Discover, Research, Innovation and Education

Friday, April 13
&
Saturday, April 14, 2018

Edmonton Clinic Health Academy
University of Alberta
## Friday, April 13, 2018
### Discovery & Research Sessions
#### ECHA L1-430

<table>
<thead>
<tr>
<th>Time</th>
<th>Presenter</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00</td>
<td><strong>Welcome – Dr. Michael Mengel</strong></td>
<td></td>
</tr>
<tr>
<td>8:05 - 8:20</td>
<td>Hannah Forbes</td>
<td>MLS</td>
</tr>
<tr>
<td>8:20 - 8:35</td>
<td>Theora Gray</td>
<td>MLS</td>
</tr>
<tr>
<td>8:35 – 8:50</td>
<td>Charlotte McWilliams</td>
<td>MLS</td>
</tr>
<tr>
<td>8:50 – 9:05</td>
<td>Kristalee Watson</td>
<td>Resident</td>
</tr>
<tr>
<td>9:05 – 9:20</td>
<td>Peter Dromparis</td>
<td>Resident</td>
</tr>
<tr>
<td>9:20 – 9:35</td>
<td>Bhumi Bhatt</td>
<td>PhD</td>
</tr>
<tr>
<td>9:35 – 9:50</td>
<td><strong>Break</strong></td>
<td></td>
</tr>
<tr>
<td>9:50 – 10:05</td>
<td>Evelyn Asiedu</td>
<td>PhD</td>
</tr>
<tr>
<td>10:05 – 10:20</td>
<td>Lacey Haddon</td>
<td>PhD</td>
</tr>
<tr>
<td>10:20 – 10:35</td>
<td>Sudha Bhavanam</td>
<td>PhD</td>
</tr>
<tr>
<td>10:35 – 10:50</td>
<td>Ian Vander Meulen</td>
<td>MSc</td>
</tr>
<tr>
<td>10:50 – 11:05</td>
<td>Jingyang Xu</td>
<td>MSc</td>
</tr>
<tr>
<td>11:05 – 11:20</td>
<td>Hanyong Peng</td>
<td>Post-Doctoral Research Fellow</td>
</tr>
<tr>
<td>11:20 – 11:35</td>
<td>Ran Zhuo</td>
<td>Post-Doctoral Research Fellow</td>
</tr>
<tr>
<td>11:35 – 12:45</td>
<td><strong>Lunch</strong></td>
<td></td>
</tr>
<tr>
<td>12:45 – 1:00</td>
<td>Regan Wolansky</td>
<td>Director, AHS</td>
</tr>
<tr>
<td>1:00 – 1:30</td>
<td>Dr. Penny Colbourne</td>
<td>Toxicologist, AHS</td>
</tr>
<tr>
<td>1:30 – 1:45</td>
<td>Bruce Lyon</td>
<td>Medical Lab Technologist, U of A Hospital</td>
</tr>
<tr>
<td>1:45 – 2:00</td>
<td>Rebecca Nawaz</td>
<td>Medical Lab Technologist, AHS</td>
</tr>
<tr>
<td>2:00 – 2:15</td>
<td>Dr. Anna Fuezery</td>
<td>Clinical Biochemist &amp; Medical Lead of Point-of-Care Testing, AHS</td>
</tr>
<tr>
<td>2:15 – 2:45</td>
<td>Dr. Narmin Kassam, Pam Mathura, Jennifer Crawford</td>
<td>Director of General Internal Medicine (U of A), Senior Strategic Improvement Consultant, MLT, AHS</td>
</tr>
<tr>
<td>2:45 – 3:45</td>
<td><strong>Poster Viewing</strong></td>
<td></td>
</tr>
<tr>
<td>3:45 – 4:00</td>
<td><strong>Break</strong></td>
<td></td>
</tr>
<tr>
<td>4:00 – 5:00</td>
<td><strong>Concurrent Presentation:</strong> Rachelle Tessier &amp; Jason Layton: Stroke Ambulance – <strong>ECHA L1-430</strong></td>
<td></td>
</tr>
<tr>
<td>4:00 – 5:00</td>
<td>Dr. John W. Macgregor Memorial Lecture – <strong>ECHA L1-190</strong></td>
<td></td>
</tr>
<tr>
<td>4:00 – 5:00</td>
<td><strong>Dr. Lori L. Burrows, PhD, FAAM</strong></td>
<td></td>
</tr>
<tr>
<td>4:00 – 5:00</td>
<td>Professor &amp; CIHR University Delegate</td>
<td></td>
</tr>
<tr>
<td>4:00 – 5:00</td>
<td>Dept. of Biochemistry &amp; Biomedical Sciences</td>
<td></td>
</tr>
<tr>
<td>4:00 – 5:00</td>
<td>Michael G. DeGroote Institute for Infectious Disease Research, McMaster University</td>
<td></td>
</tr>
<tr>
<td>4:00 – 5:00</td>
<td>Title: Canary in a coal mine - using biofilms to find new antimicrobials</td>
<td></td>
</tr>
<tr>
<td>5:00 – 5:15</td>
<td><strong>Oral Presentation Awards / Teacher Awards</strong></td>
<td></td>
</tr>
<tr>
<td>5:30 – 8:00</td>
<td><strong>Dinner</strong></td>
<td></td>
</tr>
</tbody>
</table>
### Saturday, April 14, 2018

**Education Sessions**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Room 1</th>
<th>Room 2</th>
<th>Room 3</th>
<th>Room 4</th>
<th>Room 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:45 – 8:00</td>
<td></td>
<td>L1 - 190</td>
<td>L1 - 135</td>
<td>L1 - 131</td>
<td>L1 - 125</td>
<td>L1 - 150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Registration &amp; Continental Breakfast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:00 – 8:50</td>
<td>Session 1</td>
<td>AP-Year in Review - Dr. Graham Jones: The forensic investigation of fentanyl and other opioid deaths in Alberta</td>
<td>Joel Rivero: CSMLS MLA Membership &amp; other updates</td>
<td>Simon Charlebois: Haiti’s 2010 earthquake humanitarian crisis</td>
<td>Lab Tour of the UAH (4th floor) - limited to the first five</td>
<td>Andrea Taylor: Mental Health</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:00 – 9:50</td>
<td>Session 2</td>
<td>Dr. Penny Colbourne: Legalization of Cannabis, Making our Way Through the Haze</td>
<td>Keith Steinbach: Emotional Intelligence</td>
<td>Graduate Student Session: LinkedIn Basics w/ Christine Gertz</td>
<td>Lab Tour of the UAH (4th floor) - limited to the first five</td>
<td>AP-Year in Review - Dr. Judith Hugh: Prosigna™ testing in Alberta: The Journey &amp; the First 50 tests</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00 – 11:00</td>
<td>Extra Session &amp; Poster Judging</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1 - 190</td>
<td></td>
<td>Poster Viewing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AP-Year in Review - Dr. Erin Chapman: Updates in Head &amp; Neck Cancer Staging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Event</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 11:15 – 12:15   | Dr. R. E. Bell Memorial Lecture – **ECHA L1-190**  
Dr. Verna Yiu  
President & CEO, Alberta Health Services, Professor of Pediatrics, Division of Pediatric Nephrology |
| 12:15 – 12:30   | Poster Presentation Awards                                           |
| 12:30 – 1:30    | Lunch                                                                 |

### Session 3 : 1:30 – 2:20

<table>
<thead>
<tr>
<th>Room</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 - 190</td>
<td>Jamie Tereposky: Volunteering as a MLT in Bangladesh</td>
</tr>
<tr>
<td>L1 - 131</td>
<td>Graduate Student Session: Stress, Self-Care &amp; Boundaries w/ Lisa Purdy</td>
</tr>
<tr>
<td>L1 - 125</td>
<td>Lab Tour of the UAH (4th floor) - limited to the first five</td>
</tr>
<tr>
<td>L1 - 150</td>
<td>Dr. Graham Jones: Hemlock, the pong-pong tree and rosary pea seeds</td>
</tr>
<tr>
<td>L1 - 140</td>
<td>Shanaz Iyer: Meditation</td>
</tr>
</tbody>
</table>

### Session 4 : 2:30 – 3:20

<table>
<thead>
<tr>
<th>Room</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 - 125</td>
<td>Lab Tour of the UAH (4th floor) - limited to the first five</td>
</tr>
<tr>
<td>L1 - 150</td>
<td>Dr. Brian Wong: Granulocyte Transfusion</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:20 – 3:45</td>
<td>Closing Remarks – <strong>L1-190</strong></td>
</tr>
</tbody>
</table>
Oral Presentations
Title: Development of Aptamer Targeting Viable but Nonculurable *Escherichia coli* O157:H7

Authors: Hannah Forbes, Yanming Liu and Xing-Fang Li

**Background and Aim:**
A number of bacteria in stressful environments change to a viable but nonculturable state (VBNC). Pathogens such as *Escherichia coli* O157:H7 at VBNC state can maintain virulence but are not easily detected with conventional methods. Aptamers are DNA sequences that have high affinity to specific targets. To develop an affinity assay to detect VBNC *E. coli* O157:H7, we are generating a pair of aptamers specifically targeting these cells. These will be used to develop an Enzyme-Linked Aptamer-Sorbent Assay (ELASA).

**Material and Methods:**
VBNC *E. coli* O157:H7 cells were generated through incubation of culturable cells in chlorinated tap water. Selection was performed by incubating an ssDNA library with VBNC *E.coli* O157:H7 cells. The ssDNA tightly bound onto the cell surfaces were covalently cross-linked using formaldehyde. Unbound and weakly bound ssDNA sequences were removed by turbo DNase I and volume dilution. Tightly bound ssDNA was quantified by qPCR and amplified by emulsion polymerase chain reaction (ePCR) followed by ssDNA generation. The resulting ssDNA pool will be used for counter-selection and next round selection.

**Results:**
After one round of selection the ssDNA pool was lowered to ~10^6 DNA sequences from ~10^16, indicating methods used can dramatically remove unbound and weakly bound ssDNA while cross-linked ssDNA was protected. Amplification cycle numbers were optimized, 32 cycles for ePCR with 17-20 cycles for extension produced enough products for extraction.

**Conclusions:**
Additional rounds of selection and counter selection are required to select 2 aptamers specific to VBNC *E.coli* O157:H7 for developing an affinity assay to detect this pathogen.
Title: Regulation of Chemotactic Factors in the Human Fetal Membranes

Authors: Theora Gray*, Xin Fang*, and David M. Olson*

Affiliations: Department of Obstetrics & Gynecology, Pediatrics, 220 Heritage Medical Research Centre, University of Alberta, Edmonton, Alberta, Canada, T6G 2S2.

Background and Aim: Parturition is an inflammatory event characterized by the infiltration of leukocytes to uterine tissues. Infiltration is dependent upon chemotactic factors produced by the human fetal membranes (amnion, chorion, and attached decidua), but they have not been identified. The aim of this study is to identify which chemoattractants are upregulated in human fetal membranes.

Materials and Methods: Explants punched from intact fetal membranes from 10 term not in labour (TNL) placentas were cultured. Five different chemokines or inflammatory mediators were added to the culture media to stimulate an inflammatory environment. Control explants received medium without added chemokines. mRNA from control tissues was pooled. RT-PCR validated mRNA abundance in the tissues. Statistical analysis using Prism software tested normality using log-transformed data, and one-way analysis of variance (ANOVA) tested significance of treatments (p≤0.05).

Results: Seven genetic targets were screened by RT-PCR, which identified 5 chemokine candidates (CXCL1, CXCL8, CXCL10, CCL2, and CCL5) based upon their degree of mRNA expression when compared to a control. The majority of results demonstrated dose response relationships. Lipoteichoic acid (LTA) simulated a dose response relationship in CCL2, CXCL1, CXCL8, CXCL10, and IL-6 and was significant with CCL2 (p<0.01). Lipopolysaccharides (LPS) established dose-response significance for CCL5 (p<0.05 and p<0.01), CXCL8 (p<0.05), and CXCL10 (p<0.05). CCL2 was decreased compared to the control when stimulated with IL-6; however, the results are significant (p<0.05, p<0.01).

Conclusions: The most abundantly activated chemokines at the maternal-fetal interface are CCL2, CXCL1, CXCL8, and CXCL10. These chemokines could be used diagnostically or as a therapeutic target in cases of pre-term labour.
**Title:** Design and Validation of a qRT-PCR Assay to Differentiate Rotarix ® Vaccine Strain from Wild Type G1P[8] Rotaviruses

**Authors:** Charlotte McWilliams, Ran Zhuo, Xiaoli Pang

**Background and Aim:**
Rotavirus infection is a leading cause of severe gastroenteritis in infants and young children worldwide. In June 2015, Alberta implemented the Rotarix vaccine which consists of a live attenuated G1P[8] rotavirus strain, providing protection to the most common G1P[8] genotype and other common genotypes. Children asymptomatic of gastroenteritis participating in a province-wide gastroenteritis surveillance study who were vaccinated with Rotarix were tested positive for G1P[8] rotavirus but the source of the G1P[8] strain was ambiguous. The aim of this project was to design and validate a qRT-PCR method to differentiate between Rotarix and wild type G1P[8] strains so asymptomatic infections could be distinguished from vaccine-shedding.

**Material and Methods:**
The design of this assay was based on Guatam et al. 2013. Primers and a Locked Nucleic Acid (LNA) probe targeting a region of the NSP2 gene which has the most sequence diversity between the vaccine and the wild type G1P[8] strain were designed. The LNA probe is a shorter oligonucleotide with the same Tm as the probe designed by Guatam et al so it has the advantage of higher specificity and better quenching.

**Results:**
The new NSP2 assay was specific to Rotarix vaccine strain. No cross reaction to wild type G1P[8] or other strains of rotaviruses nor to other enteric viruses tested: norovirus, adenovirus, saprovirus and astrovirus was observed. The limit of detection of this assay was 10 copies/reaction.

**Conclusions:**
The new NSP2 assay was successful at differentiating between wildtype G1P[8] Rotaviruses and Rotarix vaccine G1P[8] strains.

**Reference:**
Gautam R, Esona MD, Mijatovic-Rustempasic S, Ian Tam K, Gentsch JR, Bowen MD. Real-time RT-PCR assays to differentiate wild-type group A rotavirus strains from rotarix(®) and RotaTeq(®) vaccine strains in stool samples. Human vaccines & immunotherapeutics 2013;10(3):767-777
Background and Aim:
Non-covalent DNA catalytic reactions offer unique ways for isothermally and amplified detection of nucleic acids without the use of enzymes. Several DNA catalytic methods have been developed, including hybridization chain reaction (HCR), entropy driven catalysis (EDC), and catalytic hairpin reaction (CHR). Nevertheless, these methods have some common limitations such as slow reaction kinetics, low amplification efficiency, and exhaustive substrate preparation and purifications. This study aims to develop a new non-covalent DNA catalytic reaction showing improved amplification efficiency and kinetics, and simple substrate preparation.

Material and Methods:
The catalytic reaction uses a substrate of three-way junction (TWJ), which creates tension at the junction and destabilizes the base stacking interaction between the DNA strands. Therefore, the presence of TWJ facilitates and accelerates the toehold-mediated strand displacement reaction which is fundamental to DNA catalytic reactions. Additionally, the preparation of the TWJ substrates is simple, only requiring annealing of three DNA strands at appropriate ratios without any additional purification. Initiator DNA mediates the release of fluorophore from fluorophore-quencher labeled TWJ substrate and fluorescence signaling.

Results:
The one-layer TWJ-mediated catalytic reaction was able to achieve 30-fold signal amplification within one hour, which represents a 13.6-fold increase in amplification efficiency compared to reactions using substrates without TWJ. 1950-fold signal amplification was obtained when the reaction was used in a two-layer format, which allowed detection of as low as 0.4 pM DNA target.

Conclusions:
The TWJ-mediated DNA catalytic reaction shows promising applications for detection of nucleic acids in test tubes and cancer cells, and construction of DNA circuits.
Title: Assessing the ionic liquids 1-ethyl-3-methylimidazolium and 1-ethyl-1-methylpyrrolidinium bromide as novel N-nitrosamine precursors

Authors: Ian Vander Meulen, Ping Jiang, Xing-Fang Li

Background and Aim:
Ionic liquids (ILs) are increasingly used in industrial processes as “green chemicals” because of unique properties of low volatility and customizability. ILs can enable novel processes and/or replace conventional organic solvents in a variety of applications. Widespread use may increase the risk of accidental release of IL-containing industrial wastes into environmental waters. Most ILs are highly water soluble, and have estimated environmental half-lives of several days to a month. IL cations often consist of aromatic or alkyl quaternary amines that resemble previously confirmed N-nitrosamine (NAs) precursors. NAs are confirmed animal carcinogens and classified as probable human carcinogens. NAs are also potent, estimated to have negative health effects at ng/L concentrations. Therefore, this study sought to evaluate two ILs, 1-ethyl-1-methylpyrrolidinium bromide (EMPyr) and 1-ethyl-3-methylimidazolium bromide (EMIm), for their nitrosamine formation potential.

Material and Methods:
Each IL species was reacted with pre-formed monochloramine under standardized conditions. After 24h, samples were extracted from water by liquid-liquid extraction using dichloromethane. The extracts were analyzed to determine nitrosamine concentrations using HPLC-MS/MS. Quantification of NAs was achieved using a deuterated internal standard.

Results:
Both EMIm and EMPyr can produce NAs: EMIm generating N-nitrosomethylethylamine, while EMPyr producing N-nitrosopyrrolidine. EMPyr was a more productive precursor under all conditions evaluated, with a yield on the same order of magnitude as polydiallyldimethylammonium chloride, a confirmed nitrosodimethylamine precursor.

Conclusions:
This study emphasizes the importance of prevention of environmental discharge of ILs to water bodies, but also highlights a need for further evaluation of potential lifecycle impacts of ILs prior to their wide ranging applications.
Title: A novel model to investigate hormone response in luminal A breast cancers

Authors: Lacey Haddon, Hosna Jabbari, Xiuying Hu, and Judith Hugh

Background and Aim:
Estrogen receptor (ER)+ breast cancer is divided into two subtypes, luminal A and luminal B, which differ in their ER expression and response to hormone therapy. Luminal A patients often have higher ER levels and an excellent response to hormone therapy. Currently, molecular testing is only 75% accurate at differentiating between the luminal subtypes, causing many luminal A patients to receive unnecessary chemotherapy. Since ER is a major regulator of gene transcription in breast cancer, we hypothesized that increased ER expression enables novel ER-DNA interactions that promote the formation of chromatin structures, called DNA loops, which regulate the response to hormone therapy.

Material and Methods:
The luminal A-like model was generated by transducing a luminal B cell line with an inducible ESR1 plasmid (MCF7-ER). MCF7-ER cells were treated +/- 10nM E2 and measured for genome-wide changes in ER-DNA-binding and differential gene expression. ER peaks were compared against published datasets for ER-mediated DNA loops mapped in E2-treated MCF-7s.

Results:
Increased ER expression promotes novel ER-binding at several proliferative genes and corresponds to previously mapped DNA loops. Differential expression analysis showed a basal increase in these genes in MCF7-ER cells that switches to repression in the presence of hormone.

Conclusions:
Our results show that increased ER expression, a luminal A phenotype, mediates changes in gene transcription through DNA looping. DNA loops can be visualized by fluorescence in situ hybridization (FISH) and offers a novel biological mechanism that can be used as an innovative diagnostic test for the luminal subtypes.
**Title:** Cancer Cachexia: Piwi-interacting RNAs (piRNAs) expressed in human skeletal muscle as potential post-transcriptional gene regulators

**Authors:** Bhumi Bhatt¹, Ashok Narasimhan¹, Sunita Ghosh², Vera Mazurak³, Vickie Baracos²,⁴ and Sambasivarao Damaraju¹,⁴

Departments of Laboratory Medicine and Pathology¹, Oncology² and Agricultural Food and Nutritional Science³, University of Alberta and Cross Cancer Institute⁴, Edmonton, Alberta

**Background and Aim:**
Cancer cachexia (CC) is a multifactorial syndrome, characterized by ongoing loss of skeletal muscle with or without fat loss, affects 50% of cancer patients and contribute to ~20% cancer-related deaths. piRNAs (26-32nt) are small non-coding RNAs similar to miRNAs, regulate gene expression by binding to 3'UTR of target mRNA. However, their expression in muscle, role and mechanism of action are not addressed in CC.

**Material and Methods:**
Study subjects (n=42) were classified as cachectic and non-cachectic cases based on international consensus diagnostic framework (%weight loss and/or sarcopenic status and BMI). RNA from muscle biopsies is sequenced for small RNAs (Illumina-MiSeq). Aligned reads mapped to piRNAdb, raw data filtered using a cut-off >5 read counts in at least 80% samples and normalized using RPKM. piRNAs exhibiting a fold-change >1.2 and p<0.1(one-way ANOVA) were considered differentially expressed (DE). Bioinformatic analysis was performed using Partek Genomics Suite and target identification by miRanda.

**Results:**
1168 piRNAs were expressed in muscle; 28 piRNAs were retained following filtering criteria. We identified 8 DE piRNAs (3 were up-regulated and 5 downregulated in cases). hsa-piR-27081 is up-regulated and predicted to target GLUL, has been validated by qRT-PCR in independent cohorts (n=45) and their expression levels showed concordance to that of sequencing data.

**Conclusions:**
We identified DE piRNAs in cachectic cases and validated hsa-piR-27081 from independent muscle biopsies. GLUL is a regulator of muscle protein synthesis and upon silencing could potentially contribute to muscle catabolism. This needs to be further interrogated using cell-line model systems.
Title:  Base Mine Lake 2038: Using analytical chemistry to predict the future of oil sands end pit lakes.

Authors:  Evelyn Asiedu, Jonathan Martin, Ania Ulrich

Background and Aim:  
After many rounds of recycling, the resultant water byproduct of oil sands extraction is toxic due to the elevated concentrations of dissolved organic compounds from bitumen. Currently, over 1 billion m³ of oil sands process-affected water (OSPW) awaits remediation prior to its return to the environment. A leading strategy for OSPW reclamation employs end pit lakes (EPL) whereby OSPW is stored in evacuated mines and diluted until toxicity is reduced through natural processes. In 2012, Base Mine Lake (BML) became the first oil sands EPL. The objective of this project was to assess the feasibility of the EPL strategy by examining the microbial biodegradation kinetics of the dissolved organics in BML.

Material and Methods:  
Laboratory microcosms containing BML OSPW were monitored for over one year (n=3, T=21°C) alongside negative controls. Furthermore, the organic profile of BML was compared to field samples representing aged and fresh OSPW. Ultrahigh resolution high pressure liquid chromatography- Orbitrap mass spectrometry was applied to all samples.

Results:  
After 1 year, microcosms supplemented with nutrients revealed endogenous microorganisms were capable of degrading simple carbon sources but not bitumen-derived organics. Principal components analysis was able to distinguish OSPW of different ages based on relative contribution of chemical classes. The oldest samples had the lowest intensities (concentrations) of organics.

Conclusions:  
Dissolved organics in OSPW are persistent in the laboratory over 1 year, but concentrations may slowly decrease in the field over time. To ensure that the toxicity of BML decreases in the future, intervention strategies may be needed to accelerate in situ biodegradation.
**Title:** Time and Perfusate Dependent Molecular Response in Ex Vivo Lung Perfusion

**Authors:** P Dromparis¹, NS Aboelnazar², S Wagner¹, S Himmat², CW White², S Hatami², J Luc², DH Freed², J Nagendran², M Mengel¹, and BA Adam¹

¹Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada
²Department of Surgery, Division of Cardiac Surgery, University of Alberta, Edmonton, AB, Canada

**Background and Aim:**
Lung transplantation has limited organ utilization and high rates of graft dysfunction, in part due to acute lung injury (ALI). Cold static preservation (CSP) aims to minimize ALI but is limited by short preservation periods and an inability to assess organ quality. Ex vivo lung perfusion (EVLP) is a promising alternative that may allow for injury assessment and repair; however the molecular mechanisms are unclear. This study explores ALI gene response in EVLP.

**Material and Methods:**
EVLP lungs (n=57) were perfused with acellular solution (AC), packed red blood cells (pRBCs) or whole blood (WB), ventilated with either positive or negative pressure. Biopsies were taken in vivo (n=25) and after 0hrs (n=68), 6hrs (n=8) and 12hrs (n=68) and compared to CSP (n=11). Standard functional and histologic parameters were assessed. ALI genes (48) were quantified with NanoString.

**Results:**
28 “repair” genes were upregulated and 6 “injury” genes were downregulated after EVLP. Repair and injury gene sets correlated with functional and histologic parameters (Figure 1). Gene set expression differed in EVLP (p<0.001) but not CSP (p>0.05). Repair gene set expression peaked at 6hrs (p<0.001), whereas injury gene set expression decreased only at 12hr (p<0.001). pRBCs and WB perfusates showed greater injury gene set reduction compared to AC (p=0.008 and p=0.038, respectively). Gene set expression was similar between ventilation strategies (p>0.05).

**Conclusions:**
EVLP alters the molecular phenotype in a manner that is quantifiable, time and perfusate dependent, and correlates with histologic and functional parameters. Gene expression may provide more precise, objective and mechanistic approach for assessing donor lungs.
Table 1

<table>
<thead>
<tr>
<th></th>
<th>'Repair' 28-gene set</th>
<th></th>
<th>'Injury' 6-gene set</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman's rank correlation coefficient</td>
<td>p-value</td>
<td>Spearman's rank correlation coefficient</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Functional parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO₂/FiO₂ ratio</td>
<td>0.302</td>
<td>&lt;0.001</td>
<td>-0.501</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lung compliance</td>
<td>0.200</td>
<td>0.027</td>
<td>-0.477</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulmonary artery pressure</td>
<td>-0.078</td>
<td>0.395</td>
<td>0.321</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulmonary vascular resistance</td>
<td>-0.148</td>
<td>0.105</td>
<td>0.410</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulmonary airway pressure</td>
<td>-0.189</td>
<td>0.037</td>
<td>0.269</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Histological parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>0.196</td>
<td>0.011</td>
<td>-0.140</td>
<td>0.069</td>
</tr>
<tr>
<td>Perivascular neutrophils</td>
<td>0.130</td>
<td>0.091</td>
<td>0.047</td>
<td>0.541</td>
</tr>
<tr>
<td>Alveolar inflammation</td>
<td>-0.133</td>
<td>0.085</td>
<td>0.150</td>
<td>0.052</td>
</tr>
<tr>
<td>Interstitial neutrophils</td>
<td>-0.199</td>
<td>0.009</td>
<td>0.128</td>
<td>0.096</td>
</tr>
<tr>
<td>Interstitial edema</td>
<td>-0.329</td>
<td>&lt;0.001</td>
<td>0.124</td>
<td>0.108</td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>-0.365</td>
<td>&lt;0.001</td>
<td>0.215</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Title: Molecular risk prediction in ANCA-associated crescentic glomerulonephritis: added value over clinical and histologic parameters

Authors: Kristalee Watson1, Benjamin Adam1, Peter Dromparis1, Ainslie Hildebrand2, Michael Mengel1
1Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada
2Department of Medicine, Division of Nephrology, University of Alberta, Edmonton, AB, Canada

Background and Aim:
Molecular methods have the potential to improve classification, activity grading, and chronicity staging of native kidney diseases. However, current empirically-derived consensus classification systems based on biopsy features suffer from non-specificity, arbitrary rules, and limited reproducibility. We employed the NanoString® platform to quantify gene expression in formalin-fixed paraffin-embedded (FFPE) native kidney biopsies with anti-neutrophil cytoplasmic antibody (ANCA)-associated crescentic glomerulonephritis (GN) with the aim of identifying quantifiable molecular parameters that can predict end stage renal disease (ESRD).

Material and Methods:
The expression of a 54-gene set for inflammation, crescent-associated and nephron injury genes was quantified using the NanoString® platform on RNA isolated from FFPE native kidney biopsies with ANCA-associated crescentic GN (n=74). Clinical, histomorphologic, and gene expression parameters were analyzed for significance in predicting the development of ESRD (dialysis-dependence or renal transplant).

Results:
Multivariate Cox regression demonstrated lower patient age, higher percentage global glomerulosclerosis and higher expression of crescent-associated genes were independently predictive of developing ESRD (p=0.002, p=0.003, and p<0.001, respectively; Table 1). The addition of crescentic gene expression to age and global glomerulosclerosis better predicts the development of ESRD versus age and global glomerulosclerosis alone in logistic regression prediction models (AUC 85.1 versus 71.9, respectively, p=0.023); this is illustrated in Kaplan-Meier curves for renal survival (Figure 1).

Table 1. Prognostic significance of clinical, histologic and molecular parameters on end-stage renal disease in ANCA-associated crescentic glomerulonephritis (n=74*).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Male</td>
<td>1 [Reference]</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.366 (0.592-3.153)</td>
<td>0.465</td>
</tr>
<tr>
<td>Age, yr</td>
<td>0.972 (0.948-0.997)</td>
<td>0.026</td>
</tr>
<tr>
<td>Glomerulosclerosis dependency at presentation</td>
<td>0.012 (0.913-0.327)</td>
<td>0.916</td>
</tr>
<tr>
<td>Serum creatinine, µmol/L</td>
<td>1.002 (1.001-1.003)</td>
<td>0.005</td>
</tr>
<tr>
<td>Urine protein/creatinine ratio, mg/mmol</td>
<td>1.001 (1.000-1.003)</td>
<td>0.060</td>
</tr>
<tr>
<td>ANCA screen</td>
<td>1 [Reference]</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>cANCA positive</td>
<td>0.256 (0.074-0.880)</td>
<td>0.031</td>
</tr>
<tr>
<td>Anti-MPO titre, mean fluorescence intensity units</td>
<td>1.001 (1.000-1.002)</td>
<td>0.106</td>
</tr>
<tr>
<td>Anti-PR3 titre, mean fluorescence intensity units</td>
<td>1.002 (0.999-1.005)</td>
<td>0.158</td>
</tr>
<tr>
<td>Histologic parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-sclerotic non-crescentic glomeruli, %</td>
<td>0.985 (0.968-1.002)</td>
<td>0.075</td>
</tr>
<tr>
<td>Global glomerulosclerosis, %</td>
<td>1.029 (1.010-1.048)</td>
<td>0.002</td>
</tr>
<tr>
<td>Total crescents, %</td>
<td>1.003 (0.989-1.018)</td>
<td>0.684</td>
</tr>
<tr>
<td>Cellular crescents, %</td>
<td>1.004 (0.988-1.019)</td>
<td>0.650</td>
</tr>
<tr>
<td>Fibrocellular crescents, %</td>
<td>1.011 (0.978-1.045)</td>
<td>0.385</td>
</tr>
<tr>
<td>Fibrous crescents, %</td>
<td>0.916 (0.805-1.042)</td>
<td>0.183</td>
</tr>
<tr>
<td>Intersitial fibrosis and tubular atrophy, score 2-3</td>
<td>2.798 (0.937-8.349)</td>
<td>0.065</td>
</tr>
<tr>
<td>Gene expression †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All genes</td>
<td>1.014 (1.003-1.025)</td>
<td>0.013</td>
</tr>
<tr>
<td>Crescentic gene set</td>
<td>1.003 (1.001-1.005)</td>
<td>0.005</td>
</tr>
<tr>
<td>Endothelial gene set</td>
<td>1.013 (0.999-1.027)</td>
<td>0.064</td>
</tr>
<tr>
<td>Inflammation gene set</td>
<td>1.002 (0.997-1.008)</td>
<td>0.414</td>
</tr>
<tr>
<td>Macrophage gene set</td>
<td>1.013 (0.996-1.036)</td>
<td>0.267</td>
</tr>
<tr>
<td>Nephron injury gene set</td>
<td>1.004 (1.001-1.007)</td>
<td>0.005</td>
</tr>
<tr>
<td>NK cell gene set</td>
<td>1.013 (0.995-1.031)</td>
<td>0.151</td>
</tr>
</tbody>
</table>

HR, hazard ratio; 95% CI, 95% confidence interval.
*22/74 patients developed end-stage renal disease.
†Gene expression units: geometric mean of normalized counts.
Figure 1. Kaplan-Meier renal survival curves for ANCA-associated crescentic glomerulonephritis, separated into low- and high-risk groups based on: (A) full logistic regression model with age, percentage global glomerulosclerosis, and crescentic gene expression; and (B) reduced model using age and percentage glomerulosclerosis alone.

Conclusions:
Molecular assessment of FFPE native kidney biopsies with ANCA-associated crescentic GN provides quantifiable information that independently predicts the development of ESRD. Molecular information can be incorporated with clinical and histological parameters into risk prediction models.
Title: Identifying enteropathogens in children with acute gastroenteritis through enhanced detection: a prospective cohort study

Authors: Ran Zhuo, Brendon D Parsons, Bonita E Lee, Linda Chui, Stephen B Freedman, Samina Ali, Karen Lowerison, Xiao-Li Pang on behalf of the APPETITE Team
1Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada
2Department of Pediatrics, University of Alberta, Edmonton, AB, Canada
3Provincial Laboratory for Public Health (ProvLab, Alberta Health Services), Edmonton, AB, Canada
4Sections of Pediatric Emergency Medicine and Gastroenterology, Alberta Children’s Hospital and Alberta Children’s Hospital Research Institute, University of Calgary, Calgary, AB, Canada
5Department of Pediatrics, Faculty of Medicine & Dentistry, Women and Children’s Health Research Institute, University of Alberta, Edmonton, Alberta, Canada
6Cumming School of Medicine, University of Calgary, Calgary, AB, Canada.

Background and Aim:
Acute gastroenteritis (AGE) is a common childhood infection yet sample submission rates are low. Therefore, current pathogen-specific disease burden of AGE is likely underestimated. The Alberta Provincial Pediatric Enteric Infection Team (APPETITE) study is a prospective province-wide study capable of estimating the relative distribution of enteropathogens causing diarrhea and/or vomiting in Albertan children.

Material and Methods:
Children (≤18 yrs) with AGE and asymptomatic controls were recruited through two pediatric emergency departments, a nursing hotline: Health Link Alberta and a public health clinic. Rectal swabs and stool samples were collected and tested for 5 viruses, 9 bacteria and 3 parasites using enteric bacterial culture, a lab-developed gastroenteritis virus panel RT-qPCR and the commercially available Luminex xTAG Gastroenteritis Pathogen Panel.

Results:
From Dec 2014 to Jan 2018, 72.9% (n=2,199) of the 3,018 symptomatic patients tested positive for ≥ 1 enteropathogen. Norovirus was the most commonly detected pathogen (n=807, 26.7%), followed by adenovirus (n=563, 18.7%) and rotavirus (n=507, 16.8%). Clostridium difficile was detected in 14.4% (n=436) of symptomatic children and in 11.6% (n=166) of the control individuals. Sapovirus (n=297, 9.8%) and astrovirus (n=106, 3.5%) were less prevalent. Excluding C. difficile, 5.9% (n=176) patients were infected by a bacterial pathogen and 0.5% (n=16) by a parasitic pathogen. Although 38% (n=547) of the 1,434 asymptomatic children tested positive for ≥ 1 pathogen, 11.2% (n=161) tested positive for rotavirus shortly following rotavirus vaccine administration.

Conclusions:
This study allows us to understand the relative proportional etiologies of enteropathogens in children with AGE.
Title: Methylated phenylarsenical metabolites discovered in chicken liver

Authors: Hanyong Peng, Bin Hu, Qingqing Liu, Jinhua Li, Xing-Fang Li, Hongquan Zhang, and X. Chris Le

Background and Aim:
Arsenic (As) is recognized as one of the most important environmental agents causing cancers of the skin, bladder, and lung. The practice of feeding 3-nitro-4-hydroxyphenylarsonic acid (Roxarsone, ROX) to poultry and swine lasted for more than 60 years before the European Union and the United States stopped its use. Many other countries continue to use phenylarsenicals in the poultry industry. Ingestion of these poultry meat and meat products results in exposure to residual arsenic. However, it remains unclear how ROX may be metabolized and potentially produce new arsenic species of toxicological significance. We aim to gain an understanding of possible metabolism of ROX from a controlled feeding study that involved 1,600 chickens of two common commercial strains.

Material and Methods:
The extracts of chicken liver were subjected to identification and quantification analyses by anion exchange HPLC separation and both inductively coupled plasma mass spectrometry (ICPMS) and a series of electrospray ionization mass spectrometry (ESI-MS).

Results:
Methyl-3-nitro-4-hydroxyphenylarsonic acid (methyl-ROX), methyl-3-amino-4-hydroxyphenylarsonic acid (methyl-3-AHPAA), and methyl-3-acetamido-4-hydroxyphenylarsonic acid (methyl-N-AHPAA) were identified in chicken livers. The concentrations of methyl-ROX were as high as 90 µg/kg, even after a 5-day clearance period. In vitro enzymatic reactions suggest methylation is from enzymatic addition of a methyl group to a trivalent phenylarsenical. IC50 values for the trivalent phenylarsenicals were 300-30,000 times lower than those for the pentavalent phenylarsenicals.

Conclusions:
The identification and determination of the three new toxicologically relevant methyl phenylarsenical metabolites are important for human exposure and health effect assessments.
Evaluation of T regulatory cell depletion on *Mycobacterium tuberculosis* (*Mtb*) H37Ra infection and immune responses of human PBMCs in an *in vitro* model of human PBMC-*Mtb* infection

**Authors:** Sudha Bhavanam, Gina R. Rayat, Monika Keelan, Dennis Kunimoto, Steven J. Drews

**Background and Aim:**
*Mycobacterium tuberculosis* (*Mtb*) is a major global health problem. In 2015, approximately 2 million deaths caused by *Mtb* infection were reported. T regulatory cells (Tregs) are a subset of T lymphocytes whose main function is to prevent autoimmunity but pathogens also take advantage of this for their survival. This study evaluated the impact of Tregs on *Mtb* infection by measuring the mycobacterial loads and immune responses of human peripheral blood mononuclear cells (PBMCs) infected with *Mtb* H37Ra.

**Material and Methods:**
Tregs were depleted from human PBMCs using a column that specifically selects CD4^+^CD127^low^CD25^+^ T lymphocytes. Both Tregs depleted (PBMCs-Tregs) and Tregs undepleted (PBMCs+Tregs) PBMCs were infected with *Mtb* H37Ra *in vitro*. The size of cell aggregates, mycobacterial load (expressed as colony forming unit (CFU)), and levels of cytokines at 0-8 days of infection were determined by microscopy, CFU assay, and enzyme linked-immunosorbent assay, respectively.

**Results:**
Cell aggregates were smaller in the PBMCs-Tregs compared to PBMCs+Tregs at day 8 post-infection. *Mtb* CFUs were higher in the PBMCs-Tregs compared to PBMCs+Tregs at days 3-8. Levels of IL-17 (3 and 5 days) and the Th1 cytokines: IFN-γ (at days 3 and 5), TNF-α, and IL-6 (at day 3) were lower in PBMCs-Tregs compared to PBMCs+Tregs. Levels of Th2 cytokines IL-10 and IL-4 were higher at day 3 in the PBMCs-Tregs compared to PBMCs+Tregs.

**Conclusions:**
Tregs depletion in human PBMCs infected with *Mtb* H37Ra leads to a shift from a Th1 to a Th2 cytokine-rich environment that supports survival of *Mtb*.
Poster Presentations
Title: Evaluation of an automated chemiluminescent assay for the serological detection of measles, mumps, rubella and varicella-zoster.

Authors: Adesewa Adeleye, Carmen Charlton

Background and Aim:
Clinical testing for measles, mumps, rubella and varicella antibodies is currently done at the Provincial Laboratory for Public Health using the Enzygnost ELISA assays in combination with the BEP 2000 (Siemens, Germany) platform. This system however, is not fully integrated into the laboratory information system and requires manual data entry. This study aimed to evaluate the performance of the Liaison XL (DiaSorin, Italy), a chemiluminescent immunoassay method, as compared to the current system in use for clinical testing of these viral antibodies.

Material and Methods:
For each marker being validated, 50 positive and 75 negative specimens were retrospectively selected from a pool of previously tested patient specimens and analyzed using the Liaison XL. The results produced were then compared to the previous Enzygnost result and diagnostic sensitivity and specificity were calculated.

Results:
For Varicella IgG, specificity and sensitivity were 96% and 95% respectively while for IgM they were 100% and 61%. Specificity and sensitivity were 94% and 97% respectively for Measles IgG and for IgM it was 100% and 59%. For Mumps IgG, specificity and sensitivity were 98% and 100% respectively while for IgM they were 94% and 74%. Lastly for Rubella IgM, specificity and sensitivity were 99% and 68% respectively.

Conclusions:
The Liaison XL showed excellent specificity and sensitivity for the 3 IgG assays evaluated however the IgM assays showed low sensitivity. The high number of false negatives mandates further investigation through an arbitrator test to determine the true result for those specimens.

Table 1: Calculated parameters for seven assays on the DiaSorin Liaison XL as compared to the Siemens ELISA.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
<th>PPVa (%)</th>
<th>NPVb (%)</th>
<th>Agreementc (%)</th>
<th>Kappa statistic</th>
<th>p valued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varicella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>96</td>
<td>95</td>
<td>97</td>
<td>92</td>
<td>96</td>
<td>0.92</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IgM</td>
<td>100</td>
<td>61</td>
<td>100</td>
<td>64</td>
<td>77</td>
<td>0.57</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

| Measles |
| IgG    | 94              | 97              | 96      | 96      | 96             | 0.92           | <0.01    |
| IgM    | 100             | 59              | 100     | 70      | 79             | 0.59           | <0.01    |

| Mumps |
| IgG    | 98              | 100             | 99      | 100     | 99             | 0.98           | <0.01    |
| IgM    | 94              | 74              | 95      | 71      | 82             | 0.65           | <0.01    |

| Rubella |
| IgM    | 99              | 68              | 94      | 91      | 92             | 0.76           | <0.01    |

aPPV = Positive Predictive Value
bNPV= Negative Predictive Value
Agreement between previous Siemens Enzygnost results and Liaison XL results
dp value for the calculated Kappa statistic
**Title:** Evaluation of cryptococcal antigen lateral flow assays for detection of *Cryptococcus* infection

**Authors:** Lillian Feng and Tanis C. Dingle (supervisor)

**Background and Aim:**
*Cryptococcus neoformans* and *Cryptococcus gattii* are opportunistic yeasts that are found in soil and bird droppings. Inhalation of these yeasts can cause pulmonary disease or meningitis in immunocompromised patients. Diagnosis of cryptococcal infection is typically by Gram stain, culture, and a cryptococcal antigen test. However, the current latex agglutination cryptococcal antigen test in the Provincial Microbiology Laboratory is laborious and time-consuming. The aim of this study was to evaluate two new lateral flow assays by Immuno-Mycologics (IMMY) and Bio-Rad in comparison to the current Meridian Bioscience latex agglutination method.

**Material and Methods:**
This project is a comparison of three Cryptococcal antigen methods: Meridian Bioscience CALAS, IMMY CrAg LFA, and Bio-Rad RDT Crypto PS. True patient positives are difficult to obtain in this region so artificial samples were created by inoculating human serum and CSF with commercial cryptococcal antigen. Archived true-positive and true-negative patient samples were also tested.

**Results:**
Comparison of IMMY and Bio-Rad assays to the Meridian Bioscience assay

<table>
<thead>
<tr>
<th></th>
<th>Number of samples with Meridian Bioscience (reference method) result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculated CSF Samples</td>
</tr>
<tr>
<td>Test results</td>
<td>Positive</td>
</tr>
<tr>
<td>IMMY</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Bio-Rad</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>

**Conclusions:**
Both the IMMY and Bio-Rad assays showed perfect overall agreement with the current Meridian Bioscience method with all artificially inoculated samples. The two discrepancies between the positive Meridian Bioscience patient samples and the negative IMMY and Bio-Rad patient samples could not be resolved in favour of either test method. Overall, the two new assays demonstrate high accuracy, as well as ease of use and markedly reduced tech time.
Title: Combined Antibody Titre Investigation in Prenatal Patients

Authors: M Farrell BHSc MLT\(^1\), G Clarke MD FRCPC\(^1,2\), G Barr MLT\(^2\), J Hannon MD FRCPC\(^1,2\)

\(^1\)Department of Laboratory Medicine and Pathology, University of Alberta, \(^2\)Canadian Blood Services, Edmonton, Canada

Background and Aim:
For prenatal patients with multiple antibodies, separate titrations for each antibody are generally performed. Assessing antibody concentrations separately ignores in vivo conditions and may not be representative of the combined effect multiple antibodies can have on the fetus. This study examined the difference between combined and separate titre levels when multiple antibodies were present.

Material and Methods:
Thirty-one samples containing combinations of 2 different antibodies were examined. For each sample two titrations by the standard method (separate titre levels for each antibody) and one single combined titration method was performed. The separate method was achieved by titering individual antibodies separately against reagent cells that expressed only one antigen of the combination of antibodies. The combined titre method was performed using reagent cells that expressed both antigens against which the antibodies were directed.

Results:
Overall 19/31 samples (61.3%) showed an increased titre with the combined titration method. Of the 12 samples that showed no increase, 10 contained a separate titre of < 1 for either one or both antibodies. Of the samples where both antibodies had a separate titre of \(\geq 1\), 15/17 (88.2%) showed an increased titre level with the combined titration method.

Conclusions:
In cases where two antibodies are present, titrations performed by a combined method will produce titre levels equal to or higher than a standard separate antibody titration method. Based on these results, a combined titration will reach a critical titre level as early as, or earlier in gestation than antibodies monitored by a single titration method.
Title: Developing aptamers to detect culturable *Escherichia coli* 0157:H7 infections in a matter of hours

Authors: Seunghyun Lee, Yanming Liu, Xing-Fang Li

Background and Aim: *Escherichia coli* O157:H7 is a major global health issue, leading to human illnesses and even death. Currently, culture method is being utilized which takes several days for a diagnosis. We propose to generate a pair of aptamers, which are short single stranded oligonucleotides, specific to culturable *E. coli* O157:H7. We will ultimately develop an Enzyme-Linked Aptamer-Sorbent Assay(ELASA), which will take less than a day for a diagnosis, to replace the culture method.

Material and Methods: Aptamers are generated by a selection technique called Systematic Evolution of Ligands via Exponential Enrichment. In each selection round, four steps were performed: 1. *E. coli* O157:H7 cells were incubated with a ssDNA library; 2. Cell-ssDNA complex were treated in 1% formaldehyde for cross-linking tightly bound ssDNA onto cell surface; 3. To remove unwanted ssDNA, the cells were treated by Turbo DNase treatment twice followed by a large volume dilution; 4. Finally, the cells were quantified by real-time PCR and then amplified by emulsion PCR followed by ssDNA generation.

Results: After the first round of selection, $10^8$ molecules remained in the pool. Formaldehyde treatment could only cross-link tightly bound ssDNA to cells while the cross-link between cells did not happen because no cell aggregate was observed. Covalent crosslink between tightly bound ssDNA and cells surface could prevent any loss from stringent washes and treatment. One round of counter-selection using dead *E. coli* O157:H7 has been completed.

Conclusions: Aptamer specific to culturable *E. coli* O157:H7 will be enriched through two more rounds of selection and two rounds of counter-selection.
**Title:** Defining Positive Thresholds in Flow Cytometry Crossmatches: Not so Normal

**Authors:** Wayne Ly¹, Patricia Campbell¹²³, Luis Hidalgo¹²³, Deanna Manna², Lydia Kohut², Anne Halpin¹²³⁴

1. Department of Laboratory Medicine and Pathology  
2. Alberta Health Services  
3. Alberta Transplant Institute  
4. Canadian National Transplant Research Program

**Background/Aim:** Histocompatibility laboratories use flow cytometry crossmatch (FCXM) to assess donor-recipient compatibility. Positive thresholds must be determined, as this is a laboratory-developed test. Our aim was to evaluate methods to establish FCXM thresholds using different lot numbers of this assay's key reagents: FITC-labelled anti-human IgG (FITC-IgG) and the negative control serum (NS).

**Material/Methods:** Sera (n=24) from healthy/non-transfused donors were screened for human leukocyte antigen (HLA) antibodies. Using these sera, T- and B-cell FCXMs were performed using a previously-validated method (n=24 cells). Data normality was measured: Shapiro-Wilk test, normal Q-Q and histogram plots (SPSS Statistics 24). Outliers were excluded by mean±3SD and Tukey’s methods. Expected positive FCXMs were performed using current/new lot numbers of FITC-IgG and NS; multiple positive thresholds were evaluated.

**Results:** Negative FCXM results are not normally distributed (Figure 1). Although NS were screened for HLA antibodies, many FCXM showed non-specific reactivity. Tukey’s method excluded more outliers than applying mean±3SD, but may exclude valid results (Table 1). Based on known/expected positive results, a threshold of mean+3SD appears best for PBL T-cells (Table 1). B-cell thresholds for HLA class II antibodies and threshold analysis of spleen and frozen spleen cells is still ongoing.

**Conclusions:** Evaluating data for normality is important to select valid methods for determining thresholds and removing outliers; these statistics may be misleading if data are skewed. Careful evaluation of individual lots is important as differences in normal samples may yield specific challenges in threshold studies. A more thorough screening of NS may also be required.
Figure 1. Normal Q-Q plots of FCXM median channel shifts, after excluding outliers, from expected negative serum samples crossmatched against peripheral blood T cells (A), peripheral blood B cells (B), fresh spleen T cells (C), fresh spleen B cells (D), frozen spleen T cells (E), and frozen spleen B cells (F). P-values indicate results of Shapiro-Wilk normality test. PBL = peripheral blood
Table 1. Comparison of thresholds for the new FITC-IgG. Outliers are excluded using mean±3SD or Tukey’s fences. Cell types used include PBL (n=11), spleen (n=9), and frozen spleen (n=4). Each of these cells is crossmatched with 24 NS samples. All thresholds will be applied to expected/known positive results to test validity.

<table>
<thead>
<tr>
<th>Outliers excluded using:</th>
<th>PBL (T cells)</th>
<th>Spleen (T cells)</th>
<th>Frozen spleen (T cells)</th>
<th>PBL (B cells)</th>
<th>Spleen (B cells)</th>
<th>Frozen spleen (B cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± 3 SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># of XM excluded</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mean</td>
<td>17.48</td>
<td>22.09</td>
<td>23.28</td>
<td>21.59</td>
<td>25.42</td>
<td>34.90</td>
</tr>
<tr>
<td>SD</td>
<td>23.26</td>
<td>25.24</td>
<td>35.07</td>
<td>24.65</td>
<td>32.73</td>
<td>44.84</td>
</tr>
<tr>
<td>Threshold (mean + 3 SD)</td>
<td>87.26</td>
<td>97.80</td>
<td>128.48</td>
<td>95.54</td>
<td>123.62</td>
<td>169.43</td>
</tr>
<tr>
<td>Tukey’s fences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># of XM excluded</td>
<td>19</td>
<td>11</td>
<td>6</td>
<td>12</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Mean</td>
<td>13.79</td>
<td>19.98</td>
<td>18.73</td>
<td>19.37</td>
<td>23.72</td>
<td>33.35</td>
</tr>
<tr>
<td>SD</td>
<td>17.62</td>
<td>22.31</td>
<td>27.59</td>
<td>21.55</td>
<td>30.62</td>
<td>42.48</td>
</tr>
<tr>
<td>Thresholds:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97.5th percentile</td>
<td>53.80</td>
<td>71.00</td>
<td>76.78</td>
<td>69.00</td>
<td>96.60</td>
<td>127.50</td>
</tr>
<tr>
<td>mean + 1 SD</td>
<td>31.41</td>
<td>42.28</td>
<td>46.32</td>
<td>40.91</td>
<td>54.33</td>
<td>75.83</td>
</tr>
<tr>
<td>mean + 1.5 SD</td>
<td>40.22</td>
<td>53.44</td>
<td>60.12</td>
<td>51.69</td>
<td>69.64</td>
<td>97.07</td>
</tr>
<tr>
<td>mean + 2 SD</td>
<td>49.03</td>
<td>64.59</td>
<td>73.91</td>
<td>62.46</td>
<td>84.95</td>
<td>118.32</td>
</tr>
<tr>
<td>mean + 2.5 SD</td>
<td>57.85</td>
<td>75.74</td>
<td>87.70</td>
<td>73.23</td>
<td>100.26</td>
<td>139.56</td>
</tr>
<tr>
<td>mean + 3 SD</td>
<td>66.66</td>
<td>86.90</td>
<td>101.50</td>
<td>84.01</td>
<td>115.56</td>
<td>160.80</td>
</tr>
<tr>
<td>mean + 3.5 SD</td>
<td>75.47</td>
<td>98.05</td>
<td>115.29</td>
<td>94.78</td>
<td>130.87</td>
<td>182.04</td>
</tr>
<tr>
<td>mean + 4 SD</td>
<td>84.28</td>
<td>109.20</td>
<td>129.09</td>
<td>105.55</td>
<td>146.18</td>
<td>203.28</td>
</tr>
<tr>
<td>mean + 4.5 SD</td>
<td>93.09</td>
<td>120.36</td>
<td>142.88</td>
<td>116.32</td>
<td>161.49</td>
<td>224.52</td>
</tr>
<tr>
<td>mean + 5 SD</td>
<td>101.90</td>
<td>131.51</td>
<td>156.68</td>
<td>127.10</td>
<td>176.79</td>
<td>245.76</td>
</tr>
</tbody>
</table>

PBL = peripheral blood, XM = crossmatches, SD = standard deviation
Title: Simulation to Instill Foundations of Lab Practice in Medical Laboratory Science Students

Authors: Rachel McKellar, Lisa Purdy, Roberta Martindale, Amanda VanSpronsen

Background and Aim: Simulation is an effective learning methodology employed in health sciences education. This project introduced formal simulation pedagogy into the Medical Laboratory Science (MLS) curriculum. The aim of this project was to create both meaningful evaluation of and resources for MLS simulation.

Material and Methods: A stakeholder assessment was performed through document review and surveys of MLS students and teaching technologists in the Edmonton Zone. Survey results confirmed that both groups recognize simulation as a beneficial educational method that could increase student confidence.

Based on qualitative analysis of stakeholder feedback, simulated scenarios were developed for MLS students to increase experience with critical-thinking and troubleshooting skills. A 'MLS Simulation Group Assessment Rubric' (MLS-SGAR), modelled after the Creighton Competency Evaluation Rubric\(^1\) and the Simulation Thinking Rubric\(^2\), was developed and used to evaluate student performance. The scenario, rubric and post-survey were piloted with program alumni before use with the baseline group. 15 students in their final year, with little to no simulation experience, served as this baseline group.

Results: Students were evaluated using the MLS-SGAR. Most were categorized as “concrete thinking”, where they had necessary skills but often lacked initiative and communication. Most students (83%) agreed the simulation allowed them to practice troubleshooting and critical thinking skills in a realistic way. Two-thirds (66.67%) of participants preferred a group simulation format. All participants agreed that the simulation allowed them to analyze their own behaviors.

Conclusions: Resources for simulation were developed for use throughout MLS education. Simulation is providing opportunities for MLS students to apply critical-thinking and troubleshooting skills.

References:

**Title:** Association between n-3 long-chain polyunsaturated fatty acid status and breast cancer

**Authors:** Robert Palencia¹, Susan Goruk², Mohammadreza Pakseresht²,³, Jennifer Vena³, Catherine J. Field²

Departments of ¹Medical Laboratory Sciences, ²Agricultural, Food and Nutritional Science, University of Alberta, ³CancerControl Alberta, Alberta Health Services

**Background and Aim:**
There is evidence that breast cancer risk is related to modifiable lifestyle factors including dietary intake. Animal studies have demonstrated that feeding diets high in omega-3 polyunsaturated fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) reduce the growth and progression of mammary tumours. The purpose of this project is to determine if there is an association between fatty acid status and breast cancer.

**Material and Methods:**
A nested case-control study was conducted using the Alberta Tomorrow Project cohort (2008-2015). Adults (35-69y) with no previous history of cancer were enrolled and a plasma sample banked. Ninety-five participants who developed breast cancer were considered as cases. After matching for age and BMI 133 women who did not develop breast cancer (controls) were also included in this study. Lipids were extracted from plasma samples using the modified Folch method and phospholipids were isolated by thin layer chromatography. The phospholipids were methylated and the relative percent of each fatty acid identified using gas liquid chromatography.

**Results:**
Women who developed breast cancer had less plasma EPA (0.96±0.40% vs 1.13±0.69%, P<0.05) and more total omega-6 fatty acids (33.4±1.9% vs 32.8±2.2%, P<0.05) compared to the control group.

**Conclusions:**
Results suggest that a higher long chain omega-3 fatty acid status and lower omega-6 status is associated with lower risk of breast cancer but a larger sample is needed to confirm this (i.e. enabling risk analysis and controlling for other confounding risk factors). Future analysis should focus on establishing if there is the relationship between dietary intake, plasma status and breast cancer risk.
Title: Validation of cardiac valve allograft decontamination and bioburden reduction

Authors: Christina Crossie*, Jelena L. Holovati, and Graeme Dowling

Background and Aim:
Bacterial contamination of recovered tissue allografts poses a serious threat to transplant recipients. Cardiac valve (CV) allografts are typically disinfected in an antibiotic cocktail at 4°C; however, antibiotic availability and recent changes to Canadian health standards have resulted in a requirement for further validation of disinfection practices at the AHS Comprehensive Tissue Centre.

Material and Methods:
Disinfection solution, composed of vancomycin (50 μg/mL), tobramycin (80 μg/mL), and cefoxitin (240 μg/mL) in RPMI and was inoculated with $10^5$ CFU/mL of five challenge organisms considered medically significant in tissue banking at 4°C and 21°C. Following 24 hours of incubation, the remaining CFUs were counted and log reduction was calculated. To further evaluate the appropriateness of a disinfection incubation, range of 24 hours ± 2 hours, four CVs were bisected and inoculated with $10^6$ CFU of challenge organisms before being placed in the disinfection solution. At each time point one half of the allograft was removed and washed in Lactated Ringer’s solution before recovering the remaining bacteria in sterile saline by sonication and mechanical shaking. The final recovery solution was filtered and tested for bacterial growth.

Results:
The initial results demonstrate that 4°C incubation reduced the bacterial growth between log 1.00 and log 2.00 whereas incubation at 21°C reduced the bioburden by greater than log 4.00.

Conclusions:
The study established that incubation of CVs in the updated disinfection solution for 24 hours ± 2 hours at 21°C was sufficient to reduce the bioburden above the minimum required standard of 4.00 logs.
Title: Sickle Cell Exchange Process Review

Authors: Querengesser E., Poseluzny D., Lyon B., Gerges H., Nahirniak S., Bolster L.

Background and Aim:
Exchange transfusions have been gaining traction as an effective treatment to combat complications associated with Sickle Cell Disease and other hemoglobinopathies. This project aimed to characterize the transfusion medicine and hematology aspects of the patients within the Red Cell Exchange (RCE) program. This information will be used to supplement a larger program quality assurance review.

Material and Methods:
Ethical considerations were assessed by the A pRoject Ethics Community Consensus Initiative (ARECCI) guidelines and screening tool. A database was created by extracting elements from the AHS Blood Bank usage records, Laboratory Information System, and Netcare for 39 individuals enrolled within the RCE program. Information collected focused on results generated between January 1st, 2017 through December 31st, 2017. This information was subsequently analyzed.

Results:
74.3% of the patients received transfusions on a chronic basis over the past year. The prevalence of alloantibodies within this cohort was 17.9%. Within the alloimmunized subpopulation, units were prophylactically matched for the c, Fy(a), S, and Jk(b) antigens 96.9%, 95.2%, 93.6%, and 92.1% of the time. Every unit for this subpopulation was phenotypically matched for the C, E, e, K, s, Fy(b), and Jk(a) antigens. Testing for blood borne pathogens occurred at least once in 46.1% of patients.

Conclusions:
The incidence of alloimmunization within the RCE program is lower than that seen in the overall sickle cell population and no new alloantibodies were detected. Units were routinely prophylactically matched with minimal discrepancies in the recommended depth of unit typing. Deficiencies in blood borne pathogen testing were also detected through the project.
**Title:** In search of an improved test for Cystic Fibrosis-Related Diabetes (CFRD) screening: the utility of fructosamine and Hemoglobin A1c

**Authors:** Shelby Sissons¹, Grace Lam², Mathew Estey³, Jan Dayton⁴, Kimberley Mulchey⁵, Maeve P. Smith⁵, Neil E. Brown⁵, Winnie M. Leung⁵

¹ Division of Medical Laboratory Science, Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta
² Division of Internal Medicine, Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta
³ Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta
⁴ DynaLIFE Medical Labs
⁵ Division of Pulmonary Medicine, Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta

**Background and Aim:**
Prevalence of Cystic Fibrosis Related Diabetes (CFRD) is increasing. Early detection of CFRD and identifying abnormal glycemic control are important, as they are associated with declining lung function and general health. However compliance with the gold standard oral glucose tolerance test (OGTT) is poor. This study evaluates fructosamine and HbA1c as alternative screening tests for CFRD, and their relationship with lung function.

**Material and Methods:**
Fructosamine, Hemoglobin A1c and percent-predicted forced expiratory volume in 1 s (FEV1) were measured in patients undergoing a 2-hour OGTT for CFRD screening. Fractional serum fructosamine (FSF) was calculated as fructosamine/total protein.

**Results:**
ROC analysis suggested that FSF can identify both patients with impaired glucose tolerance (IGT) (AUC = 0.740, p= 0.0018) and those that classify as having CFRD (AUC = 0.745, p= 0.0373). Patients with an FSF <3.66μmol/g had a higher FEV1 (median 82.3 %) compared to those with FSF >3.66 μmol/g (median % 63.3; p= 0.0538). Likewise, HbA1c could identify patients with IGT (AUC = 0.686, p= 0.0343) and those with CFRD (AUC = 0.750, p= 0.0302). Patients with an HbA1c <5.7% had a significantly higher FEV1 (median 76.3%) compared to those with HbA1c > 5.7% (median 60.9 %; p= 0.0313).

**Conclusions:**
FSF and HbA1c reliably identified patients with both IGT and CFRD. Lung function in patients with FSF >3.66 μmol/g and HbA1c >5.7% appears to decline, suggesting these markers reflect relevant outcomes in cystic fibrosis. These simple blood tests show potential as effective tools in CFRD screening.
**Title:** Specific and Sensitive Detection of DNA mutation using DNAzyme

**Authors:** Bi Han Su, Hongquan Zhang, Albert Zuehlke

**Background and Aim:**
It is evident that Hepatitis C Virus (HCV) lead to increased risk of liver cancer thus it is imperative to have a reliable DNA detection method for diagnosis. An autonomous DNA nanomachine is constructed by using DNAzyme and once triggered by the target DNA, the DNA motor system would assemble. The aim of this study is to determine whether this target induced DNA motor system is able to detect DNA mutation with high sensitivity and specificity.

**Material and Methods:**
Gold nanoparticles (AuNP) were first loaded with FAM fluorophore substrates. Then it was further conjugated with affinity ligands, which are a DNA probe that is complementary with the HCV gene. A DNAzyme swing arm was also conjugated with another DNA probe complementary to a different portion of the HCV gene. The assembly of the conjugated AuNP with DNAzyme occurs when both probes sandwich and hybridized the HCV gene. The hybridization would bring DNAzyme to close proximity to FAM substrate and thus activating the DNA motors system and generate fluorescent signals. If one of these probes did not hybridized with the HCV gene, DNA motors would not assemble.

Comparison of FAM fluorescence at 535nm between HCV gene and single mismatched variant were done, duplicates samples were run to confirm reproducibility.

**Results:**

![Graph 1.0 progress curve of DNA motor system in response to 200pM of HCV gene and variant](image)

**Conclusions:**
This demonstrated that DNA motor system is able to differentiate HCV gene from a single mismatch variant which confirms it is able to detect DNA mutation with high sensitivity and specificity.
**Title:** Why use two when one may do? - Prevalence and Impact of Transfusing Multiple Red Blood Cell Units

**Authors:** C. Tampus, H. Blain, H. Gerges, S. Nahirniak

**Background and Aim:**
Choosing Wisely Canada recommends “Don’t transfuse more than one red cell unit at a time when transfusion is required in stable, non-bleeding patients.” The main objective of this project is to evaluate the prevalence and impact of transfusing multiple red blood cell units in hemodynamically stable, non-bleeding adult patients. This information will allow directed education and algorithmic screening implementation to reduce the number of inappropriate red cell transfusions if identified for further improvement in red blood cell utilization.

**Material and Methods:**
Following ARECCI ethics guidelines and transfusion medicine (TM) service requirements, this retrospective audit evaluated the data of non-bleeding, adult inpatients at the University of Alberta Hospital for the first week of January 2018. Evaluation was performed by a TM physician according to START guidelines. The clinical status, Hemoglobin concentration pre- and post-transfusion and presence of symptoms plus the number of red cells transfused, indications for transfusion, and TM adverse events were obtained from the SunQuest Laboratory Information System, Netcare, and Patient Charts.

**Results:**
A total of 136 requests for 312 red cell units were placed. Of these, 43 orders (32%) do not meet the criteria on the START’s Prescreening Guidelines. Preliminary analysis to date has identified 6 orders (4%) that are likely inappropriate after conducting chart review. So far, only 2 patients (1.5%) had a documented adverse event.

**Conclusions:**
Based on the results of this project, a new transfusion screening algorithm should be developed and implemented to ensure RBC transfusion appropriateness in stable inpatients. This will improve patient safety and proper red cell utilization.
Title: Standardizing medical laboratory technologist training: A competency-based approach

Authors: Tomas D., Villatoro V., Lyon B., Granley C.

Background and Aim:
Accurate, reliable test results are critical for providing the best patient care, and in order to achieve this, medical laboratory technologists (MLTs) need to be competently trained. Often, medical laboratories will rely on checklists to ensure all the required competencies are met; however, the burden is then placed on the trainer to ensure all the required knowledge and skills are taught in a short period of time. This means there is a possibility of concepts being missed or not adequately covered during the training process.
This project set out to gather current perceptions of MLT training in the Edmonton zone, and use this information to implement an effective and standardized approach to MLT training.

Material and Methods:
MLT perceptions of current training processes were gathered using an online Google Forms questionnaire. The results of this questionnaire were then used to inform changes implemented at selected sites. Electronic documents were created to be used alongside the current checklists as an attempt to standardize training, support trainers, and allow for the ability to sign off trainees electronically. After implementing the new training process, MLTs were surveyed to evaluate the changes.

Results:
Our initial survey indicated that technologists use the current training checklists, however, there was uncertainty about how to use them or what was expected to be taught to trainees. By utilizing an electronic checklist with links to pertinent training information included, the training technologists indicated that a more standard approach to training was possible.

Conclusions:
Medical laboratory technologists responded positively to the new training process, with interest in exploring their use further in the future. This method can be utilized by other laboratories to standardize their training processes to deliver the best possible patient care.
**Title:** Evaluation of a competitive allele-specific TaqMan® PCR assay for monitoring the EGFR T790M mutation in lung cancer patients using cell-free DNA

**Authors:** Carly Vanderschaaf, John Coffin, Iyare Izevbaye

**Background and Aim:**
The development of the T790M mutation in EGFR positive lung cancer patients is one of the most common causes of secondary resistance to treatment with tyrosine kinase inhibitors. New developments in the ability to use less invasive cell-free circulating tumour DNA (cfDNA) from plasma samples rather than formalin fixed paraffin embedded (FFPE) biopsy specimens for monitoring tumour genetics has highlighted the need to investigate more cost effective, sensitive, and flexible assays than currently used EGFR methods. This study evaluated a competitive allele-specific TaqMan® PCR assay (castPCR) for the detection and monitoring of the T790M mutation.

**Material and Methods:**
Previously identified T790M positive patients and commercially prepared FFPE and cfDNA samples were used in this study. Method optimization, accuracy, precision, and limit of detection were explored for both sample types. In addition to demonstrating the performance of the castPCR method and identifying the benefits of using cfDNA to patient care, a cost analysis was completed to highlight the financial benefits of using the new assay.
Title: Development of a Complement-Dependent Cytotoxicity (CDC) Flow Cytometry Assay

Authors: Kaytlin Wu, Dr. Luis Hidalgo, Anne Halpin, Dr. Esme Dijke

Background and Aim:
CDC assays are the gold standard for determining donor-specific HLA antibodies associated with high risk antibody-mediated transplant rejection. These assays suffer from low sensitivity, subjective scoring, and limited reproducibility. We aimed to develop a next-generation CDC flow cytometry assay that benefits from the enhanced sensitivity and reproducibility of flow cytometry and usage of a fixable viability dye.

Material and Methods:
Lymphocytes purified from peripheral blood mononuclear cells (PBMCs) of healthy volunteers were incubated with and without increasing dilutions of serum containing HLA antibodies, followed by standard or low-toxicity rabbit complement. Cell death was defined by the cell percentage stained with viability dye. Cells were fixed with paraformaldehyde and analyzed by flow cytometry.

Results:
A calculated number of cells were killed by heating/cooling; viability dye was titrated to determine optimal concentration to detect cell death. Low-toxicity complement caused less background toxicity than standard complement. Use of T and B-cell labelling antibodies did not affect CDC. Sera characterized for HLA specificities were tested against lymphocytes from HLA-typed volunteers. Cell death was readily detected following incubations with sera but lacking rabbit complement (T–20.3%, B–32.0%). EDTA-treated sera had lower background toxicity (T–3.8%, B–16.3%).

Conclusions:
We developed a CDC flow cytometry assay to measure T and B-cell cytotoxicity following incubation with sera containing HLA antibodies. Upcoming projects will test this assay using sera screened for the complement-fixing ability of HLA antibodies. Our assay is currently optimized for PBMCs; splenocyte-derived lymphocytes will also be investigated.
Title: Stability of Plasma Cardiac Troponin I in the BD Barricor Blood Collection Tube

Authors: Kevin K Yoo, Anna K Füzéry, Joshua E Raizman, Albert KY Tsui

Background and Aim: In Edmonton we recently introduced the new Barricor tube to improve sample quality for cardiac troponin I (cTnI) measurement. Since the Barricor tube is a recent development by BD Canada, stability data on cTnI analyzed on Beckman Coulter DxI 800 are limited. We evaluated the tubes to develop evidence-based specimen storage and handling recommendations for cTnI across Edmonton.

Material and Methods: Plasma samples (n=93) that arrived to the University of Alberta Hospital laboratory were divided into three cTnI concentration ranges based on the proposed 99th percentile of the Beckman Coulter AccuTnI +3 assay (0.04 µg/L) and current clinical decision points: 0.02-0.04, 0.04-0.1 and 0.1-0.5 µg/L. Groups of 10 samples from each range were stored in three different environments: room temperature, 4°C and -20°C. Samples stored at room temperature were analyzed at 5 different time points up to 48h. Samples stored at 4°C were analyzed up to 7 days. Samples stored at -20°C were analyzed at 2 h. Data were analyzed using ANOVA. Significance was defined as p<0.05.

Results: There were no statistically or clinically significant differences between the baseline cTnI result and any of the later time points for room temperature and 4°C storage. For the -20°C, there was more variability among the 10 samples at 2 h.

Conclusions: Our study suggests that cTnI in a Barricor is stable for up to 48h at both room temperature and at 4°C.
Title: Taking the law into your own hands: Using simulation to assess medical laboratory science student competency in chain of custody procedures.

Authors: Yurkiw N, Purdy L, Martindale R, Van Spronsen A, Villatoro V, O’Brien-Smith E, Crawford J, Garchinski A

Background and Aim: In the latest revision of the CSMLS General MLT competency profile, students are required to “perform sample collections and chain of custody procedures relating to specimens with legal implications”. To address this change, a simulation was created by the Medical Laboratory Science (MLS) program at the University of Alberta. This project evaluated student procedural understanding pre-, during and post- simulation and evaluated student perception of simulation as an educational technique.

Material and Methods: A series of surveys were created to assess student knowledge and understanding. The control group included senior MLS students who will be assessed by the previous competency profile. The study group included MLS students currently in clinical training who will be assessed by the new competency profile. Both groups received surveys. The study group was then invited to participate in the simulation.

Results: Observations of the study group’s performance in the simulation revealed areas of concern. There were procedural actions that were consistently missed by participants. Such areas were discussed with the participants during the simulation debrief. Post simulation, students reported feeling more comfortable performing chain of custody procedures and dealing with specimens with legal implications.

Conclusions: Simulation gave students an opportunity to work through a procedure that is rare for them to encounter during clinical training. Pre-simulation learning materials, the simulation, and current standard operating procedures will be revised to reflect the observations and feedback collected during the simulations. Recommendations will be made to institutions pursuing similar simulation initiatives.
**Title:** Identification of a molecular repair response in ex vivo perfused porcine hearts

**Authors:** Nicole Herbers¹, Sanaz Hatami², Jayan Nagendran², Michael Mengel¹, Darren Freed², Benjamin Adam¹
¹Department of Laboratory Medicine and Pathology, University of Alberta
²Division of Cardiac Surgery, Department of Surgery, University of Alberta

**Background and Aim:**
Cardiac transplantation is a life-saving intervention for advanced heart failure but is limited by a shortage of suitable donor organs. Ex vivo heart perfusion (EVHP) represents a promising alternative for organ preservation and repair. We aimed to assess the feasibility of using gene expression to monitor cardiac tissue injury and repair during EVHP.

**Material and Methods:**
Heart samples were obtained from 24 pigs either in vivo (IV, n=5) or after 12 hours of ex vivo heart (EVHP, n=15) or combined heart and liver (H+L, n=4) perfusion. Functional parameters were recorded during EVHP. Histology was assessed for features of cardiac injury. NanoString was used to measure 68 genes related to cardiac injury and repair. Molecular data were assessed for differential expression and correlated with function and histology.

**Results:**
43 genes were significantly up-regulated and 8 genes were significantly down-regulated in EVHP vs. IV (FDR<0.05) (Figure 1). As an aggregate ‘repair’ gene set, the up-regulated genes exhibited higher expression in EVHP vs. IV (p<0.001), EVHP vs. H+L (p=0.002), and H+L vs. IV (p=0.02). As an aggregate ‘injury’ gene set, the down-regulated genes showed lower expression in EVHP vs. IV (p<0.001), EVHP vs. H+L (p=0.004), and H+L vs. IV (p=0.02). No statistically-significant correlation was observed between gene set expression and function or histology.

**Conclusions:**
These data suggest that EVHP induces a molecular repair response that is independent from functional and histological parameters. This response appears to be abrogated in high-demand H+L perfusion. This represents a novel approach for measuring donor heart quality during EVHP.
Title: Optimizing Susceptibility Testing Frequency for Bacteremic Patients

Authors: Johith Jacob and Tanis C. Dingle

Background and Aim:
Bacteremia is a major cause of sepsis and mortality in critically ill patients. Studies have shown delayed effective antibiotic administration correlate with septic patient mortality. Optimizing susceptibility testing frequency for bacteremic patients can improve efficient use of laboratory resources. At the Edmonton ProvLab, susceptibility testing for most blood isolates occurs every 3 days except for a limited number of organisms where daily susceptibility testing occurs daily. The aim of this study was to determine optimal testing frequency in an effort to make improvements to current protocol.

Material and Methods:
Susceptibility data of blood isolates from 2008-2016 at UAH was gathered from the Laboratory Information System (LIS). Patient susceptibility data with repeat bacteremia was analyzed for major susceptibility change (MSC: sensitive to resistant or vice versa) within 7 days.

Results:
From >600 patients with repeat bacteremia within 7 days, 49 patients showed MSCs. Repeat bacteremia within 7 days occurred more with Gram-Positive bacteria but MSCs occurred more with Gram-Negative bacteria. Furthermore, patients with MSCs within 1-2 days occurred at ≤1% with MRSA and MSSA while those with CoNS, Enterococcus faecalis, Enterococcus faecium, Enterobacter cloaceae, and Escherichia coli occurred at ≥3%.

Conclusions:
For most bacteria, 3-day susceptibility testing frequency of repeat blood isolates is appropriate. Protocol for repeat blood isolates with MRSA/MSSA as well as CoNS and SPICE organisms (1 day) should be maintained. However, repeat blood isolates with Enterococcus sp. and E. coli should be revised to daily testing.
**Title:** What's Normal? An Evaluation of Pediatric T Cell Markers in Healthy Controls

**Authors:** Morgan J Sosniuk¹, Anne Halpin¹,²,⁵,⁶,⁷, Juanita Wizniak⁷, Artur Szkotak⁶,⁷, Simon Urschel¹,²,⁵, Lavinia Ionescu¹,²,⁵ and Lori West¹,²,³,⁴,⁵,⁶. ¹Dept of Pediatrics, U of Alberta, Edmonton, Canada; ²Alberta Transplant Institute, Edmonton, Canada; ³Dept of MMI, U of Alberta, Edmonton, Canada; ⁴Dept of Surgery, U of Alberta, Edmonton, Canada; ⁵Canadian National Transplant Research Program, National, Canada; ⁶Dept of Laboratory Medicine and Pathology, U of Alberta, Edmonton, Canada and ⁷Laboratory Medicine and Pathology, Alberta Health Services, Edmonton, Canada.

**Background and Aim:**
Pediatric studies involve challenges including limited normal controls and small sample volumes. Standardized flow cytometry (FC) lymphocyte immunophenotyping panels are commercially available and normal adult ranges are published, however, comparable pediatric control data are rare. We aimed to establish a reference dataset for pediatric samples using standardized, comprehensive FC panels. We collaborated with the clinical laboratory to test normal pediatric samples with the goal of establishing comprehensive reference data and sharing information/results on this alternate staining method.

**Material and Methods:**
700uL blood from healthy children (n=10) aged 66 days-16 years was used to perform DuraClone FC phenotyping. Samples were tested in five, 10-colour T-/B-cell panels. Acquisition was performed by Navios cytometer (Beckman Coulter). The markers programmed cell death protein-1 (PD-1), a measure of T-cell exhaustion, and γδ T-cells and associated markers Vd1 and Vd2, were specifically analysed as reference data are lacking.

**Results:**
Preliminary analyses showed age-related trends. PD-1+ CD4 T-cells increases with age (R²=0.701); this association was weaker for CD8 T-cells. CD3+ T-cell number, including γδ T-cells, decreases with age; % of each population remains constant. There is a trend toward decreasing Vd1:Vd2 γδ T-cell ratio with age.

**Conclusions:**
Duraclone immunophenotyping is rapid and uses small blood volumes. These results begin to establish pediatric reference data and show promise for clinical laboratory application due to the potential to decrease technologist time as well as variation due to technologist-to-technologist variation. This method also lends itself to automated gating and analysis strategies, which are currently being explored.
**Title:** Characterization of Shiga Toxin-Producing *Escherichia coli* O157 Using Molecular Methods

**Authors:** Jonas Szelewicki, Brendon Parsons, Shuai Zhi, Linda Chui

**Background and Aim:**
Shiga toxin-producing Escherichia coli (STEC) produce toxins encoded by stx<sub>1</sub> and stx<sub>2</sub> genes that cause infection ranging from mild diarrhea to potentially life-threatening complications including hemolytic uremic syndrome, especially in young children. Stx genes are subclassified into ten subtypes, two of which (Stx2a, Stx2c) are often correlated with higher pathogenicity. It was hypothesized that there would be a correlation between presence of stx genes and clinical outcome. The objective of the study was to identify and characterize shiga toxin genes from 103 clinical E. coli O157 isolates from Alberta in 2016 and correlate gene subtypes with disease outcome, with patient epidemiological data included in the analysis.

**Material and Methods:**
Following DNA extraction from isolates, real time-PCR amplification assays were used to detect and subtype stx genes.

**Results:**
Our results showed that infection with E. coli O157 that carry the toxin gene stx<sub>2a</sub> or a combination of Stx1a and 2a subtypes produced the most severe clinical outcome. The majority of these patients were children, with a higher incidence of hospitalizations among the young and the elderly. The number of reported cases of E. coli O157 was highest in the summer months and in southern Alberta.

**Conclusions:**
In conclusion, this study shows the importance of detecting shiga toxin genes and their subtypes using molecular methods so early intervention including terminating the use of antibiotics and expanded hydration therapy might improve disease outcome in STEC patients.
Scope of Practice Descriptions Vary for Pathologists’ Assistants in Alberta, Manitoba, and Ontario

Authors: Chan, Ainsley; Keelan, Monika; Mather, Cheryl; Lee, Danielle

Background and Aim:
Pathologists’ Assistants (PAs) working in surgical pathology are highly skilled medical laboratory personnel who describe and dissect surgical resection specimens to contribute diagnostic and prognostic information. However, as a newly certified profession in Canada, there is no universally accepted national standard describing PAs’ responsibilities. The main objective of this study is to identify differences in practices and language to move toward establishing a national competency profile for the PA profession by first interviewing program directors of the Master’s degree PA (MPA) training programs and PAs in Canada.

Material and Methods:
4 MPA program directors, 1 assistant program director (PA) and 1 program coordinator (PA) (at the Universities of Alberta, Calgary, Manitoba, and Western Ontario) were interviewed to determine routine activities and professional competencies of PAs working in surgical pathology in Alberta, Manitoba, and Ontario. Interview transcripts were qualitatively analyzed to determine similarities and differences in job expectations, site requirements, and program learning objectives.

Results:
There is strong consensus regarding minimum training, proficiencies, and continuing education expectations for PAs; however, descriptions of specific workplace duties and laboratory infrastructure vary across Alberta, Manitoba, and Ontario.

Conclusions:
Disparities in scopes of practice for PAs across Alberta, Manitoba, and Ontario identified during interviews with program directors and PAs of MPA programs supports further research to elucidate these differences across a broader range of working Canadian PAs. Standardizing language and PA responsibilities will provide a basis for developing a national competency profile to define and increase recognition for this unique health care profession in Canada.
**Title:** Evaluation of Human Identity Determination Ordering Practices in the Edmonton Zone

**Authors:** Dhalla, Fatema; Mather, Cheryl

**Background and Aim:**
Human Identity Determination (HID) is a molecular technique to test whether two or more tissues match at a genetic level. Used as a quality assurance (QA) tool, HID testing can prevent patient harm from specimen mix-ups or tissue contamination. The aim of this study was to evaluate the ordering of HID testing in the Edmonton Zone and localize stages of error to drive process improvement.

**Material and Methods:**
In this study, all HID cases in the Edmonton Zone from 2014 to 2017 were reviewed to determine the reason for testing, assess error stage, determine patient impact; and, for cases of tissue contamination, determine whether QA was appropriately documented.

**Results:**
HID test requests increased from 2 cases in 2014 to 21 cases in 2017. The number of cases requested for tissue contamination, specimen mix-up, and tissue confirmation increased at similar rates. Most cases were requested due to errors during the analytic stage of testing, and the majority of cases were caught before the occurrence of patient harm. Of the tests requested due to suspected contamination, 13/15 had confirmed contaminants. However, only two of these cases had correct QA documentation, and QA follow up was documented in just two cases.

**Conclusions:**
HID requests have increased dramatically, but no single cause for this rise was identified. HID test results are being utilized as an effective QA tool in regard to patient harm prevention, but post-test QA procedures are not being appropriately completed or documented in order to trigger downstream process improvements.
Title: Process-Affected Water in the Oil Sands Industry: Toxicity Attribution and Evaluating Ageing as a Remediation Strategy

Authors: Ian Gault, Dr. Birget Moe, Dr. Angela Sun, University of Alberta; Dr. Jonathan Martin, University of Stockholm

Background and Aim:
Oil sands process-affected water (OSPW) is used to separate bitumen from sand in the surface-mining oil sands industry of Alberta. OSPW contains a complex dissolved organic mixture that is toxic, persistent, and largely uncharacterized. One reclamation strategy consists of ageing OSPW in end-pit lakes such that sedimentation and biodegradation of the organics through ageing will eventually allow for safe environmental integration. This investigation contributes to predicting the effectiveness of this strategy by testing the toxicity of candidate chemical classes in various fresh and aged OSPW.

Material and Methods:
Chemical fractionation and high resolution orbitrap mass spectrometry were used on OSPW, with cytotoxicity measured through real-time cell analysis of human liver carcinoma cells (HepG2) and endocrine disruption measured using the yeast estrogenic/androgenic screens.

Results:
An isolated fraction containing naphthenic acids (NAs) was responsible for the cytotoxicity observed, while the non-acidic fraction, speculated to contain steroidal chemicals, was not cytotoxic. Estrogen/androgen receptor antagonists were in all fractions, with both NAs and the non-acidic fractions active near environmentally-relevant concentrations. Toxicity of OSPW in an end-pit lake decreased over time, but an older, geographically-separate sample of 23-years had a unique time-dependent biphasic toxicity profile.

Conclusions:
Cytotoxicity decreased with ageing, with NAs responsible, but the biphasic response from the recalcitrant organics should be explored. Furthermore, the ubiquitous presence of estrogen/androgen receptor antagonists indicate that multiple chemical classes may be steroidal. By identifying the most active chemicals in OSPW there can be a better understanding of observed toxic effects and allow for monitoring to ensure releases are protective of downstream uses.
Title: Determination of Arsenic Species in Fish from Aboriginal Communities Before and After the Northern Alberta Wildfires

Authors: Karen S. Hoy, Xiufen Lu, Qingqing Liu and X. Chris Le

Background and Aim:
On average, 70% of home decks or porches destroyed in the 2016 Northern Alberta wildfires contained pressure-treated wood preserved with chromated copper arsenate (CCA). Upon burning CCA treated wood, arsenic and other metals were released and deposited in the surrounding environment. Arsenic species have varying toxicities. For example, consumption of high abundances of arsenobetaine is relatively non-toxic; however, elevated concentrations of inorganic arsenic are known to cause skin and lung cancers. Therefore, it is critical to distinguish between and quantify common arsenic species in food to assess consumption risks. Aboriginal communities are concerned about the effect wildfires have on the quality of their commonly consumed traditional foods, such as game meat, fish, berries and cattails. The objective is to quantify and compare common arsenic species present in fish samples from a 10-year First Nations Food Nutrition and Environment Study (FNFNES) with post-wildfire samples.

Material and Methods:
Fish samples were homogenized and replicate aliquots were extracted using a water-methanol mixture, followed by protease digestion and microwave-assisted extraction. Arsenic species were determined using high performance liquid chromatography (HPLC) separation with detection via inductively coupled plasma mass spectrometry (ICPMS) and electrospray ionization mass spectrometry (ESIMS).

Results:
HPLC enables separation of arsenite, arsenate, monomethylarsonic acid, dimethylarsinic acid, and arsenobetaine. Simultaneous detection using ESIMS and ICPMS offers information for the identification and quantitative determination of arsenic species. Parallel analysis of the samples using ICPMS without HPLC separation provides total arsenic concentration. A comparison between the total arsenic concentration and the sum of individual arsenic species contributes to an assessment of the accuracy of the methods. Our results from the analysis of arsenic species in fish will also be compared with the values previously reported for other food items.

Conclusions:
This study will aid in assessing the health risks associated with exposure to environmental contaminants, including arsenic, by Aboriginal communities.
Title: Cryopreserved Buffy Coat-derived Monocytes for the Assessment of Alloantibody Clinical Significance

Authors: Betty J. Kipke1, Donald R. Branch3, 4, Jason P. Acker1, 2, Jelena L. Holovati 1, 2

1University of Alberta, Edmonton, AB, Canada, 2Canadian Blood Services, Edmonton, AB, Canada, 3University of Toronto, Toronto, ON, Canada, 4Canadian Blood Services, Toronto, ON, Canada.

Background and Aim:
Monocyte monolayer assay (MMA) is a compatibility testing method for evaluating the clinical significance of red blood cell (RBC) alloantibodies. Time-consuming monocyte isolation procedures and requirement for fresh monocytes have limited application of the MMA. The aim of this study was to develop and assess the utility and efficacy of cryopreserved buffy-coat (BC)-derived monocytes for MMA application.

Material and Methods:
Peripheral blood mononuclear cells (PBMCs) were isolated from BC, pooled and cryopreserved in liquid nitrogen. Monocytes from pooled PBMCs were incubated with either anti-D-sensitized, anti-Scianna2 (Sc2)-sensitized, anti-AnWj-sensitized or anti-Jra-sensitized RBCs. MMA phagocytic index (PI) and membrane integrity were determined microscopically.

Results:
Cryopreserved PBMCs showed 96 ± 1% viability post-thaw. Our previous study showed no statistically significant difference in phagocytosis of anti-D sensitized RBCs by cryopreserved BC monocytes vs fresh monocytes. When pooled cryopreserved BC monocytes were challenged with previously established clinically significant alloantibody-sensitized RBCs, the results were consistent with previous findings: an average PI of 9 ± 2 for anti-Sc2, 64 ± 5 and 56 ± 5 for anti-AnWj and 11 ± 3 and 22 ± 10 for anti-Jra-sensitized RBCs. Alloantibodies tested showed a weak (1+) IAT reactivity.

Conclusions:
This study shows that cryopreservation preserved monocyte viability and phagocytosis function for MMA. As previously reported with fresh monocytes MMA assay, the three alloantibodies tested with cryopreserved BC monocytes were shown to have a phagocytic index of clinical significance (PI>5%). The use of cryopreserved BC-monocytes has the ability to achieve consistent and predictable results from MMA by minimizing inter-assay variations.
**Title:** DNAzyme-based Applications for Nucleic Acid and Protein Detection

**Authors:** Hanyong Peng, Ashley M. Newbigging, Zhixin Wang, Jeffrey Tao, Wenchuan Deng, X. Chris Le, and Hongquan Zhang

**Background and Aim:**
“DNA enzymes” (DNAzymes) are unique synthetic DNA structures with functionalities that mimic proteinaceous enzymes. “Peroxidase-mimicking” DNAzymes bind to hemin and perform redox reactions to generate a signal, while “RNA-cleaving DNAzymes” cleave hybridized nucleic acid substrates. Both DNAzymes have been, and continue to be, utilized to develop nanodevices for biomarker detection. Our aim is to discuss the variety of analytical formats that incorporate DNAzymes.

**Materials and Methods:**
Literature was reviewed for recent assays that incorporated DNAzymes as signal transducers, amplification substrates, and/or catalytic beacons either conjugated to nanomaterials or free in solution.

**Results:**
Strategies usually begin with an inactivated DNAzyme, where the catalytic core is caged in a hairpin loop or unbound to the substrate to prevent the generation of background signals. Target proteins or nucleic acids then disrupt the hairpin structure or facilitate the folding of the catalytic core to activate the DNAzyme. Once activated, peroxidase-mimicking DNAzymes reduce reactive chemicals to generate optical or electrochemical responses. RNA-cleaving DNAzymes cleave molecular beacons to generate fluorescence signals or cleave substrate strands to initiate non-enzymatic DNA amplification processes. As nucleic acids themselves, DNAzymes can also be split into two halves that reassemble in the presence of target molecules. To amplify signal generation, DNAzymes can be subject to DNA amplification techniques and conjugated to nanomaterial surfaces.

**Conclusions:**
DNAzymes are advantageous over their protein counterparts for biomolecular detection. DNAzymes are thermally stable and easy to synthesize due to their nucleic acid composition. Recent work introducing DNAzyme technology into living cells opens promising opportunities for biomarker detection in tissue samples and patients.
Title: Arsenic speciation in human urine for identification of potential genetic determinants of arsenic-induced diseases

Authors: Jagdeesh S. Uppal, Dr. X. Chris Le

Background and Aim: Exposure to high concentrations of arsenic has been shown to contribute to illnesses such as cardiovascular disease, neurological diseases, respiratory problems, and cancer. Because there is no animal study that is perfectly suitable for studying the mechanisms of arsenic toxicity and carcinogenicity, human epidemiological studies are vital for identifying risk factors and how diseases can be prevented. One of the risk factors is genetic susceptibility to arsenic. We currently collaborate on an epidemiologic study in an arsenic-affected population in India to determine whether differences in genetics affect the methylation and excretion of arsenic species. An analytical requirement of this study is the determination of arsenic species, including inorganic and methylated arsenic species, in urine samples from the study population of 1800 people.

Material and Methods: To quantify individual arsenic species in urine, we have developed a method using high performance liquid chromatography (HPLC) separation with inductively coupled plasma mass spectrometry (ICPMS) and electrospray ionization (ESI) tandem mass spectrometry (MS/MS) detection.

Results: Quantification of inorganic arsenite, arsenate, monomethylarsonic acid, and dimethylarsinic acid has been achieved using HPLC-ICPMS. The simultaneous detection with both ESIMS and ICPMS further assisted the identification of new arsenic metabolites.

Conclusions: The results of arsenic species in the participants’ urine, along with their health outcome and genetic polymorphism data collected by our collaborators, will be incorporated into statistical models to analyze any associations between urinary arsenic speciation and genetic variations. The overall study will identify potential genetic determinants of arsenic-induced diseases, which is particularly important for health protection of susceptible populations.
Title: Regulation of P53 and its E3/E4 Ubiquitin Ligases by Wip1

Authors: Yasser Abuetabh and Roger Leng

Background and Aim:
UBE4B is a potential oncogene implicating the outcomes of several cancers, including brain, breast and liver cancers. Recently, our lab demonstrated that UBE4B promotes degradation of phospho-p53 (S15 and S392) in response to IR, suggesting UBE4B plays a role in regulating p53 after DNA damage. However, UBE4B regulation is still largely unknown. Wild-type p53-induced protein 1 phosphatase (Wip1) is an essential main known phosphatase that targets phosphorylated p53. Moreover, Wip1 is found to target most of the negative and positive regulators of p53, including ATM, ATR, Hdm2 and Chk1/2. Thus, we wondered if Wip1 could also target UBE4B.

Material and Methods:
We investigated the endogenous UBE4B protein levels in response to Wip1 over-expression or silencing in different cancer cell lines with or without IR exposure.

Results:
Our data showed that UBE4B and Wip1 were induced mainly in wild-type p53 expressing cell lines in response to IR. Moreover, Wip1 overexpression increased the level of UBE4B protein, while a reduction in Wip1 via Wip1-siRNA resulted in decreased UBE4B protein level. Our preliminary data demonstrated that Wip1 co-immunoprecipitated with UBE4B.

Conclusions:
Our preliminary data demonstrated that Wip1 could directly or indirectly impact the expression of UBE4B. Our aim is to explore this potential relationship between Wip1 and UBE4B. Consequently, revealing the possible regulators of UBE4B may lead to development of novel therapeutic strategies to battle cancer.
Title: Investigating Halobenzoquinone Formation Potential of Aromatic Amino Acids and the Impact of Iodide and Bromide During Drinking Water Chlorination

Authors: Lindsay K Jamaiff Blackstock, Ping Jiang, Guang Huang, Wei Wang, Xing-Fang Li

Background and Aim:
Disinfection byproducts (DBPs) result from organic matter reacting with disinfectants used to prevent waterborne disease transmission. Epidemiologic studies show a link between long-term DBP exposure and increased bladder cancer risk. Halobenzoquinones (HBQs) are a class of highly cytotoxic DBPs that have been identified in drinking water. Recent studies identified a trend in the toxicity of halogenated DBPs and HBQs (iodinated > brominated > chlorinated). Water sources high in bromide and iodide ions (i.e., impacted by industry or natural geological deposits) have been found to produce higher proportions of Br- and I-DBP analogues. Determining which organic materials are precursors to HBQs is essential to reduce their formation in drinking water. Amino acids are ubiquitously present in natural water bodies and aromatic amino acids (AAAs) consist of sub-structures similar to HBQs.

Material and Methods:
Here AAAs are investigated as precursors to HBQs under water chlorination conditions. Furthermore, the impact of bromide and iodide concentrations on Br- and I-HBQ congener formation will be studied. A high-performance liquid chromatography tandem mass spectrometry method was developed to identify and quantify AAAs and HBQs.

Results:
After chlorination of model solutions, all three AAAs produced 2,6-dichloro-1,4-benzoquinone (DCBQ) and/or 2,3,6-trichloro-1,4-benzoquinone. Furthermore, in the presence of bromide, 2,6-dibromo-1,4-benzoquinone was formed in addition to DCBQ.

Conclusions:
HBQ formation from chlorination of AAAs is dependent on pH, reaction time, relative reactant concentrations, and AAA structures. Further studies are needed to investigate the impact of iodide on I-HBQ formation. This work will provide insight on future strategies to reduce exposure to HBQs in drinking water.
**Title:** Regulation of tumor suppressor PTEN by #14 microRNA in breast cancer cells

**Authors:** Chengsen Chai and Roger Leng

**Background and Aim:**
In cancer cells, tumor suppressor PTEN was one of most frequently inactivated genes. MicroRNA involved regulation of genes by binding to the target messenger RNAs and impeding their translation. In this project, we aim to find and confirm new microRNAs that bind to PTEN mRNA and lead to PTEN inactivity.

**Material and Methods:**
20 predicted microRNAs targeting PTEN were constructed and transfected into breast cancer cell MCF7. Endogenous PTEN level were examined in whole cell lysates by immunoblotting. Next co-transfection of microRNA and its target PTEN ORF&3'UTR was performed in HEK 293 cell to check the effect of microRNAs on exogenous PTEN. By luciferase assay, the binding sites of microRNAs on target mRNA were confirmed.

**Results:**
MicroRNA #14 highly expressed in breast cancer cell lines and down-regulated both endogenous and exogenous PTEN level. MicroRNA #14 bound to PTEN mRNA 3'UTR negatively regulating target protein translation. In term of its biological function, microRNA #14 promoted BCa cell proliferation, cell cycle arrest and cell migration.

**Conclusions:**
MicroRNA #14 was found to negatively regulate PTEN and highly expressed in breast cancer cells, which played an oncogenic role.
**Title:** Expression, purification and characterization of Cas13a protein in bacteria

**Authors:** Wei Feng, Hanyong Peng, Hongquan Zhang, X.Chris Le

**Background and Aim:**
The CRISPR-based diagnostics (CRISPR-Dx) generates great interest in recent years. The CRISPR/Cas13a has been demonstrated for its RNA-guided RNase function. We can envision enormous research potential in exploring its capability in point-of-care diagnosis and genetic disease treatment. However, the purification of Cas13a protein is a difficult but essential first step, since no commercial product is currently available. In this work, we aim to express the Cas13a protein, simplify the conventional purification procedures, and improve the purity and yield of the Cas13a protein.

**Material and Methods:**
Various parameters were studied in expressing the His-tagged Cas13a, including the amount of Isopropyl β-D-1-thiogalactopyranoside (IPTG), the duration and temperature of induction. Cells were lysed by ultrasonication, and cell lysates were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then affinity chromatography (Ni-NTA) was used to purify the His-tagged Cas13a protein from a crude cell lysate. Different Ni-NTA columns were compared for their capture efficiency. The captured protein was released using a gradient of imidazole.

**Results:**
Gel electrophoresis results indicated the presence of the Cas13a protein. The protein band was consistent with the expected size of the Cas13a protein (171 kDa). No corresponding protein band was observable from the control cells that were not induced with IPTG. After the affinity column separation, the purity of the Cas13a protein was 79%.

**Conclusions:** Cas13a were successfully produced. The purified Cas13a proteins can be used for the development of new assays for nucleic acid targets.
Title: Influence of Selenium on Arsenic Uptake and Efflux by Human Hepatoma Cells and Primary Hepatocytes

Authors: Gurnit Kaur1, Olena Ponomarenko2, Kelly L. Summers2,3, Nataliya V. Dolgova2, Olga Antipova4, Donna N. Douglas5, Norman M. Kneteman5, Ingrid J. Pickering2,3, Graham N. George2,3, Elaine M. Leslie1,6

1Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, University of Alberta, Canada
2Department of Geological Sciences, University of Saskatchewan, Canada.
3Department of Chemistry, University of Saskatchewan, Canada
4Advanced Photon Source, Argonne National Laboratory, Argonne, USA.
5Department of Surgery, University of Alberta, Canada
6Department of Physiology, University of Alberta, Canada

Background and Aim: Hundreds of millions of people worldwide are exposed to the proven human carcinogen arsenic at levels exceeding the World Health Organization guideline. Animal models have shown that selenium and arsenic are mutually protective via increasing the biliary excretion of each other. Despite ongoing human clinical trials, the influence of selenium on human hepatic handling of arsenic is not yet adequately understood. We hypothesized that selenium would increase the uptake and efflux of arsenite (AsIII) in human hepatoma (HepG2) cells and hepatocytes.

Material and Methods: In order to test this hypothesis, we studied the influence of selenite (SeIV) and selenide (SeII-), on (i) arsenic uptake by suspended HepG2 cells and human hepatocytes, and on (ii) arsenic efflux from sandwich-cultured human hepatocytes (SCHH). After SCHH were treated with 73AsIII (± SeIV or SeII-) for 24 hours, efflux of 73As across basolateral and apical surfaces was measured.

Results: Contrary to our hypothesis, SCHH biliary efflux of 73As in the presence of SeIV was reduced by 11 to 100% across 5 preparations, whereas basolateral efflux was reduced by 20 to 47%. Uptake of 73AsIII by suspended HepG2 and human hepatocytes was also inhibited by SeIV, but increased uptake was observed in the presence of SeII-. X-ray fluorescence imaging of HepG2 cells also suggested that AsIII accumulation in the presence of SeII- was 3-4 times higher than in the presence of SeIV.

Conclusions: These results are consistent with the rapid reduction of SeIV to SeII- in erythrocytes, with SeII- being the main form transported to the liver.
Title: Quantification of biofilm formation in heat resistant *Escherichia coli* isolates implicated in human infection

Authors: Angela Ma, Linda Chui

Background and Aim:
The ability of bacteria to form biofilms on various surfaces facilitates increased resistance against environmental stressors. Biofilms in food processing environments may result in persistent survival of foodborne pathogens. If heat resistant *E. coli* isolates are also capable of forming biofilm, they may pose a significant threat to food safety due to their resistance to inactivation by high temperatures used in routine cleaning procedures.

Material and Methods:
Biofilm formation by heat resistant isolates (clinical n=3; environmental n=3) was determined. A 96 well plate was used as an anchor and immersed in wells of 10^8* bacterial cells/ml with Luria Bertani (LB) broth serially diluted from 100-10%. Plates were incubated for 24 hours at temperatures of 4°C, 24°C, and 37°C followed by staining with 1% crystal violet solution. Dye adhering to the plates was dissolved in 95% ethanol and the optical density at 595 nm was measured.

Results:
Plates incubated at 4°C resulted in no biofilm formation by all isolates. Of the three clinical heat resistant isolates, only one formed biofilms at 24°C and 37°C. 40% diluted LB broth was the most effective concentration for promoting biofilm formation at 24°C; whereas at 37°C, biofilm formation correlated directly with increasing LB broth concentration.

Conclusions:
Heat resistant clinical isolates are less efficient in biofilm formation compared to the environmental strains as shown in this study. Biofilm formation by heat resistant *E. coli* may serve as highly persistent reservoirs for cross contamination in food processing. The identification of pathogenic *E. coli* possessing so many survival traits against environmental stressors prompts the potential reevaluation of pathogen inactivation measures.
**Title:** CIP-1, a calcium sensor protein, encodes an important component of the p53-mediated stress response pathway.

**Authors:** Ao Ma and Roger Leng

**Background and Aim:**
p53 is a crucial tumor suppressor gene that is related with many functions through interacting with its downstream genes such as \( p21^{WAF1} \) and MDM2. Recently, our lab discovered that a gene encoding a neuronal calcium sensor named CIP-1 is a potential novel p53 target gene. CIP-1 is involved in calcium signal transduction pathways; it has been demonstrated that CIP-1 plays roles in the genesis of tumors. My goal is to determine whether CIP-1 protein is a component of the p53-mediated stress response pathway. By characterizing CIP-1 as a novel p53 target gene in the regulation of Ca\(^{2+}\) signaling, we open up new understanding in the mechanism of tumors.

**Material and Methods:**
p53-null and wild-type p53 expressing cells were treated with \( \gamma \)-irradiation or drug to activate p53. The expression levels of p53 and CIP-1 (both in mRNA and protein) were examined by using quantitative real time-PCR and Western Blotting, respectively. Luciferase assay was performed to proof the transcription of CIP-1 can be activated by p53. The effect of CIP-1 on tumorigenesis was tested through colony formation assay under the conditions of over-expressed and knocked-down CIP-1.

**Results:**
CIP-1 mRNA and protein levels increased in response to p53 activation in various p53 WT cell lines; but not in p53-null cells. P53 was demonstrated to be able to bind to the regulatory sequence of CIP-1, and this binding can promote the transcription of CIP-1. When CIP-1 is over-expressed, fewer tumor cell colonies formed, and p53 can promote the colony formation inhibition effect of CIP-1.

**Conclusions:**
The transcription of CIP-1 can be induced by p53, hence the protein expression of CIP-1 is promoted. CIP-1 functions as a tumor suppressor, p53 can enhance its tumor suppressor effect.
Title: Enzyme-free and Isothermal Amplified HER2 Detection

Authors: Ashley M. Newbigging, Hongquan Zhang, X. Chris Le

Background and Aim: Trastuzumab, a successful HER2+ cancer treatment, targets overexpressed HER2 on cells. It is critical to assess the HER2 status of cancer cells when selecting treatment type for patients. Current methods for HER2 status determination use immunohistochemistry, which is labour-intensive and costly. To decrease the cost and technical complexity, we aim to develop a homogenous, enzyme-free isothermal amplification assay for specifically detecting HER2 cell surface biomarkers.

Material and Methods: We designed two DNA probes, each containing a HER2 aptamer domain, a linker domain, and a shared complementary domain. The two complementary domains hybridize together only when the two probes target the same HER2 molecule. Hybridization triggers the release of an initiator strand that activates a Hybridization Chain Reaction involving four hairpin DNA strands. Each hairpin contains complementary regions to two other hairpins caged within the hairpin structure. The initiator uncages an initial hairpin, which uncages the next, and so on, resulting in a long “polymer” of double stranded DNA containing repeating hairpins. Every four repeats restore the fluorescence from a fluorophore(F)-quencher(Q) beacon.

Results: Isothermal signal amplification was achieved. The strand displacement beacon provided real-time fluorescence detection. Gel electrophoresis results confirmed the products of the Hybridization Chain Reaction.

Conclusions: We developed a homogenous and isothermal assay without requiring enzymes, for the specific detection of HER2 on cells. We achieved fluorescence intensity dependent on the initiator concentration with a tunable detection limit. Changing the DNA sequences of the aptamer in the probes can enable detection of other proteins or nucleic acids.
**Title:** Protein-Initiated Strand Displacement Activating a DNAzyme Motor

**Authors:** Albert Zuehlke, Hanyong Peng, Jing Yang Xu, Wenchan Deng, and Hongquan Zhang

**Background and Aim:**
Protein biomarker detection has both health and environmental applications. Current methods are limited in accomplishing accurate protein detection in a rapid, simplified, and low-cost format. However, recent advances in nanomaterials allows for development of novel methods capable of resolving these limitations. Our proposed method allows for the detection of protein in a sensitive, specific, mix-and-read format with potential for onsite analysis.

**Material and Methods:**
The DNAzyme motors are constructed using gold nanoparticles (AuNPs) labelled with hundreds of DNA strands. The DNAzyme strands are inactivated using a blocking sequence. The more numerous fluorescein-labelled strands act as DNAzyme substrate and serve as a three-dimensional track. Fluorescein fluorescence is efficiently quenched due to the close proximity to the AuNP surface. DNAzyme activation along the high-density track relies on removal of the blocking sequence. This is accomplished by simultaneous binding of two target specific affinity probes to a single protein target. Partial hybridization occurs between the two probes that then displaces the blocking sequence through toe-hold mediated strand displacement. The DNAzyme is activated and begins binding and cleaving nearby substrate strands. The DNAzyme strand moves autonomously along the AuNP surface until substrate strands become depleted. No longer tethered to the AuNP surface, the cleaved fluorescein generates fluorescent signal reflective of the protein target’s concentration.

**Results:**
Proof of concept results using biotinylated probes and streptavidin target yields a detection limit approaching 1pM. The dynamic range for streptavidin is between 0-200pM.

**Conclusions:**
Our method provides homogeneous, isothermal, and amplifiable detection of streptavidin with potential to apply to other proteins of interest.
Title: Utilization of Serum protein and Urine protein electrophoresis in Pediatric patients.

Authors: Joy Adekanmbi 1, Dylan Thomas 1,2, Mathew Estey1,2, Don Zhang 2, Karina Rodriguez-Capote 1,2
1University of Alberta, Laboratory Medicine and Pathology, 2DynaLIFE Medical Labs, Edmonton.

Background and Aim:
Serum protein electrophoresis (SPE) and urine protein electrophoresis (UPE) are used in the diagnosis, prognosis and monitoring of monoclonal gammopathies. Thus, these tests should be requested mainly in patients older than 50 years. Though debatable, UPE is also used to assess renal damage. At DynaLIFE, these tests are performed by gel electrophoresis, which is labor intensive and time consuming. This study aims to review physician ordering patterns to promote measures directed at appropriate testing.

Material and Methods:
Retrospective analysis of SPE and UPE performed on pediatric patients (≤ 18 years) in 2014. De-identified information consisting only on age, gender, interpretative results and ordering physicians were obtained through the laboratory information system.

Results:
706 SPE and 86 UPE were performed for pediatric patients. 97% of tests ordered did not have patient’s pertinent history. SPE and UPE were ordered concurrently in 13% of patients. All SPE and UPE were negative for a monoclonal protein. 95% of UPE’s showed a normal or mild glomerular pattern, while the 5% with combined glomerular and tubular damage pattern were patients with known renal disease also been monitored by other tests of renal function. Over 60% of both SPE and UPE were ordered by general practitioners, with 40% ordered by two family physicians.

Conclusions:
Auditing SPE and UPE utilization gave insight into the need for intervention and optimization of these tests. We proposed physician education, restriction of test menu and enforcing the need for clinical history as interventions to curtail utilization of these tests.
**Title:** Ncounter Technology Copy Number Variation (Cnv) Analysis Compared to Fish for the Classification, Diagnosis and Predictive Prognosis in Brain Tumors

**Authors:** Joy Adekanmbi\(^1,2\), Karl Narvacan\(^1\), Frank van Landeghem\(^1,2\), Iyare Izevbaye\(^1,2\), Ben Adam\(^1,2\), Kim Formenti\(^1,2\)

\(^1\)University of Alberta, Laboratory medicine and Pathology, Edmonton, Alberta. \(^2\)Alberta health services.

**Background and Aim:**

nCounter is a new precise and reproducible method in which gene target is quantified directly using barcode counting. This method is robust across many sample types with minimal hands-on time and high level internal QC checks.

Our study aims to determine whether nCounter technology in the analysis of ATRX, IDH, EGFR, PTEN, MGMT and 1p/19q co-deletion using copy number variation could be a replacement for FISH which is the current gold standard in the genetic classification of brain tumors. Ultimately, the diagnostic efficiency gained from this research work aims to improve the overall quality of health care in brain tumor oncology.

**Material and Methods:**

This is a retrospective study using FFPE tissue.

1. Fluorescence in-situ Hybridization was performed as follows
   - 1p and 19q co-deletion: 40 patients
   - EGFR amplification: 32 patients

2. CNV Elements XT Assay
   - nCounter Assays was used to detect copy number variation (CNV) and counts of genetic loci in enriched DNA.

**Results:**

Table 1. Summary of diagnostic parameters for nCounter CNV analysis as compared to FISH

**ROC curve**

**Conclusions:**

Co-deletion of 1p/19q chromosomal arms typically indicate tumors of oligodendroglial origin and generally better prognosis and chemosensitivity (Boots-Sprenger et al.). On the other hand, EGFR amplification is supportive of a diagnosis of glioblastoma multiforme (GBM) or high grade astrocytoma, and predicts a poorer prognosis (Smith et al.). Having a robust, fast and reliable method in detecting the molecular genetics behind brain tumors aids in the diagnosis and prognostication for each neuro-oncological patient.
Title: Turning urine into gold: An analysis of updated red blood cell reporting categories for microscopic urinalysis

Authors: Dr. David Beyer, Joan Hanks, Dr. Mark Assmus, Dr. Trevor Schuler, Dr. Tim Wollin, Dr. Mathew Estey

Background and Aim:
Approximately 2000 screening cystoscopies are performed annually for the investigation of microscopic hematuria in Edmonton. Fifty percent of the cystoscopies are performed for patients with microscopic hematuria in the 1-5 red blood cells (RBCs) per high-powered field (HPF) category. According to the Canadian Urological Association (CUA) guidelines, microscopic hematuria is defined as >2 RBCs/HPF. Currently, the Edmonton Zone only reports a 1-5 category which may lead to an over-representation of microscopic hematuria. Patients with only 1-2 RBCs/HPF on urinalysis do not require a comprehensive workup for bladder cancer screening according to current guidelines. Our aim is to examine the proportion of patients within the 1-5 RBCs/HPF category that have clinically significant microscopic hematuria (>2RBC/HPF).

Material and Methods:
We analyzed 681 urine samples from two consecutive days that were reported as 1-5 RBCs/HPF in the Edmonton zone. Of these samples we determined the proportion that had >2 RBCs/HPF. This analysis was performed on all three urine microscopy analyzers at our regional DynaLIFE Medical Labs.

Results:
Only 41% (279/681) of urine samples categorized as 1-5RBC/HPF had clinically significant microscopic hematuria (>2RBC/HPF).

Conclusions:
By reporting the proportion of patients with clinically significant hematuria (>2RBC/HPF) in the 1-5 category we could prevent approximately 600 unnecessary comprehensive workups for bladder cancer in our region per year. Including the upper tract imaging, urine cytology, cystoscopy and specialist consultation costs (estimated 2500$/patient) this could translate into 1.5 million dollars saved annually. Additionally, we can prevent invasive procedures and psychological stress associated with a potentially unnecessary workup for bladder cancer in these patients.
**Title:**  Fibrin-Associated EBV-positive diffuse large B-cell lymphoma of prosthetic aortic valve and aortic root graft: a variant of diffuse large B-cell lymphoma associated with chronic inflammation

**Authors:**  Abdulrahim Alabdulsalam, MD, Ashish Rajput, MD, Sabin Bozso, MD, Michael Moon, MD, FRCSC, Anthea Peters, MD, FRCPC, Jean Deschenes, MD, FRCPC

**Case Report**  
A 40-year-old male with history of aortic valve and root replacement for aortic regurgitation, presented nine years after the procedure with shortness of breath and reduced exercise tolerance. Imaging studies revealed a presumed thrombus extending from aortic valve leaflets up to the distal end of the graft. Immediate surgery was performed, with replacement of the aortic valve and ascending aorta grafts. On histologic examination of the thrombus, it contained aggregates of large highly atypical cells, admixed with fibrinous material, with no tissue invasion. Immunohistochemistry revealed these cells to be of B-cell origin, positive for CD20 and PAX5. They were also positive for EBV by in situ hybridization. A diagnosis of fibrin-associated EBV-positive Diffuse large B-cell lymphoma (DLBCL) was rendered and the patient underwent staging procedures which showed no other sites of disease. He received 4 cycles of systemic chemotherapy and he is currently alive without evidence of disease at 18-month interval post-surgery.

**Discussion**  
Fibrin-associated DLBCL is a newly described entity that is considered a variant of DLBCL associated with chronic inflammation (DLBCL-CI), and is included in the 2016 WHO classification. Thirteen cases of DLBCL involving cardiac prosthetic devices have been reported thus far, and most were designated as DLBCL-CI. Almost all of them behaved in an indolent manner with no death attributable to the lymphoma. It is important to emphasize the distinction between this type of lymphoma and DLBCL-CI as a typical case of the latter has a drastically worse prognosis.

**Figure.** (A) Luminal surface of explanted valve showing atypical lymphoid infiltrate composed of moderate to large pleomorphic cells with apoptosis, mitosis and necrotic areas (20X). (B) CD20 immunohistochemistry is positive the lesion (20x).
**Title:** Antifungal Susceptibility of Clinical *Candida* species Isolates from Northern Alberta, 2014-2017

**Authors:** Alexander Bello, Brad Jansen, Jeff Fuller, and Tanis Dingle

**Background and Aim:**
The development of resistance of *Candida* species to antifungal agents has been well documented in the last decade. The aim of this study was to analyze trends in susceptibility of *Candida* species in Northern Alberta from 2014 to 2017.

**Material and Methods:**
*Candida* species isolated from any site and for which susceptibility testing was performed were included in the analysis. Susceptibility rates were calculated for those species with ≥20 unique isolates and MIC$_{50}$ and MIC$_{90}$ values were determined for those species with ≥10 unique isolates. Susceptibility testing was performed according to the broth microdilution (BMD) method outlined in CLSI M27.

**Results:**
Fluconazole resistance increased for *C. albicans* (0/67, 1/210 [0.5%], 2/139 [1.4%] and 2/130 [1.5%]) and *C. glabrata* (0/22, 1/115 [0.9%], 0/81, 6/95 [6.7%]) from 2014, 2015, 2016 to 2017, respectively. Voriconazole resistance also steadily increased for *C. albicans* (0/130, 2/210 [1%], 2/139 [1.4%] to 3/130 [2.3%]). Micafungin susceptibility remained at 100% for *C. albicans* and *C. parapsilosis*, but resistance increased for *C. glabrata* (0/95, 0/115, 2/81 [2.5%], 2/95 [2.1%]). The MIC$_{90}$ values for *C. glabrata* notably increased for fluconazole (4ug/mL to 16ug/mL), voriconazole (0.12ug/mL to 0.5ug/mL) and posaconazole (0.25ug/mL to 1ug/mL) between 2014 and 2017, respectively. Azole MIC$_{90}$ values also increased for *C. parapsilosis* (voriconazole and posaconazole) and *C. tropicalis* (posaconazole and itraconazole) between 2015 and 2017.

**Conclusions:**
Overall, resistance appears to be increasing for some *Candida* species against both azoles and echinocandins. Continued surveillance will be important to determine if these resistance trends endure.
Title: Detection of Enteropathogens in Pediatric Patients with Acute Gastroenteritis: Comparison of Rectal Swabs and Stool Samples

Authors: Gandhi-Shah B¹, Zhi S¹, Qiu J¹, Parsons BD¹, Lee B², Pang X¹,³, Freedman SB⁴, Williamson-Urquhart S⁵, Farion KJ⁶, Gouin S⁷, Poonai N⁸, Hurley K⁹, Finkelstein Y¹⁰, Schuh S¹¹, Chui L¹,³ for the Pediatric Emergency Research Canada (PERC) PROGUT Study Group.

¹Departments of Laboratory Medicine and Pathology, ²Department of Pediatrics, University of Alberta; ³Provincial Laboratory for Public Health (Alberta Health Services), Edmonton, Alberta, Canada. ⁴Sections of Pediatric Emergency Medicine and Gastroenterology, Alberta Children's Hospital, Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary; ⁵Section of Pediatric Emergency Medicine, Alberta Children's Hospital, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada. ⁶Departments of Pediatrics and Emergency Medicine, Children’s Hospital of Eastern Ontario, University of Ottawa, Ottawa, ON, Canada. ⁷Division of Pediatric Emergency Medicine, Department of Pediatrics, CHU Ste-Justine, Université de Montréal, Montréal, Québec, Canada. ⁸Division of Emergency Medicine, London Health Sciences Centre, Department of Paediatrics, Schulich School of Medicine and Dentistry, Western University, London, ON, Canada. ⁹Division of Pediatric Emergency Medicine, IWK Health Centre, Halifax, NS, Canada. ¹⁰Divisions of Paediatric Emergency Medicine and Clinical Pharmacology and Toxicology, ¹¹Division of Paediatric Emergency Medicine and Research Institute, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada.

Background: Acute Gastroenteritis (AGE) is a major cause of morbidities and mortalities in pediatric patients worldwide. Current diagnostic test relies on the submission of stool samples. This study compared the performance of rectal swabs (RS) with stool samples (SS) in detecting enteropathogens in children with AGE enrolled in the Probiotic Regimen for Outpatient Gastroenteritis Utility of Treatment (PROGUT) study.

Methods: Between November 2013 and June 2017, children with AGE (3 months to 48 months old) were enrolled at six Canadian Pediatric Emergency Departments. RS collected in viral transport media and SS were frozen at -80 °C till testing was performed using the Luminex X-TAG Gastrointestinal Pathogen Panel (GPP) with 17 targets. Detection of enteropathogens in the two types of samples were compared.

Results: From 789 participants, 90% (713/789) RS and 41% (328/789) SS were collected and tested. Overall, 68% (485/713) RS and 67% (219/328) SS tested positive for one or more enteropathogens. Paired samples were collected from 32% (252/789) participants: 59% (150/252) participants had identical targets detected in both RS and SS; 7.5% (19/252) patients had different targets detected comparing the paired samples with some targets detected in SS only. Overall, 63% (160/252) RS and 67% (169/252) SS tested positive for at least one enteropathogen.

Conclusions: Rectal swab is a suitable alternative sample for the detection of enteropathogens in AGE.
**Title:** Formation and Occurrence of Iodinated Tyrosyl Dipeptides in Disinfected Drinking Water

**Authors:** Guang Huang, Ping Jiang, Lindsay K. Jmaiff Blackstock, Dayong Tian, Xing-Fang Li

**Background and Aim:**
Tyrosyl dipeptides are ubiquitous in surface water. Source waters have elevated iodide concentrations due to impacts by natural and anthropogenic drivers. Iodinated disinfection byproducts (I-DBPs) are highly toxic and can be easily formed during chloramination, used by many drinking water treatment plants to reduce the formation of regulated DBPs. Fewer studies have reported the precursors or occurrence of I-DBPs compared to chlorinated DBPs. A primary reason may be that iodinated organic compounds lack distinct isotopic patterns, making them more difficult to identify at low concentrations in authentic water.

Here, we aimed to develop a strategy that enables the identification of I-tyrosyl dipeptides resulting from specific peptides in drinking water.

**Material and Methods:**
Seven model dipeptides, tyrosylglycine (Tyr-Gly), tyrosylalanine (Tyr-Ala), tyrosylvaline (Tyr-Val), tyrosylhistidine (Tyr-His), tyrosylglutamine (Tyr-Gln), tyrosylglutamic acid (Tyr-Glu), and tyrosylphenylalanine (Tyr-Phe), reacted with freshly prepared monochloramine, and these reaction solutions were analyzed. The stability of these iodinated dipeptides was also evaluated. To enable sensitive detection of I-tyrosyl dipeptides in authentic water, we developed a high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) method with multiple-reaction monitoring (MRM) mode.

**Results:**
3-I-/3,5-di-I-tyrosyl dipeptides were identified as the major products. These I-tyrosyl dipeptides were synthesized and found to be stable in Optima water over 8 days. 3-I-/3,5-di-I-Tyr-Ala and 3-I-/3,5-di-I-Tyr-Gly were detected in authentic tap waters, but not in the raw waters. The corresponding precursors, Tyr-Ala and Tyr-Gly, were also detected in the same tap and raw water samples.

**Conclusions:**
This study demonstrates that iodinated dipeptides exist as DBPs in drinking water.
Title: Ascorbic Acid Assisted High Performance Liquid Chromatography Mass Spectrometry Differentiation of Isomeric C-chloro- and N-chloro- Tyrosyl Peptides

Authors: Ping Jiang, Guang Huang, Lindsay K. Jmaiff Blackstock, Jianye Zhang, Xing-Fang Li

Background and Aim:
Disinfection byproducts (DBPs) form unintentionally from the reaction between disinfectants and natural organic matter in raw water. Nitrogenous DBPs (NDBPs) are more toxic than corresponding carbonaceous DBPs but less frequently studied. Peptides are major components of organics in water and can readily react with chlorine to form NDBPs during chlorination. Both the amino group (N-) and side chains (C-) of a peptide are reactive sites which can yield a complex mixture of N-Cl- and C-Cl-peptides. Here we aim to develop a method to differentiate N-Cl- from C-Cl- peptides and assess their occurrence in water.

Material and Methods:
We chlorinated seven representative tyrosyl dipeptides to simulate the formation of chlorinated peptides in both model solution and raw water. A high performance liquid chromatography (HPLC) with high resolution mass spectrometry (MS) method was developed to detect the chlorinated dipeptides. The specific reductive nature of ascorbic acid towards N-Cl- was used to differentiate N-Cl- from C-Cl- peptides. Tandem MS was used to further illustrate the structure of chlorinated peptides.

Results:
We identified 4 N-Cl- and 2 C-Cl- products from each tyrosyl dipeptide. As chlorine dose increased, the number of chlorine substituents on the dipeptides increased. These chlorinated products are stable over 9 days.

Conclusions:
The chlorinated dipeptides can form during chlorination of raw water containing tyrosyl dipeptides. The ascorbic assisted HPLC-MS/MS method is effective for the differentiation of N-Cl- from C-Cl- isomers. This method can be utilized for the discovery of many other chlorinated peptide DBPs and for the study of their formation and occurrence in water.
Title: Metabolism of a Phenylarsenical Drug in Human Hepatic Cells and Identification of a New Arsenic Metabolite

Authors: Qingqing Liu, Elaine M. Leslie, Birget Moe, Hongquan Zhang, Donna N. Douglas, Norman M. Kneteman, and X. Chris Le

Background and Aim: Environmental contamination and human consumption of chickens could result in potential exposure to Roxarsone (3-nitro-4-hydroxyphenylarsonic acid), an organic arsenical that has been used as a chicken feed additive in many countries. However, little is known about the metabolism of Roxarsone in humans. The objective of this research was to investigate the metabolism of Roxarsone in human liver cells and to identify new arsenic metabolites of toxicological significance.

Material and Methods: Human primary hepatocytes and hepatocellular carcinoma HepG2 cells were treated with 20 or 100 μM Roxarsone. Arsenic species were characterized using a strategy of complementary chromatography and mass spectrometry.

Results: Roxarsone was metabolized to more than 10 arsenic species in human hepatic cells. A new metabolite was identified as a thiolated Roxarsone. The 24-hour IC50 values of thiolated Roxarsone for A549 lung cancer cells and T24 bladder cancer cells were 380 ± 80 and 42 ± 10 μM, respectively. This thiolated arsenic metabolite was more toxic than Roxarsone, whose 24-hour IC50 values for A549 and T24 cells were 9300 ± 1600 and 6800 ± 740 μM, respectively.

Conclusions: We have characterized the metabolism of Roxarsone in human liver cells and successfully identified a new phenylarsenic metabolite. The identification and toxicological studies of the new arsenic metabolite are useful for understanding the fate of arsenic species and assessing the potential impact of human exposure to Roxarsone.
Title: Designing Well-Defined Functional Monolith for Efficient Extraction of Peptides in Water

Authors: Zhongshan Liu, Guang Huang, Xing-Fang Li

Background and Aim:
The small peptides, which are hydrophilic and hard to deeply remove in drinking water, become known precursors for the formation of disinfection by-products (DBPs) with genotoxicity and cytotoxicity. Unfortunately, we still know little about how many kinds of peptides and potential DBPs in real water samples due to their complexity and very low recovery rate.

Herein, considering that peptides show zwitterionic feature, we designed a mixed-mode monolith to improve their recovery rate.

Material and Methods:
The monolith was prepared by sol-gel method and modification (Figure a), and then was cut and packed into syringe. For test, standard dipeptides solution flowed through monolithic SPE which was then eluted with methanol (5 wt% NH₃·H₂O). The contents of dipeptides in elution were determined by HPLC-MS/MS. The recovery rate is calculated by the ratio of dipeptide content in elution to that in stock standard solution.

Results:
Thanks to non-friability, the monolith was easily cut and packed into empty cartridge. Theoretically, the octadecyl and sulfonate groups were grafted like zebra stripes on the skeleton surface of monolith that features mixed modes. The recovery rates of four dipeptides on our SPE are 23.86% (Tyr-Gly), 50.71% (Tyr-Val), 55.55% (i-Tyr-Ala) and 44.76% (di-i-Tyr-Ala), respectively, which are dramatically improved comparing with commercial C18 and HLB SPE cartridges (Figure b-c).

Conclusions:
Based on the peptides characterization, we designed well-defined functional monolith and enhanced their recovery rate. It will be very helpful for identification of unknown peptides, as well as prediction of potential DBPs in real water samples.
Title: Cytotoxicity of halogenated tyrosine/tyrosylalanine and their toxicological mechanism

Authors: Dayong Tian a,b, Birget Moe a, Guang Huang a, Ping Jiang a, Xing-Fang Li a

a Division of Analytical and Environmental Toxicology, University of Alberta
b College of Chemical and Environmental Engineering, Anyang Institute of Technology

Background and Aim:
Tyrosine and tyrosylalanine exist in source water. They can readily react with free chlorine to form halogenated compounds in disinfected water (DBPs, disinfection byproducts). These halogenated chemicals were also found in human cells and might be major products of protein oxidation in human cells. The purpose of the study is to explore their cytotoxicity and toxicological mechanism.

Material and Methods:
In this study, we studied comparative cytotoxicity of halogenated tyrosine/tyrosylalanine on Chinese hamster ovary (CHO-k1) cells.

Results:
We found that 3-I-Tyr is more toxic than 3-Cl-Tyr, and 3,5-I,I-tyr-ala is significantly higher toxic than 3-I-Tyr-ala, indicating the substitution of diodo can significantly enhance the toxicity of halogenated tyrosine/tyrosylalanine, which is higher than some of EPA regulated HAAs. These chemicals yield time dependent toxicity. Second, we examined the extracts of Cl-/I-products generated by chlorination of 8 peptides with and without KI. The extracts with I− have higher cytotoxicity than that of chlorination of 8 peptides without I−. This shows that iodo-DBPs contribute to the higher cytotoxicity. Thirdly, we measured ROS generation by these DBPs, showing the disruption of intracellular redox homeostasis, related to GSH level, is a major contribution to the cytotoxicity. Finally, a QSAR model was developed and the toxicological mechanism was revealed, which make it possible to predict the cytotoxicity using physicochemical descriptors.

Conclusions:
The halogenated tyrosine/tyrosylalanine might yield higher cytotoxicity. A QSAR model was developed and the toxicological mechanism was revealed. This study provides a comprehensive insight into the toxicity of these emerging DBPs.
Title: Demethylation of N6-methyladenosine mediated by bicarbonate-activated hydrogen peroxide

Authors: Jinjun Wu, Xiang Zhou, X. Chris Le

Background and Aim:
N6-methyladenosine (m6A) represents a relatively abundant modification in eukaryotic RNA. This modification plays important roles in the regulation of gene expression. Recently, Fat mass-/obesity-associated proteins (FTO) and AlkB homologue 5 (AlkBH5) were found to be m6A demethylases, indicating a novel regulatory mechanism in mammalian cells. However, m6A has similar properties to adenosine and has a lower reactivity; and as a consequence, limited research has been focused on this nucleoside. The primary objective of this study was to develop a new chemical method enabling the oxidative demethylation of m6A by reactive oxygen species at the nucleobase level and in RNA strands.

Material and Methods:
m6A was treated with the H2O2/bicarbonate system. The products were purified using chromatography and characterized using high-resolution mass spectrometry, NMR and diphenyl-1-pyrenylphosphine (DPPP) fluorescence detection. The oligo RNA modified with m6A was analyzed using MALDI-TOF mass spectrometry. The demethylation ratio of Hela RNA was analyzed using LC-MS.

Results:
A new chemical method for the oxidative demethylation of m6A was revealed. Three intermediates, N6-hydroxymethyladenosine (hm6A), N6-formyladenosine (f6A), and N6-hydroperoxymethyladenosine (oxm6A), were characterized, and the \( \cdot \)OH radical mechanism underlying the decomposition was illustrated. We demonstrated that the ratio of the N6-methyladenosine to adenosine (m6A/A) was reduced by 11% in Hela RNA in vitro, suggesting that the demethylation reactions occurred.

Conclusions:
We have developed a novel chemical reagents-driven system for the effective oxidative demethylation of m6A and its N6-methyl analogues. The discovery of the radical demethylation mechanism and the abundant reactive oxygen species in the cells may offer new insight into the study of m6A.
Title: Ultra-sensitive discrimination of single nucleotide variations in circulating cell-free DNA using an exonuclease reaction mediated by a specific DNA terminal structure

Authors: Tongbo Wu, Wei Chen, Meiping Zhao and X. Chris Le

Background and Aim:
Sensitive detection of the single nucleotide variants in cell-free DNA (cfDNA) provides great opportunity for minimally invasive diagnosis and prognosis of cancer and related diseases. We aim to develop a novel strategy for quantification of cfDNA mutations at low abundance.

Material and Methods:
cfDNA was extracted from the plasma samples of patients. Single-stranded DNA (ssDNA) target with concerned gene mutation was obtained from the cfDNA after PCR and post-PCR treatment. A 5'-overhang fluorescent ssDNA probe was designed to hybridize with the ssDNA target and form a double-stranded DNA (dsDNA) that contained a two-nucleotide overhang structure at the 5'-end (5'-2-nt overhang). The dsDNA with this terminal structure served as the substrate of lambda exonuclease (λ exo). The enzymatic hydrolysis of this substrate released the fluorophore (FAM) from the quencher (BHQ-1), generating a fluorescence signal.

Results:
Our carefully designed probe hybridized with the mutant type target (MT) to form a 5'-2-nt overhang substrate of λ exo. Because the probe had one extra mismatch with the wild type (WT) sequence, the probe did not hybridize with the WT to form the 5'-2-nt overhang substrate. Therefore, the MT target generated much stronger fluorescence signal than the WT. The MT target could be detected at an abundance as low as 0.02%. The abundance of BRAFV600E mutation was measured to be 0.9±0.1% in the cfDNA of a thyroid cancer patient and the abundance of EGFR L858R mutation was measured to be 1.1±0.1% in the cfDNA of a lung cancer patient.

Conclusions:
We successfully developed a novel method enabling determination of trace amounts of mutations in target cfDNA from the plasma of thyroid and lung cancer patients.
Title: Poly(norepinephrine) based bi-enzyme biosensor for ultrasensitive multi-analyte determination

Authors: Chao Chen, Lanjunzi Liu, Qingji Xie, Xing-Fang Li

Background and Aim:
Discriminative detection of multiple analytes is very challenging and commonly requires bulky and sophisticated instrumentations. Electrochemical biosensors have attracted much attention because of their unmatched advantages. Most reported biosensors can only be utilized in the sensing of a single analyte, which is inefficient for real sample analysis and greatly limits the application of resultant biosensors. Thus the development of highly sensitive and discriminative techniques for detection of multi-analyte is important and meaningful for environmental monitoring.

Material and Methods:
The enzyme electrodes were prepared as illustrated in Scheme 1.

Results:
A high-performance amperometric biosensor was prepared by immobilizing HRP and GOx simultaneously with PNE polymeric biocomposites (PBCs) formed via HRP-catalyzed NE in the presence of H₂O₂ and realized the sensitively and selectively determination of glucose, H₂O₂, Cr(III) and Cr(VI) (shown in Table 1). The thus-prepared biosensors exhibit excellent performance in multi-analyte biosensing.

Table 1. Performances of proposed biosensors for glucose, Cr(VI), H₂O₂, and Cr(III) sensing.

<table>
<thead>
<tr>
<th>Detection</th>
<th>LOD</th>
<th>Sensitivity</th>
<th>Liner Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td>0.08</td>
<td>44.39</td>
<td>(0.5~4.18)×10³</td>
</tr>
<tr>
<td>(VI)</td>
<td>2.0×10⁻⁴</td>
<td>/</td>
<td>(0.5 ~ 6.0)×10⁻³</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>29</td>
<td>2.39</td>
<td>50 ~ 30.2</td>
</tr>
<tr>
<td>Cr(III)</td>
<td>0.1</td>
<td>/</td>
<td>0.10 ~ 3.8</td>
</tr>
</tbody>
</table>

Scheme 1. Illustration of the procedures for preparing biosensors and their work principles.

Conclusions:
We have successfully developed novel biosensors and applied the techniques to the detection of glucose, H₂O₂, Cr(III) and Cr(VI). The biosensors exhibit excellent performance for the detection of multi-analytes and have potential for further development in environmental monitoring.
Title: Nephrology Immersion - A Successful Natural Experiment

Authors: Ishita Moghe, Medical Resident 1, and Kim Solez

Background and Aim:
The story described in this abstract began nine years ago in January 2009 when Medical Resident 1 began working on Nephrology research tasks with the last author when she was a first year undergraduate student. It is a “natural experiment” because the scenario was not conceived of as an experiment until the present first author undergraduate student began doing similar Nephrology research tasks with the last author in January 2018 and decided to see if the work could be structured so she would learn Nephrology while doing it despite having no medical training. This “jumping into the deep end of the pool” immersion approach is quite different from the usual from-basics-to-specifics progressive acquisition of knowledge approach to medical education. A necessary component is that the student must “suspend belief” that she has truly mastered the subject until she is in medical school and learns the surrounding context of related subjects necessary to completely understand nephrology. However, if this sort of nephrology immersion education can succeed safely and comfortably, such students preloaded with Nephrology knowledge before medical school are a solution to the looming recruitment problems in Nephrology, because such students are very likely to choose a career in Nephrology as the path of least resistance in medical school. Medical Resident 1 has chosen a career in Nephrology. Immersion is an effective education technique in many other areas of human endeavour. It will probably also work here and have many advantages for the first author as she pursues a medical career.

Material and Methods:

Results:

Conclusions:
**Title:** Examination of the future scope and impact of human cell atlas technologies (individual cell RNA sequencing) on renal pathology and urinalysis

**Authors:** Ishita Moghe and Kim Solez

**Background and Aim:** Next-generation DNA- and RNA-sequencing technologies have been decreasing in cost and increasing in computing power at an impressive rate (Shapiro, Biezuner, & Linnarsson, 2013). This technology will allow us to distinguish between morphologically identical (but genetically and functionally distinct) cells (Regev et al., 2017), as well as examine the genetic components in ubiquitous kidney disorders, such as IgA nephropathy and Chronic Kidney Disease (CKD). Single-cell technology is now able to do individual cell RNA sequencing in quantities of up to a million cells at a time, which opens the door for whole-organism sequencing using the single-cell approach; this is the target of the Human Cell Atlas (HCA) initiative. We plan to describe the future scope and impact of this new technology on kidney biopsy interpretation and urinalysis. Villani et al. Science. 2017 356(6335) used single-cell RNA sequencing to gain a previously inaccessible view into human blood dendritic cells. The number of blood dendritic and monocytic cell types jumped from the traditional 6 to 12 in their study, which in itself is meaningful for kidney medicine due to the highly analogous nature of the white blood cell immune system and the kidney (both having 26 cell types traditionally). We predict that kidney cells will experience a similar increase from the 26 cell types currently classified to 52. The discovery and detection of new renal cell types will drastically affect the future of kidney pathology over the next decade, and it seems that this process has already begun, at least conceptually. https://youtu.be/oS3SzQ49A9A

**Material and Methods:**

**Results:**

**Conclusions:**