delivered to an audience of 450,000 on the steps of Rathaus Schöneberg, and excerpted here: “Two thousand years ago the proudest boast was *civis Romanus sum* [“I am a Roman citizen”]. Today, in the world of freedom, the proudest boast is “Ich bin ein Berliner!”... All free men, wherever they may live, are citizens of Berlin, and, therefore, as a free man, I take pride in the words “Ich bin ein Berliner!” (Wikipedia)

*Contributed by Sharon Geaghan*

## **Pediatric and Maternal-Fetal Division Awards Presented at the AACC Annual Meeting in July 2011**

### **Award for Outstanding Contributions to Pediatric and Maternal-Fetal Clinical Chemistry**

*Below is a reproduction of the allocution that was made on July 26, 2011, when Dr Vijay Laxmi Grey received the award (during the Molecular Pathology, Pediatric and Maternal-Fetal, Industry and Clinical Translational Joint Mixer). Allocution was made by Dr Nathalie Lepage, Chair, Pediatric and Maternal-Fetal Division.*

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It is my pleasure to honor Dr. Vijay Laxmi Grey as recipient of the 2011 PMF Division award for "Outstanding Contributions to the Pediatric and Maternal-Fetal Division".

Dr. Grey is currently a Pediatric Clinical Chemist in the Hamilton Regional Medicine Laboratory Program and Professor in the Department of Pathology and Molecular Medicine, and Associate member in the Department of Pediatrics at McMaster University. She is a fellow of the Canadian Academy of Clinical Biochemistry.

Dr Grey's contributions to Pediatric Laboratory Medicine and the Pediatric and Maternal-Fetal Division are exemplary and well deserving of this award. Dr Grey has been a member of the Pediatric Maternal and Fetal Division (PMF) of the American Association of Clinical Chemistry (AACC) since 1994, and served as Editor of the PMF Division newsletter (2000-2004) and Division Chair (2006-2008). She serves on the Pediatric Reference Range Committee of the AACC. She is currently Vice Chair of the International Federation of Clinical Chemistry (IFCC) Task Force on Pediatric Laboratory medicine and was Co-chair of the 2011-XII<sup>th</sup> International Congress of Pediatric Laboratory Medicine. She has also served on the Board of the Canadian Academy of Clinical Chemistry and was Chair (2006-2007). In 2008, she received the Canadian Academy of Clinical Chemistry National Award for outstanding contributions to clinical chemistry. She is active in the Pediatric Focus group of the Canadian Society of Clinical Chemists. Dr. Grey is an editorial board member of the *Clinical Biochemistry* journal.

Dr Grey is active in research in Nutrition and Cystic Fibrosis, and also many aspects of Clinical Biochemistry research. She is currently an investigator in the CALIPER (Canadian Laboratory Initiative on Pediatric Reference Interval Database) project aimed at the establishment of a laboratory reference interval database for biomarkers of pediatric disease.

I would like to present some of my personal interactions with Vijay.

When I first met her, she was one very respected pediatric clinical biochemist in Canada, and I was new to the field. When I asked her: who could be my contact person for sweat chloride testing, she answered "me". Who could be my contact person for vitamin measurement, she answered "me". Who could be my contact person for inulin measurement, and she also answered "me"!!

She was definitely a wealth of information, filled with technical details and clinical utility.

Then she suggested that a good implication for me would be to join the PMF division. She indicated that I would meet great people, I would be able to increase my knowledge of pediatric clinical biochemistry and I would be able to learn about hot topics in the field.

She was right on all aspects. I encourage all of you to join our division, for the same reasons.

As you would have realized by now, it is my pleasure and honor to give the Pediatric and Maternal Fetal division award for outstanding contributions to Pediatric and Maternal Fetal clinical chemistry to Dr Vijay Grey!!



(From L-R, Dr. Lepage and Dr. Grey)

### **AACC Division Poster Winners**

Congratulations of the winners of the Pediatric and Maternal Fetal Division 2011 poster session awards. In case you missed their posters at the meeting, both awardees have submitted summaries of their work.

#### **Best poster: Doaa Hashad, MD, Alexandria University**

#### **Free fetal DNA in prenatal detection of fetal RhD status and gender by molecular analysis of maternal plasma**

**Background:** Current methods of fetal genetic testing for prenatal diagnosis typically involve obtaining samples using invasive techniques that place the fetus at risk of injury or death and may further sensitize the mother against fetal red cell antigens. One potential noninvasive approach involves analysis of cell free fetal DNA (cffDNA) in maternal blood.

In many countries, anti D-immunoglobulin injections are offered to D-negative pregnant women to reduce the chances of prenatal immunization even though up to 40% of these women will have a D-negative fetus. Therefore, mass application of RhD noninvasive prenatal diagnosis of all fetuses carried by RhD-negative women is highly desirable so that unnecessary anti-D administration is avoided.

Demonstration of sex determining region of the Y-chromosome (SRY) in maternal plasma during pregnancy indicates existence of a male fetus which is of particular importance when a fetus may be affected by an X-linked disorder such as hemophilia.

**Aim:** To evaluate the use of cell free fetal DNA (cffDNA) in plasma of RhD-negative women for non-invasive early detection of fetal RhD status and gender.

**Materials and methods:** A total of 90 maternal plasma samples, prospectively collected from 90 RhD-negative pregnant women were analyzed by real time PCR for diagnosis of fetal RhD status and gender. Forty five samples were taken in the first trimester and forty five samples in the second trimester of pregnancy.

**Results:** The gestational age at time of blood sampling ranged from 7 to 24 weeks. Among 90 fetuses, 61 were RhD-positive and 29 were RhD-negative according to phenotypic diagnosis by standard serological tests. As regards sex, 37 were males and 53 were females.

The molecular analysis of cffDNA from the maternal plasma to identify fetuses that are RhD-positive in RhD-negative women had an overall diagnostic accuracy of 94.4%. PCR based detection of RhD status diagnosed RhD-positive fetuses in 59 samples (sensitivity 96.7%) and RhD-negative fetuses in 26 samples (specificity 89.7%) with 2 false negative results (NPV 92.9%) and 3 false positive results (PPV 95.2%).

In the first trimester, the sensitivity was 93.5%, with two false negative results at the 8<sup>th</sup> and 10<sup>th</sup> weeks of gestation (NPV 85.7%) and the specificity was 85.7%, with two false positive results at 8<sup>th</sup> and 11<sup>th</sup> weeks of gestation (PPV 93.5%) and the diagnostic accuracy was 91.1%.

In the second trimester, the sensitivity increased to (100%) with no false negative results (NPV 100%). The specificity increased to 93.3% with one false positive result at 20<sup>th</sup> week of gestation (PPV 96.7%). The diagnostic accuracy increased to 97.8% denoting that real time-PCR is more accurate and sensitive in diagnosing RhD-positive fetuses in the second trimester.

The increase in the sensitivity of real time –PCR technique for the diagnosis of fetal RhD status using cffDNA in the second trimester was statistically significant in comparison to the first trimester ( $Z=2.74$ ,  $p=0.032$ ). Also, the increase in the NPV in the second trimester was highly significant in comparison to the first trimester ( $Z=4.08$ ,  $p=0.01$ ).

The overall diagnostic accuracy of real time PCR-assay using cffDNA from maternal plasma in determining fetal sex was 98.89%. PCR results diagnosed male fetuses in 36 samples (sensitivity 97.3%) and female fetuses in 53 samples (specificity 100%) with one false negative result (NPV 98.1%) and no false positive results (PPV 100%).

The sensitivity in the first trimester was 95.2% with one false negative result (NPV 96%) at the 7<sup>th</sup> week of gestation with diagnostic accuracy of 97.8%. However, the sensitivity increased thereafter reaching 100% in the second trimester of pregnancy with diagnostic accuracy of 100%.

This denotes that real time-PCR was relatively accurate and sensitive in diagnosing fetal sex as early as the 7<sup>th</sup> week of gestation with increasing sensitivity in the second trimester of pregnancy.

**Conclusion:** The use of cffDNA in prenatal noninvasive early detection of fetal RhD status and gender by real time-PCR is highly sensitive and accurate as early as the 11<sup>th</sup> week of gestation for RhD status and the 7<sup>th</sup> week of gestation for fetal sex.

**Student and Young Faculty Award: Chunfang Liu, Ph.D, Fudan University**

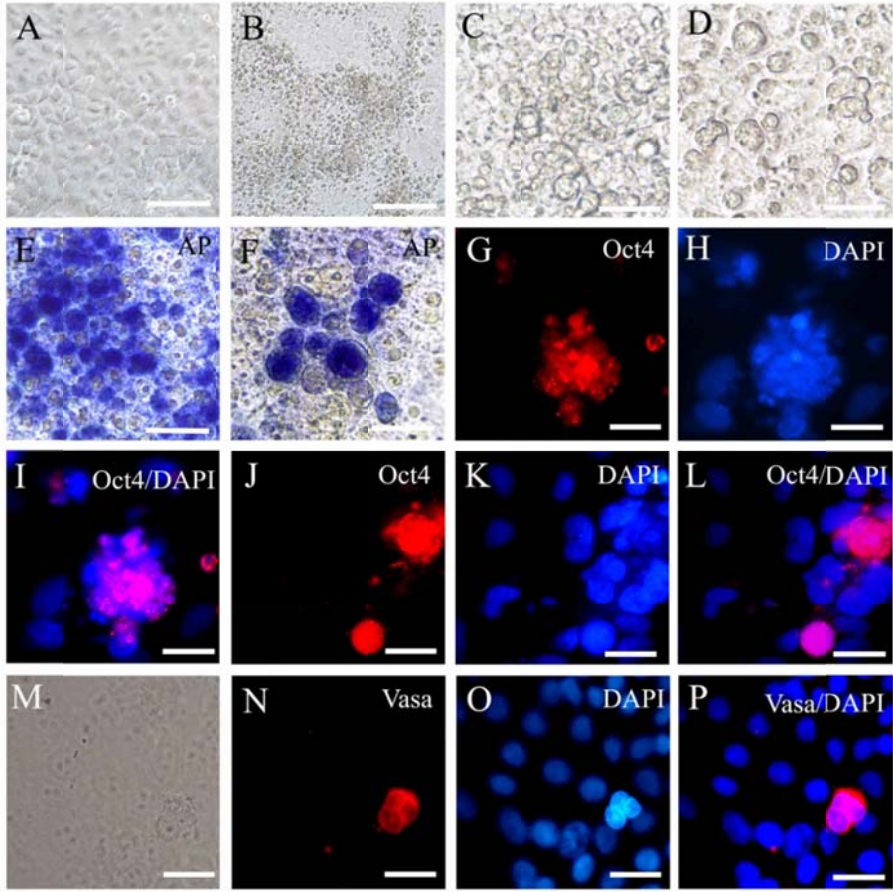
#### **Generation of oocyte-like cells from human hepatoblastoma cell line, HuH-6.**

It has long been understood that the processes of germ cell and tumor development share important similarities. The corresponding features between cancer cells and the germ cell/gamete/trophoblast differentiation pathways include: immortalization (involved in transformation), invasion, induction of meiosis (leading to aneuploidy) and migration (contributes to metastasis). It has been reported that the genes specific to germline development can be activated in many tumors. Recently, Janic et al. further revealed that germline traits are necessary for tumor growth, while the inactivation of germline genes have tumor-suppressing effects in drosophila, suggesting that the germline traits of tumors are associated with some of the tumor's malignant characteristics. Therefore, we want to address whether germline formation is activated in tumors. In this study, we used human hepatoblastoma cell line, HuH6 cells as a model.

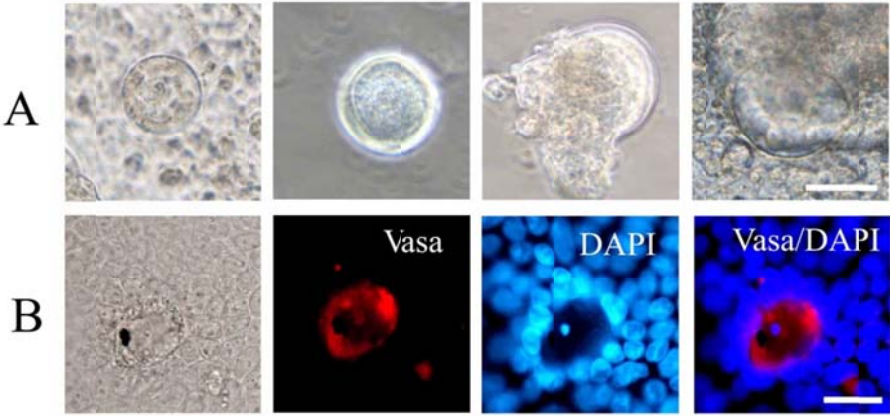
For this study, HuH6 cells were cultured continuously for 2-6 weeks without subculture to attain full confluence and a high cell density. In specific culture conditions, HuH6 cells generated spontaneously distinct small round cells with a large nucleus-to-cytoplasm ratio, similar to early germ cells in morphology (Fig. 1A-D). The germ cell-like cells expressed markers associated with germ cells, including alkaline phosphatase (AP) (Fig. 1E, F), Oct4 (Fig. 1G-L) and Vasa (Fig. 1M-P). The early germ cell-like cells could further develop into oocyte-like cells in vitro. The oocyte-like cells could grow up to 35 $\mu$ m in size and expressed Vasa which is specific to germ cells (Fig. 2). Thereby these findings indicated that the formation of germ cell-like cells are actually activated in human hepatoblastoma cells.

The appearance of germ cell-like cells in hepatoblastomas will contribute to explain why cancers possess strong germline features. The germ cells derived from cancer cells might contribute to the tumor's central malignant characteristics. Therefore, the formation of germ-like cells in tumors possibly provided novel targets to tumor biology, diagnosis and therapy.

This study was supported by Natural Science Foundation of China (No. 30801321).



**Figure 1. Formation and markers expression of germ cell-like cells.** (A) Morphology of Huh6 cells in culture. (B) Small round cells appeared above Huh6 cells. (C, D) High magnification. (E-P) Markers expression of germ cell-like cells. Bar scales = 40  $\mu\text{m}$  (A), 80  $\mu\text{m}$  (B), 20 $\mu\text{m}$  (C-P).



**Figure 2. Formation and characterization of oocyte-like cells.** (A) Phase contrast image of oocyte-like cells at different stage. (B) Expression of germline specific marker, Vasa. Bar scales = 10  $\mu$ m



## **Update on Pediatric Reference Interval Initiative**

A group gathered at the AACC Annual Meeting in Atlanta to discuss progress in various programs generating pediatric reference ranges. A summary of one of the reports is below.

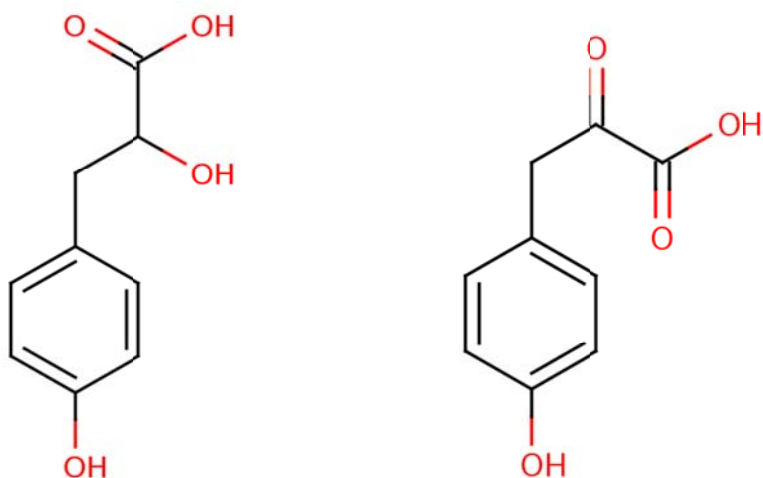
### **The PRRC's Supplemental Methodological Study (SMS)**

In January the PRRC (Mike Bennett, chair, Dennis Dietzen, Stan Lo, Vijay Grey and Patti Jones) made a proposal to the National Children's Study (NCS) for a Supplemental Methodological Study. The SMS proposal was intended as a proof of principle type study to work through the details of working with the NCS and obtaining samples from them. The SMS proposed to obtain dried blood spot (DBS) samples from the NCS for the purpose of determining pediatric reference intervals for amino acids and some steroid hormones. The SMS was written this way specifically because: 1) DBS are samples that are known to be currently available from the NCS, 2) these specific biomarkers are available by traceable, tandem MS assays at Committee Members' institutions, allowing cost to be controlled and kept low. In February the NCS granted the SMS provisional approval. Since that time the PRRC has been working through documentation, paperwork and necessary data-handling facilities required by the NCS and by the involved institutions in St. Louis and Dallas. Dennis Dietzen and Patti Jones are currently working to adapt serum MS/MS assays for amino acids and steroid respectively, into assays which can utilize DBS. In October, the PRRC received notification that samples have been pulled from the repository and are being shipped! The next steps will be to analyze the samples and then analyze the data derived from those samples.

*Submitted by Patti Jones*

## Interview With an Organic Acid

By Dennis Dietzen



The twins. Left: *p*-hydroxyphenyllactate; Right: *p*-hydroxyphenylpyruvate.

### FAST FACTS

	<i>p</i> -hydroxyphenyllactate	<i>p</i> -hydroxyphenylpyruvate
IUPAC Name	2-hydroxy-3-(4-hydroxyphenyl)propanoate	4-hydroxy- $\alpha$ -oxo-benzenepropanoate
Formula	C <sub>9</sub> H <sub>10</sub> O <sub>4</sub>	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>
Molecular weight	182.17 g/mol	180.16 g/mol
Melting point	143°C	220°C
Water solubility	5 mg/mL	1.5 mg/mL

Today you are in for a treat. We are in a quiet out of the way place in the liver away from all of the major metabolic pathways where we have garnered a buy one-get one free discussion with the twins, *p*-hydroxyphenyllactate (PHPL) and *p*-hydroxyphenylpyruvate (PHPP). These molecules are typically not good for much but you need to keep an eye on them as they are known snitches when it comes to illicit metabolic behavior.

The M: Would you describe yourselves for us, please?

PHPP: My most distinguishing feature is the hydroxyphenyl ring. Some people call it a phenolic ring.

PHPL: Yea, and some people call it aromatic! Not that you are smelly or anything.

PHPP: Real mature, sis. As I was saying, the rest of me is pretty simple. Across from my phenolic hydroxyl, I am really just good ol propanoic acid with a ketone group on my second carbon. I liked it better when I had an amino group there but I am getting used to its absence.

PHPL: I hate it when my sister does all the talking. She thinks she is so much more important than I am. I have a phenolic group and a propanoic acid chain just like her but after I lost my nitrogen I got “reduced.” No it is not some weight loss program. I just prefer to be a secondary alcohol rather than a ketone.

The M: Where do you ladies come from originally?

PHPP: We both are derived from tyrosine and distantly related to phenylalanine. Our hydroxyl group comes compliments of phenylalanine hydroxylase and then that greedy guy, tyrosine aminotransferase, takes our nitrogen away.

PHPL: I really don't think about how I got to be the way I am but a lot of people think that lactate dehydrogenase (man, that guy is everywhere) is responsible for making me an alcohol. I am just a little more complex and sophisticated than my sister.

PHPP: Oh yea, but you lead to a metabolic dead end!

The M: Now ladies. Let's calm down a bit here. Ms. PHPP, where do you ultimately end up?

PHPP: I usually don't hang around for very long. Once I get deaminated I usually go see to the corner enzyme shop and look up my friend 4-hydroxyphenylpyruvate dioxygenase. We are such good friends we share a name. After I meet with my own personal dioxygenase, I subsequently meet with homogentisate 1,2 dioxygenase, maleoylacetoacetate isomerase, fumarylacetoacetate hydrolase. Once I run this gauntlet I can help in the Krebs cycle and in ketogenesis. If any of these guys aren't around I just tend to sit around, accumulate, and hang out with PHPL.

PHPL: Yea, I am always the last one on her list. No respect.

The M: Wow, really PHPP. You are so pleasant that I can't imagine anyone not making it a priority to see you.

PHPP: It's pretty rare but it happens. We even have names for those times that it happens. When fumarylacetoacetate hydrolase is missing, we call that tyrosinemia type 1. This is bad news. The liver and kidneys don't handle this very well. When homogentisate 1,2 dioxygenase is missing, we call that alkaptonuria. Finally, when my best friend, PHPP dioxygenase fails to show up, that's called type III tyrosinemia.

The M: Really? Your best buddy just blows you off. Doesn't show up at all?

PHPP: Well, to be fair, sometimes PHPP dioxygenase is just slow. This almost always occurs shortly after birth. I tend to accumulate to a small degree but I tend to disappear pretty quickly too. This also has a name. It's called transient tyrosinemia.

The M: There is one other thing I am wondering about. I have heard that tyrosine aminotransferase is sometimes out to lunch and not around. In that case, it seems like it would be hard for you to even exist, and yet you still manage to accumulate. That's just weird. How does that happen?

PHPL: She's got that covered, too. She is just perfect. Always Mom's favorite.

PHPP: Oh stop it. When tyrosine aminotransferase is missing this is called type II tyrosinemia. In this case, I made a deal with aspartate aminotransferase. He can usually handle the amine group from tyrosine and guess what? You get me!

PHPL: Me, too!

PHPP: It's not very efficient, but it's better than nothing at all.

The M: This has been a really great discussion. I really appreciate your time. If I need to find you again, how would I go about it?

PHPL: Let me handle this one. The way to find us is by doing this thing called an organic acid profile. We get extracted with a bunch of our unrelated friends, get derivatized and then shot through a gas chromatograph. We usually hang out on your typical GC column longer than anybody else and we are late to the party. Since PHPP is a ketone, she is sometimes a little unstable and hard to pick up. We can usually keep her around by oximating her. She really doesn't like to admit her flaws. Despite our similarities, we have different tolerance to temperature and you can tell our mass spectra apart pretty easily.

The M: So, to summarize then, you guys are really around whenever tyrosine can't find its way home. That's really kind of touching but it must be hard to have all that responsibility.

PHPP: It's the least we can do to let folks know when there is a problem. We are happy to help.

The M: Thanks again for the chat.

PHPL: Sure thing.

## Excerpts From the Literature

Articles of interest compiled by the editorial board.

### **Mild infantile hypercalcaemia. JPeds (2011) 159;215 (MPM)**

Hypercalcaemia is an uncommon problem in children's chemistry, at least in comparison to adult chemistry. Investigation of hypercalcaemia in children is often difficult and usually unrewarding. Williams syndrome, immobilisation, renal disease, and inherited calcium sensing mutations are all possibilities.

The authors describe all 32 infants (children aged less than two years) referred to the hypercalcaemia clinic between July 2002 and September 2008 for evaluation of hypercalcaemia. Extensive investigations were undertaken including serum calcium, phosphate, PTH, 25(OH) Vitamin D, 1,25(OH)<sub>2</sub> Vitamin D as well as urine calcium and creatinine. Williams syndrome genetic screening, renal ultrasound and family calcium levels were also obtained. In 26 of the infants, familial hypocalciuric hypercalcaemia mutations, calcitonin, IGF-1 and urine citrate/creatinine ratios were obtained. Children were followed for four months. Dietary manipulation to decrease calcium intake was instituted in all infants. Calcium measured on the Vitros 950 platform. One infant was found to have Williams syndrome, two were found to have multicystic renal dysplasia, and two were found to have familial hypocalciuric hypercalcaemia. The two with FHH mutations shared the mutation with their mothers who were normocalcaemic.

The serum calcium concentration range at presentation was 2.65–3.53 mmol/L or 10.6-14.1 mg%. These clinicians were working with reference intervals for serum calcium of 2.10-2.65 mmol/L or 8.4-10.6 mg% for all ages.

The reference intervals for serum calcium using the Vitros platform published in Clinical Biochemistry (2010) 43:1039 as part of the Caliper scheme are 2.39-3.05 mmol/L or 9.55-12.20 mg% for children under one year of age. They then change to 2.36-2.83 mmol/L and 8.9-11.30 mg% for children aged 1-5 years. Boys have a slightly lower LRL. These are supported in other Caliper work with the Abbott and Roche platforms. Additionally it should be noted that calcium concentrations decrease rapidly in the first year of life.

To properly evaluate these children with the Caliper reference intervals the patients' ages are needed. Conceivably only three of these children may have been hypercalcaemic at presentation. Depending on the infants' ages, no more than nine would have had serum calcium concentrations above the URL.

The authors state that 59% of the infants had hypercalciuria, using a reference interval for urine calcium/creatinine of less than 0.7 (mmol/mmol). Another reference interval published in the Annals of Clinical Biochemistry (2006) 43:398 for urine calcium/creatinine ratio suggests values less than 1.5 mmol/mmol for children under one year and 1.25 mmol/mmol for those under two years of age.

With different, more appropriate reference intervals for serum calcium, a greater rate of specific diagnosis may have been obtained.

This is an interesting paper that particularly highlights the need for the development of useful age appropriate reference intervals and their application in the evaluation of disorders of infants.

**Phenylalanine is strongly associated with oxidative stress. *Molecular Genetics and Metabolism* 2011, 103:220-5. (UG)**

Phenylketonuria (PKU) is an autosomal recessive disorder most commonly caused by the deficiency of enzyme phenylalanine hydroxylase. Treatment for PKU is a phenylalanine restricted diet. Phenylalanine concentrations in the brain correlate with neurological signs and brain dysfunction in PKU patients. Oxidative stress has been implemented in the pathophysiology of many diseases including neurological diseases. Recent studies have noted that oxidative stress is high in patients with PKU. In this study the oxidative stress status was investigated in 40 adolescent and adult patients (28 females and 12 males, with age 15-50 years) with PKU. Oxidative stress markers, antioxidant enzyme activities in red blood cells, and blood antioxidant levels were studied. Nitric oxide production, as a measure of oxidative stress, was also measured. Markers of oxidative stress, thiobarbituric acid and malondialdehyde levels were significantly higher in PKU patient as compared to controls. Plasma antioxidant reactivity was significantly lower in patient group as compared to controls. The activities of superoxide dismutase and catalase were higher in erythrocytes, and correlated with phenylalanine levels. However, the activity of glutathione peroxidase was lower in erythrocytes and negatively correlated with phenylalanine levels. Plasma antioxidants beta-carotene and coenzyme Q10 was significantly lower in patients with PKU. Nitrite/nitrate, the markers of nitric oxide, were higher in patients with PKU. Since nitric oxide has a regulatory affect on the neurological function it is possible that such abnormalities in the nitric oxide metabolism could be implemented at least in part in neurological dysfunction in PKU. The authors conclude that oxidase stress status is closely linked with serum phenylalanine levels. The authors proposed that the serum phenylalanine concentration should be maintained below 700-800  $\mu\text{moles/L}$  even later in life.

**The American Thyroid Association Taskforce on Thyroid Disease During Pregnancy and Postpartum 2011 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, et al. *Thyroid* 2011; 21:1081-1124.**

**Haddow JE. The new American Thyroid Association guidelines for thyroid disease during pregnancy and postpartum: a blueprint for improving prenatal care. (Editorial) *Thyroid* 2011;21:1047-8.**

**Glinoe D. Personal consideration s on the 2011 American Thyroid Association and the 2007 Endocrine Society pregnancy and thyroid disease guidelines. (Editorial) *Thyroid* 2011; 21:2049-51. (SL)**

Recently, new guidelines (Stagnaro-Green et al) from the American Thyroid Association to address thyroid disease during pregnancy and postpartum have been published. Accompanying the guidelines are two editorials (Haddow and Glinoe). The most efficient way to consume all the information found in the guidelines for this reviewer was to read the editorials first. Glineor's editorial was particularly helpful as it concisely describes the major differences between the first set of clinical practice guidelines published in 2007, to the current guidelines. Specific mentioning of laboratory related testing in different sections of the guidelines was quite helpful. For example, the guidelines recommend narrowing the TSH reference range for pregnant individuals and establishing trimester-specific ranges

for TSH and FT4. FT4 reference ranges will need to be method-specific. Haddow highlights several of the suggested clinical management changes and their potential for acceptance or implementation. For example, in determining subclinical hypothyroidism and overt hypothyroidism, the guideline recommends TSH while describing the limitations of FT4 measurements. The new guidelines contain an enormous amount of useful information. Thankfully it is well written and easy to understand. Enjoy!

## **Newly Elected Members of the Board**

Congratulations to the newly elected members and current members who have assumed new positions:

### **Chair-**

Sharon Geaghan

### **Chair Elect-**

David Carpentieri

### **Past Chair-**

Nathalie Lepage

### **Secretary-**

Shannon Haymond

### **Treasurer-**

Sihe Wang

### **Nominating Committee-**

Nathalie Lepage (Chair)

### **Members at Large-**

Christina Lockwood

Linda Rogers

Dean Carlow

Jon Nakamoto

### **Newsletter Editor-**

Angela Ferguson

### **Newsletter Editorial Board-**

Uttam Garg

Stanley Lo

Michael Metz

### **Webmaster-**

Jumoke Oladipo



## **In Memoriam**

Dr Susan Tange-Cosio died on July 4, 2011, in Prince George, BC. A graduate of the University of Minnesota, Susan joined the faculty of McGill University in 1980. In 1988, she became the Director of Biochemistry at the Montreal Children's Hospital where she remained until 2002. Susan set a high standard for performance in the laboratory and challenged her colleagues. She was a strong individual, and knew what she wanted but she was fun to be with and had a good sense of humor.

She was a member of the Canadian Society of Clinical Chemistry (CSCC) and the American Association of Clinical Chemistry (AACC). Susan's dedication to pediatric laboratory medicine guided her career. She was active in the Pediatric Maternal Fetal Division for many years and founded the Pediatric Focus group of the CSCC. In 2003, Dr. Tange was awarded the Pediatric Maternal-Fetal Division award for Outstanding contributions to Pediatric Clinical Chemistry.

*Submitted by David Blank and Vijay Grey*