DRIvE 2024

Presented by UNIVERSITY OF ALBERTA | ALBERTA PRECISION LABORATORIES — Leaders in Laboratory Medicine

Friday April 26

DISCOVERY AND RESEARCH		
0800-0825	Breakfast and Registration	
0825-0830	Welcome	
Oral Research Presentations (Maple Leaf Room)		
0830-0845	Alam, Sarjana	Exploring new cryoprotectant agents to develop an optimized cryopreservation protocol for therapeutic regulatory T cells
0845-0900	Semeria Maitret, Tamara	Preliminary memory response profile of rubella-specific T cells: A pilot experiment
0900-0915	Wang, Huidong	Locked nucleic acid-assisted DNA catalytic reaction
0915-0930	Rais, Yasmine	Identification and quantification of human relaxin proteins by immunoaffinity-mass spectrometry
0930-0945	Tao, Jeffrey	Development of a DNAzyme walker for the detection of base excision repair enzymes within living cancer cells
0945-1000	Kasongo, Joelle	Investigating sex differences in first-time HIV antibody-positive patients receiving follow-up testing in Alberta, Canada
1000-1015	Break	
1015-1030	Trigg, Amberley	The effect of indole-3 propionic acid on potential pathobionts isolated from non-inflamed sections of pediatric patients with Inflammatory Bowel Disease
1030-1045	Jabilona, Kerrylei	Accurate Identification of Mycobacteria by the Bruker Sirius MALDI-TOF MS Instrument
1045-1100	Long, Stephanie	Investigation of new methods of cold agglutinin titre testing for Alberta Precision Laboratories
1100-1115	Feng, Wei	Understanding the reaction kinetics of CRISPR-Cas systems for sensitivity RNA detection
1115-1130	Enwere, Emeka	Using Machine Learning to Predict Oncotype DX Risk-of-recurrence Categories in Early-Stage Breast Cancer
1130-1145	Yin, Charles	CAR T Cell Exhaustion But Not Ex Vivo Cytotoxicity Is Predictive of Patient Clinical Response: An Interim Analysis of ACIT001/EXC002, a Phase Ib/II Trial of Decentralized Production of CAR T Cells for Treatment of Relapsed/Refractory Aggressive NHL and ALL
1145-1200	Mracek, JJ	Perceptions of Death in Autopsy Providers: A Qualitative Sociological Study
1200-1230	Poster Set Up and Poster Viewing (Aurora Room)	
1230-1330	Buffet Lunch (Maple Leaf Room)	

INNOVATION	
1330-1400	Unlocking the Potential of High Sensitivity Troponin: A Journey in in Implementation and Impact Dr. Albert Tsui, PhD
1400–1430	Recent advances in molecular cancer diagnostics in Alberta Dr. Soufiane El Hallani, PhD
1430-1500	Bringing innovative testing strategies to remote communities: APL's ultramarathon Dr. Kaila Crawford, Associate Medical Director, APL
1500-1510	Break
1510-1610	**DR. JOHN W. MACGREGOR MEMORIAL LECTURE** Spying On Cell Signaling And Metabolism With Genetically Encoded Biosensors Dr. Robert Campbell, University Of Tokyo
1610-1620	Oral Presentation Awards
1620-1830	Reception and Cash Bar

DRIvE 2024

Presented by UNIVERSITY OF ALBERTA | ALBERTA PRECISION LABORATORIES — Leaders in Laboratory Medicine

Saturday April 27

EDUCATION SESSIONS				
0830-0900	Registration And Breakfast			
0900-0945	Evergreen	Maple	Glacier	Prairie
	AP Year In Review Dr. Peter Dromparis - Updates in Colitis-associated Dysplasia	Industry Partner Presentation (Roche Diagnostics): Roche Vision for Innovation in Clinical Laboratory Medicine Dr. Mathieu Provençal	Clinical Mass Spectrometry in the context of Newborn Screening and Biochemical Genetics Grace Van Der Gugten	New Programs in LMP Graduate Studies: Transfusion and Molecular Specializations Chris Ward and Dr. Karen Matejka
0945-1030	Evergreen	Maple		Prairie
	AP Year In Review Dr. Jackie Chan - Thoracic Pathology update	Precision Cellular Therapies For Disease Treatment – The Role Of The Laboratory In Manufacturing Innovative Cell Therapy Products! Dr. Nicole Prokopishyn		Resume or CV - How can I make these documents competitive Dr. Renee Polziehn
1030-1045	Break			
1045-1130	Evergreen	Maple	Glacier	Prairie
	AP Year In Review Dr. Jodi Carter - Breast Pathology Update	New Lab Test: Fecal Calprotectin Dr. Dustin Proctor & Dr. Ola Ismail	Tracking Enteric Bacteria Infections in the 21st Century: Alberta's Perspective Dr. Linda Chui	A series of short topics: Stress refresher and EDI primer Lisa Purdy
1130-1230	Boxed Lunch with Poster Viewing and Judging (Aurora Room And Foyer)			
1230-1330	**DR R.E. BELL MEMORIAL LECTURE** Behavioral Economics, Automation, And Emotion In Health And Health Care Dr. David Asch, University Of Pennsylvania			
1300-1320	Minute To Win It Contest Winner Announcements			
1320-1335	Poster Presentation Awards, Grad Student Awards and Closing Remarks			

ALBERTA PRECISION LABORATORIES
Leaders in Laboratory Medicine

UNIVERSITY OF ALBERTA

DRIvE Days, HLA Workshop, April 26, 2024

All times in Mountain time zone

Time	Presenter	Title
8:25-8:30	Ben Adam	**General welcome to DRIvE Days (Main room, no Zoom)**
8:35-8:45	Anne Halpin University of Alberta, APL	**Welcome to HLA Workshop and introductions in the room** **Land Acknowledgement**
8:45-9:15	Kieran Halloran University of Alberta	**Crossing HLA antibody in Lung Transplantation: Teamwork Between the Clinical Program and the HLA Laboratory**
9:15-10:00	Karen Sherwood University of British Columbia	**Nanopore Technology: Coming to an HLA Laboratory Near You!**
10:00-10:20	**Coffee Break**	
10:20-10:40	Danielle Christian Transplant Laboratory South, APL	**Technologist Case Study: HSC Transplant Work Up Without a Matched Donor: The Role of the HLA Laboratory**
10:40-11:00	Melanie Thiessen Transplant Laboratory North, APL	**Technologist Case Study: Forget Me Not: What do the B Cells Remember**
11:00-11:30		**Vendor/Sponsor Updates**
11:30-12:30	Cathi Murphey Southwest Immunodiagnostics	**ABO Genotyping: A New Frontier in ABO Histocompatibility**
12:30-13:30	**Buffet Lunch (Maple Leaf Room)**	
1:30-2:15	Donna Veldhoen Transplant recipient and patient partner	**Transplant and Beyond from the Patient Perspective**
2:15-3:00	Anne Halpin University of Alberta, APL	**Right Under Our Noses: ASHI HLA Learning Resources**
3:10-4:10	**DR. JOHN W. MACGREGOR MEMORIAL LECTURE** **Spying On Cell Signaling And Metabolism With Genetically Encoded Biosensors** **Dr. Robert Campbell, University Of Tokyo** *Concurrent Program*	
4:10-4:20	**Awards for DRIvE Days Abstracts** *concurrent program*	
4:20-6:30	**Reception and Cash Bar**	

DRIvE 2024
Oral Research Presentations - April 26 2024

Time	Name	Title	Category
0830-0845	Alam, Sarjana	Exploring new cryoprotectant agents to develop an optimized cryopreservation protocol for therapeutic regulatory T cells	Summer Student/MSc
0845-0900	Semeria Maitret, Tamara	Preliminary memory response profile of rubella-specific T cells: A pilot experiment	Summer Student/MSc
0900-0915	Wang, Huidong	Locked nucleic acid-assisted DNA catalytic reaction	Summer Student/MSc
0915-0930	Rais, Yasmine	Identification and quantification of human relaxin proteins by immunoaffinity-mass spectrometry	PhD
0930-0945	Tao, Jeffrey	Development of a DNAzyme walker for the detection of base excision repair enzymes within living cancer cells	PhD
0945-1000	Kasongo, Joelle	Investigating sex differences in first-time HIV antibody-positive patients receiving follow-up testing in Alberta, Canada	PhD
1000-1015	Break		
1015-1030	Trigg, Amberley	The effect of indole-3 propionic acid on potential pathobionts isolated from non-inflamed sections of pediatric patients with Inflammatory Bowel Disease	MLS Student
1030-1045	Jabilona, Kerrylei	Accurate Identification of Mycobacteria by the Bruker Sirius MALDI-TOF MS Instrument	MLS Student
1045-1100	Long, Stephanie	Investigation of new methods of cold agglutinin titre testing for Alberta Precision Laboratories	MLS Student
1100-1115	Feng, Wei	Understanding the reaction kinetics of CRISPR-Cas systems for sensitivity RNA detection	Post Doctoral/ Resident
1115-1130	Enwere, Emeka	Using Machine Learning to Predict Oncotype DX Risk-of-recurrence Categories in Early-Stage Breast Cancer	Post Doctoral/ Resident
1130-1145	Yin, Charles	CAR T Cell Exhaustion But Not Ex Vivo Cytotoxicity Is Predictive of Patient Clinical Response: An Interim Analysis of ACIT001/EXC002, a Phase Ib/II Trial of Decentralized Production of CAR T Cells for Treatment of Relapsed/Refractory Aggressive NHL and ALL	Post Doctoral/ Resident
1145-1200	Mracek, JJ	Perceptions of Death in Autopsy Providers: A Qualitative Sociological Study	Post Doctoral/ Resident

Name: Sarjana Alam – Summer Student

Title:
Exploring new cryoprotectant agents to develop an optimized cryopreservation protocol for therapeutic regulatory T cells

Authors:
Sarjana Alam [1], Rebecca Mercier [2], Lavinia Ionescu [3], Lori West [1,3,4,5], Jason P. Acker [1,2], Esme Dijke [1,4,5,6]

[1]Dept. of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada
[2]PanTHERA CryoSolutions Inc, Edmonton, Alberta
[3]Dept. of Pediatrics, University of Alberta, Edmonton, AB, Canada
[4]Alberta Transplant Institute, Edmonton, AB, Canada
[5]Canadian Donation and Transplantation Research Program
[6]Alberta Precision Laboratories, Edmonton, AB, Canada

Background and Aim:
Regulatory T cell (Treg) tolerogenic therapy is a promising innovation for treatment of transplant rejection and autoimmune disorders. Defining optimal Treg cryopreservation conditions would facilitate successful clinical implementation. To develop an optimized protocol, we assessed toxicity and cryoprotection of various cryoprotectant agents (CPA).

Material and Methods:
Tregs were isolated from peripheral blood of healthy volunteers (n=3) and expanded in culture. Cells were suspended in: 1) base solution, 2) 10% dimethyl sulfoxide (DMSO) (*standard protocol*), 3) 5% DMSO, 4) 5% DMSO with 10 mM ice recrystallization inhibitors (IRIs), and 5) 5% DMSO with 5% dextran for 0-4 hours at 4°C, 22 °C and 37°C. Aliquots of each cell suspension were cryopreserved, thawed, and cultured. Recovery and viability were assessed by an automated cell counter, and phenotype by flow cytometry.

Results:
88-91% of cells had the CD4+CD25+FOXP3+ Treg phenotype, which was maintained throughout expansion. Cells suspended in 10% DMSO had lower recovery after 2 and 4 hours at 37°C only compared to other conditions. No clear viability differences were observed between 5% DMSO alone, with IRI, or with dextran (n=3). Immediately post-thaw, cells cryopreserved in 5% DMSO with 5% Dextran showed the highest recovery and viability compared to other conditions (n=2). After 48 hours in culture, no clear differences were observed between conditions.

Conclusions:
Decreased DMSO concentration may be key to improving Treg recovery after cryopreservation. Identifying the optimal CPA for Tregs will significantly advance development of an improved cryopreservation protocol for therapeutic Tregs.

Name: Tamara Amneris Semeria Maitret – MSc

Title:
Preliminary memory response profile of rubella-specific T cells: A pilot experiment

Authors:
Tamara A. Semeria Maitret[1], Sabrina Plitt[4] & Carmen Charlton[1-3*].

1 Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada
2 Provincial Laboratory for Public Health (ProvLab), Alberta, Canada
3 Canadian Blood Bank (CBS), Edmonton, Alberta, Canada
4 School of Public Health, University of Alberta, Edmonton, Alberta, Canada

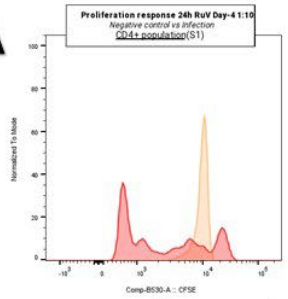
Background and Aim:
Declining antibody levels have been observed in highly vaccinated populations. In Canada, an increase in low antibody responders has been observed (15.9% in 2009-2012 to 19.9% in 2018/19). We aim to determine if memory proliferation response to rubella can be measured by flow cytometry to understand the implications of low-responders in herd immunity.

Material and Methods:
Memory CD4 and CD8 T-cells were isolated from healthy volunteers. Labeled cells were co-cultured with PBMCs (Peripheral Mononuclear Cells) previously infected with rubella virus (RuV) for 24h. Cells were recovered and stained with antibodies, viability dye, and measured by FACS (Fluorescence-activated Cell Sorting) between 4 and 8 days. Flow cytometry data was analyzed using FlowJo version 10.10.0.

Results:
Days 6, 7 and 8 showed the highest proliferation response in both T-cell populations (see Table 1), respectively. Despite CD4+ and CD8+ T-cell proliferation, the T-cell response to rubella is largely CD8+ driven when compared to negative control and 4-day of the assay (see Figure 1).

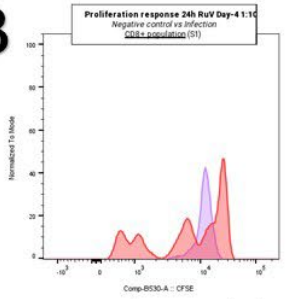
Conclusions:
After challenging T-cells with RuV, we demonstrated that the proliferation response of RuV-specific memory T-cells can be detected by flow cytometry. In addition, we identified that CD8+ T-cells mainly drove this response. It is still necessary to confirm these results with more experiments. In future, we can measure the proliferation response to have a better understanding of the immune status of low-antibody responders.

A



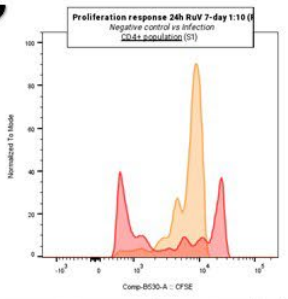
Sample Name	Subset Name	Count	Freq. of Total	Freq. of Parent
Timepoints 24h condition second part_4 day 24h rep2.fcs	Proliferation CD4+ Day 4 24h Neg C	119	0.091	2.02
24h RuV infection timepoint_24h Ruv 4-day 1-10.fcs	Proliferation CD4+ Day 4 24h 1:10	102	0.85	19.9

B



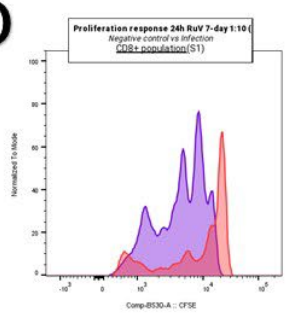
Sample Name	Subset Name	Count	Freq. of Total	Freq. of Parent
Timepoints 24h condition second part_4 day 24h rep2.fcs	Proliferation CD8+ Day 4 24h Neg C	77.0	0.059	3.99
24h RuV infection timepoint_24h Ruv 4-day 1-10.fcs	Proliferation CD8+ Day 4 24h 1:10	39.0	0.14	42.4

C



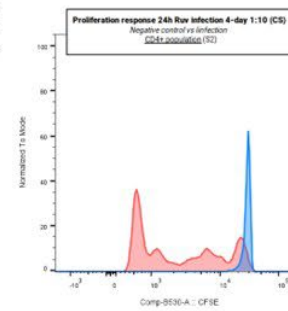
Sample Name	Subset Name	Count	Freq. of Total	Freq. of Parent
Timepoints 24h condition second part_7 day 24h rep 2.fcs	Proliferation CD4+ Day 7 24h Neg C	305	0.20	5.88
24h RuV infection timepoint_24h RuV 7-day 1-10.fcs	Proliferation CD4+ Day 7 24h 1:10	1152	2.76	57.2

D



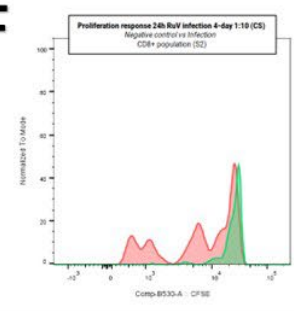
Sample Name	Subset Name	Count	Freq. of Total	Freq. of Parent
Timepoints 24h condition second part_7 day 24h rep 2.fcs	Proliferation CD8+ Day 7 24h Neg	244	0.16	14.4
24h RuV infection timepoint_24h RuV 7-day 1-10.fcs	Proliferation CD8+ Day 7 24h 1:10	434	1.05	62.7

E



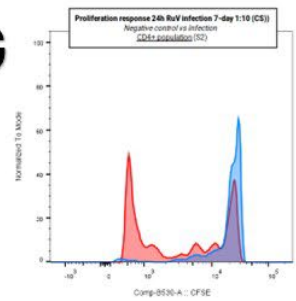
Sample Name	Subset Name	Count	Freq. of Total	Freq. of Parent
24h RuV inf 1-10--timepoints_24h Ruv 1-10 Day 4 CS.fcs	Proliferation CD4+ 24h RuV 1:10 4 day	462	0.55	9.31
Timepoints 24h condition second part_4 day 24h rep2.fcs	Proliferation CD4+ Day 4 24h Neg C	119	0.091	2.02

F



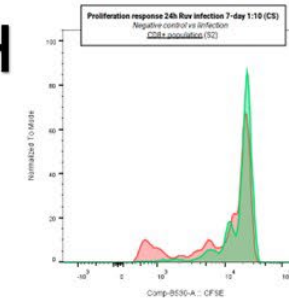
Sample Name	Subset Name	Count	Freq. of Total	Freq. of Parent
24h RuV inf 1-10--timepoints_24h Ruv 1-10 Day 4 CS.fcs	Proliferation CD8+ 24h RuV 1:10 4 day	233	0.26	14.3
Timepoints 24h condition second part_4 day 24h rep2.fcs	Proliferation CD8+ Day 4 24h Neg C	77.0	0.059	3.99

G



Sample Name	Subset Name	Count	Freq. of Total	Freq. of Parent
24h RuV inf 1-10--timepoints_24h Ruv 1-10 Day 7 CS.fcs	Proliferation CD4+ 24h RuV 1:10 7 day	835	0.66	14.0
Timepoints 24h condition second part_7 day 24h rep 2.fcs	Proliferation CD4+ Day 7 24h Neg C	208	0.33	5.74

H



Sample Name	Subset Name	Count	Freq. of Total	Freq. of Parent
24h RuV inf 1-10--timepoints_24h Ruv 1-10 Day 7 CS.fcs	Proliferation CD8+ 24h RuV 1:10 7 day	897	0.53	46.5
Timepoints 24h condition second part_7 day 24h rep 2.fcs	Proliferation CD8+ Day 7 24h Neg C	270	0.16	15.9

Figure 1. *Proliferation response of CD4+ and CD8+ T-cell populations 4-day and 7-day (n=2).* Sample 1 (A-D) histograms are separated into CD4+ (light orange) and CD8+ (lilac and purple) populations. Sample 2 (E-H) histograms are separated into CD4+ (light blue) and CD8+ (green) populations. Negative control histograms are red in all samples.

Table 1. *Proliferation response per timepoint.*

	Proliferation Response		
Timepoints (days)	S1✶ (%CD4+, %CD8+*)	S2✦ (%CD4+, %CD8+*)	Negative control (%CD4+, %CD8+*)
4	19.9, 42.4	9.31, 14.3	2.02, 3.99
5	26.9, 64.1	19.9, 36.9	3.94, 8.32
6	45.3, 82.6	15.3, 32.4	4.53, 13.2
7	57.2, 92.7	14.0, 46.9	5.74, 15.8
8	57.0, 92.9	39.7, 67.2	8.79, 27.6

*Percentage of CD4+ and CD8+ T-cell populations (Frequency of the parent – CD3+ population)

✶Sample 1

✦Sample 2

Name: Huidong Wang - MSc

Title:
Locked nucleic acid-assisted DNA catalytic reaction

Authors:
Huidong Wang, Jing Yang Xu, Hongquan Zhang

Background and Aim:
Noncovalent DNA catalytic reactions represent a recent advancement in DNA nanotechnology. Such reactions utilize cyclic toehold-mediated strand displacement reactions to produce multiple output DNA strands with only a single input nucleic acid strand. We aim to develop a simpler and more efficient noncovalent DNA catalytic reaction by improving the amplification efficiency and reaction kinetics.

Material and Methods:
We propose to enhance the binding energy of the fuel DNA by introducing locked nucleic acids (LNAs) within the fuel DNA toehold binding domain and branch migration domain, improving the amplification efficiency and reaction kinetics of noncovalent DNA catalytic reactions. To investigate and elucidate specific effects of LNAs on DNA catalytic reactions, we determined and compared the amplification efficiency and reaction kinetics.

Results:
Our findings demonstrate that the presence of LNAs enhances both the amplification efficiency and reaction kinetics. Moreover, the extent of enhancement is influenced by the LNAs number and location in the fuel DNA. Specifically, a higher number of LNAs correlates with increased amplification efficiency and faster reaction kinetics. We observed that LNAs located in the toehold binding domain play a more significant role in improving amplification efficiency compared to those in the branch migration domain. However, increasing the LNAs number in the fuel DNA also leads to a higher background signal.

Conclusions:
Our study shows the incorporation of LNAs into the fuel DNA enhances the amplification efficiency and kinetics of noncovalent DNA catalytic reactions. Future research will focus on comprehensively understanding the LNAs effects in the fuel DNA on both amplification efficiency and background generation.

Name: Yasmine Rais - PhD – Post candidacy

Title:
Identification and quantification of human relaxin proteins by immunoaffinity-mass spectrometry

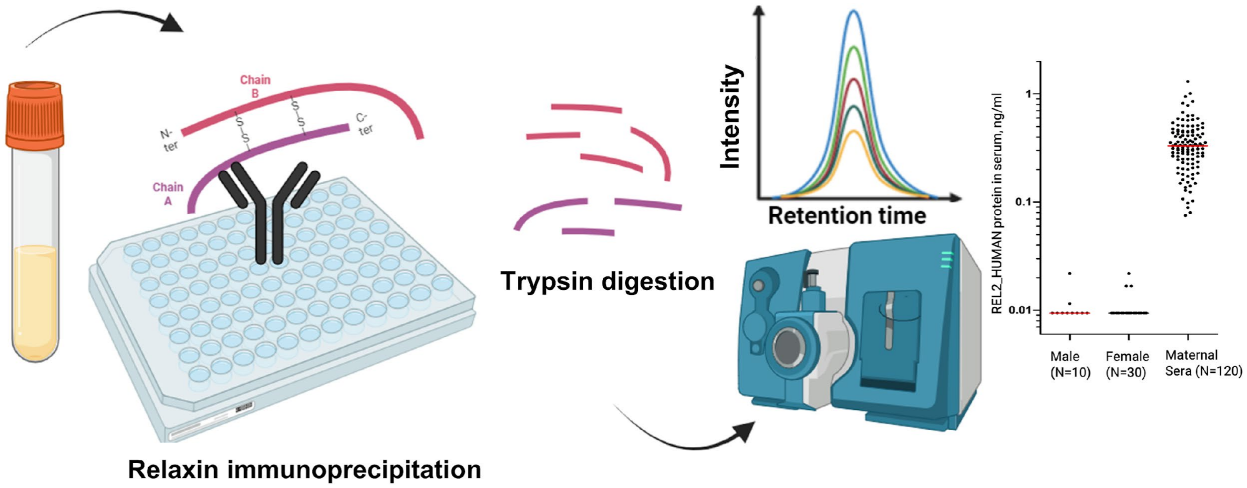
Authors:
Yasmine Rais and Andrei Drabovich

Background and Aim:
Relaxin genes belong to the Insulin/IGF/Relaxin superfamily of peptide hormones, and their physiological function is primarily associated with reproduction. Evaluation of the Human Protein Atlas revealed REL1 (RLN1 gene) as the only secreted protein which has never been evaluated as a prostate cancer marker. The literature review revealed limited and contradictory information about REL1 measurements. Our aim is to develop immunoaffinity-mass spectrometry (IA-MS) assays for human relaxins, reveal the identity and concentration of REL1 and REL2 in biological samples, and facilitate the evaluation of these proteins for diagnostic applications.

Material and Methods:
LNCaP prostate cancer cell lines were grown in RPMI-1640. RT-PCR was used to confirm gene expression. A sensitive and selective (IA-MS) assays were developed for relaxin (REL1/2) proteins. IA-MS assays were used for protein quantification. Relaxin protein was measured in 120 maternal sera samples.

Results:
Using RT-PCR, we confirmed expression of RLN1/2 transcripts in LNCaP. To investigate expression of endogenous REL1 and REL2, we developed and optimized an IA-MS assay (LOD 456.5 pg/ml and LOD 9.4 pg/ml, respectively). REL2 protein was quantified by IA-MS in blood serum, and a 21.8 pg/mL cut-off level unambiguously distinguished maternal sera (median 331 pg/mL) from the control female and male sera (median at assay LOD of 9.4 pg/mL). A biphasic expression of REL2 across the gestational weeks was observed. IA-MS assays discovered potential cross-reactivity and false-positive measurements for relaxin immunoassays.

Conclusions:
High selectivity and sensitivity of IA-MS will facilitate quantification of endogenous REL2 in clinical samples, paving the way to its comprehensive evaluation as a disease marker.



Name: Jeffrey Tao - PhD – Post candidacy

Title:
Development of a DNAzyme walker for the detection of base excision repair enzymes within living cancer cells

Authors:
Tao, Jeffrey., Zhang, Hongquan., Weinfeld, M., Le, X. Chris.

Background and Aim:
The DNAzyme walker is a nanomachine consisting of a walking DNA that traverses a track of DNA. The walking DNA steps on and off the DNA track through base pairing, fueled by DNAzyme-catalyzed hydrolysis (Figure 1A). When constructed onto gold nanoparticles (AuNPs), the DNAzyme walker can be delivered into living cancer cells and activate in response to intracellular biomarkers. We aimed to develop a DNAzyme walker that targets enzymes of the base excision repair (BER) pathway, which enhance the longevity and therapeutic resistance of cancers.

Material and Methods:
We conjugated hundreds of fluorophore-labelled track strands and multiple DNAzymes onto each 20nm-wide AuNP in solution. We then used a fluorometer to monitor the detection of apurinic/apyrimidinic endonuclease 1 (APE1), uracil DNA glycosylase (UDG), and single-stranded selective monofunctional uracil DNA glycosylase (SMUG1) in buffer and cell lysate. Lastly, we observed the intracellular detection of APE1 within living HeLa cells using confocal microscopy.

Results:
The limits of detection (LOD) of the DNAzyme walker for the BER enzymes were: APE1 – LOD_{buffer}=160 fM, LOD_{lysate}= 1 cell/100μL; UDG – LOD_{buffer}=3.2 pM, LOD_{lysate}=48 cells/100μL; SMUG1 – LOD_{buffer}=3.0 pM. APE1 was successfully detected in living HeLa cells after application of the DNAzyme walker (Figure 1B)

Conclusions:
Our research demonstrates that DNAzyme walker technology can monitor and respond to enzyme activity and disease processes such as excessive DNA repair. The DNAzyme walker has the potential to deliver chemotherapies into cancer cells while bypassing normal cells. Because upregulated BER enzymes are prevalent in many cancers, our DNAzyme walker can be activated within a diversity of cancer types.

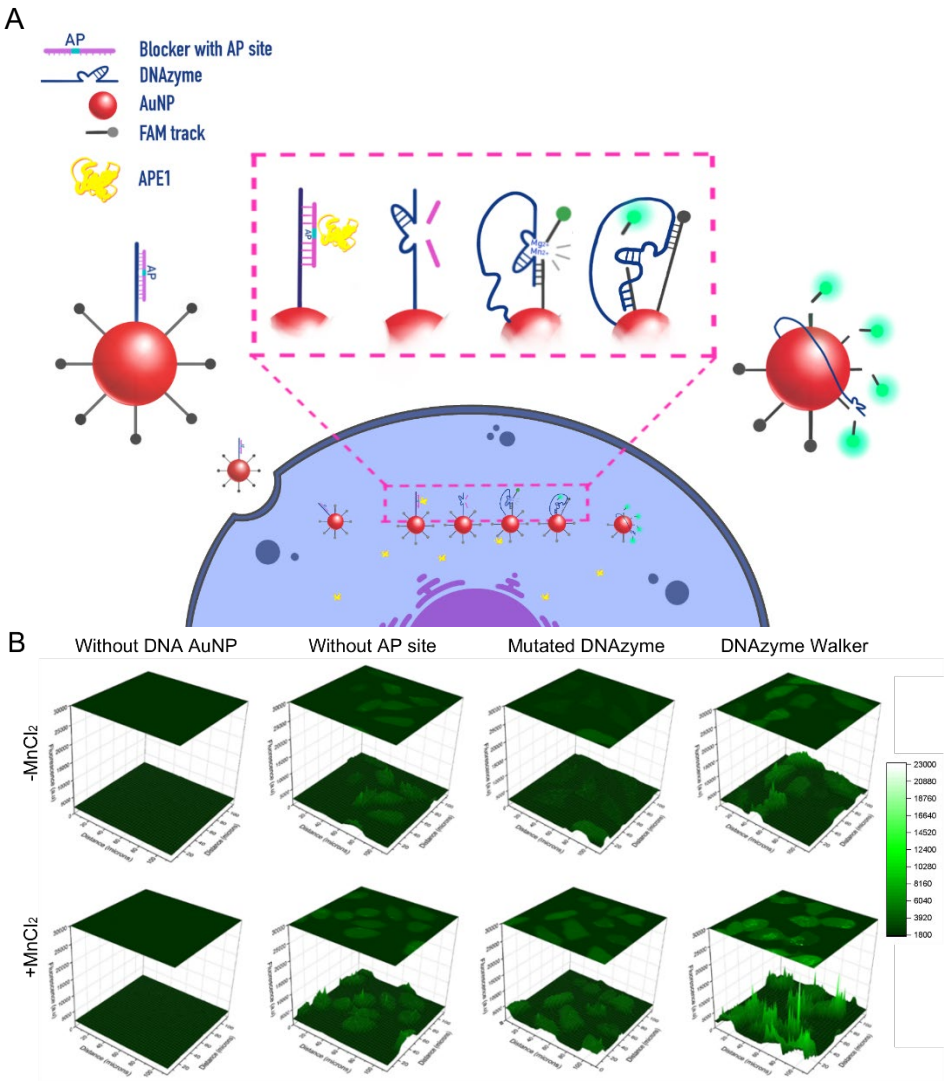


Figure 1 Detection of APE1 in living cancer cells with the DNAzyme walker. (A) Schematic of DNAzyme walker activation and operation. (B) Confocal images of APE1 detection in live HeLa cells.

Name: Joelle Kasongo - PhD – Pre candidacy

Title:
Investigating sex differences in first-time HIV antibody-positive patients receiving follow-up testing in Alberta, Canada

Authors:
N. Joelle Kasongo[1,2], L. Alexa Thompson[1, 2, 3], William Stokes[1,5,7], Sabrina S. Plitt[6], Carmen L. Charlton[1,2,3,4,7]

[1]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB
[2]Li Ka Shing Institute of Virology, University of Alberta, Edmonton, AB
[3] Women and Children's Health Research Institute (WCHRI), University of Alberta, Edmonton, AB
[4]Canadian Blood Services (CBS), Edmonton, AB
[5]Division of Infectious Diseases, Department of Medicine, University of Alberta, Edmonton, AB
[6]School of Public Health, University of Alberta, Edmonton, AB
[7]Alberta Precision Laboratories, Edmonton, AB

Background and Aim:
Diagnosis and linkage-to-care are vital components of the current HIV care continuum. Retaining patients in care prevents long-term adverse health outcomes. We aimed to compare sex differences in turnaround time (TAT) for first-time HIV antigen/antibody (Ag/Ab) positive patients being linked to Nucleic Acid Test (NAT) follow-up testing.

Material and Methods:
Patients between 2018 and 2023 who received HIV testing (Ag/Ab immunoassay, Ab differentiation, NAT) were analyzed to assess sex differences in HIV follow-up practices. Female and male patients positive for HIV Ag/Ab were used to investigate proportions of individuals receiving NAT follow-up testing and calculate TAT. Sex-stratified statistical analyses were performed using Chi-Square-test and two-sample t-test in STATAv17.

Results:
1,599,356 patients with sex variable identified were tested for HIV in Alberta. Of those, 558,181(57.8%) female and 317,222 (50.0%) male patients were screened for HIV Ag/Ab for the first time, with 471 (0.08%) females and 352 (0.11%) males testing positive by HIV Ag/Ab. 142 (30.1%) females received follow-up testing within 6 months of positive diagnosis, compared to 202 (57.4%) males. Follow-up testing was highest in female and male patients aged 41-50 years (33.1%, p=0.05; 30.7%, p=0.005 respectively). Of those receiving follow-up testing, mean TAT for females was 64 days (95% CI 55.4-72.5) compared to 66 days (95% CI 58.4-74.1) for males, (p<0.001).

Conclusions:
A higher proportion of first-time positive HIV Ag/Ab were female, however, a lower proportion are receiving follow-up testing. Interventions to focus on retaining female patients in the HIV care continuum remain a priority.

Name: Amberley Trigg – MLS Student

Title:
The effect of indole-3 propionic acid on potential pathobionts isolated from non-inflamed sections of pediatric patients with Inflammatory Bowel Diseases

Authors:
Amberley Trigg[1, 2], Nazanin Arjomand Fard[1, 3], Christopher Cheng[1, 4], Deenaz Zaidi[1, 4], Eytan Wine[1, 3, 4]

[1] CEGIIR, University of Alberta
[2] Department of Laboratory Medicine and Pathology, University of Alberta
[3] Department of Physiology, University of Alberta
[4] Department of Pediatrics, University of Alberta

Background and Aim:
Indole-3 propionic acid (IPA), is a tryptophan metabolite shown to ameliorate inflammation, intestinal integrity, and microbial dysbiosis. This study examined the effect of 1.0 mM IPA *in vitro*, above the physiologic concentration of 1-10 µM IPA. We hypothesized that a higher concentration of IPA will decrease bacterial invasion, improve tight junctions, and decrease pro-inflammatory cytokine production.

Materials and Methods:
Klebsiella variicola and *Klebsiella pneumoniae*, isolated from the uninflamed bowel of pediatric ulcerative colitis patients were used to infect Caco-2 cells pre-treated with 1.0 mM IPA. Gentamicin protection assay assessed bacterial invasion, transepithelial electrical resistance (TEER) evaluated intestinal integrity, quantitative polymerase chain reaction (qPCR) quantified gene expression and enzyme-linked immunosorbent assay (ELISA) quantified protein production of the pro-inflammatory chemokines and cytokines including interleukin (IL)-8, and tumor necrosis factor alpha (TNF-α), as well as the anti-inflammatory cytokine IL-10.

Results:
IPA treated *K. variicola* increased invasion, while IPA did not affect *K. pneumoniae* invasion. TEER values were not significantly different between IPA and no IPA groups. Gene expression of IL-8, IL-10, and TNF-α was significantly decreased for *K. variicola* infection treated with IPA. IPA increased TNF-α protein production, but did not affect IL-8, and IL-10 production with *K. variicola* infection.

Conclusions:
High IPA concentration yields variable effects on the intestinal barrier, necessitating further investigation. Notably, 1.0 mM IPA increased *K. variicola* invasion and decreased pro-inflammatory cytokine expression. Future work will focus on biofilm formation and adhesion experiments, to further examine IPA's effect on bacterial invasion potential.

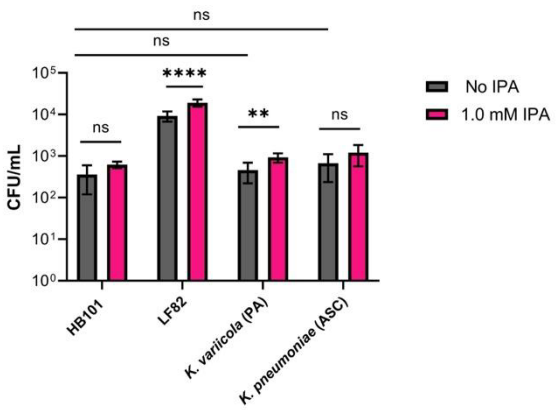


Figure 1: Gentamicin protection assay results

Name: Kerrylei Jabilona – MLS Student

Title:
Accurate Identification of Mycobacteria by the Bruker Sirius MALDI-TOF MS Instrument

Authors:
Kerrylei Jabilona, Andrea Jennings, Andrina Trifonov, Cary Shandro, Brenda Beaudin, Natalie Marshall

Background and Aim:
MALDI-TOF is a type of mass spectrometry that can rapidly identify microorganisms such as mycobacteria. At ProvLab, all identifications of mycobacteria are currently performed on one MALDI-TOF instrument. This project aims to evaluate the Bruker Sirius, a new MALDI-TOF instrument, to determine whether it can identify mycobacteria to meet both the accuracy and reproducibility standards required of a clinical laboratory.

Materials and Methods:
To assess accuracy, 532 clinical isolates were prospectively analyzed on the Bruker Sirius and reference instrument in parallel. Results were compared between instruments to determine if the identifications match and were reliable, which is indicated through a Bruker score. To evaluate reproducibility, 156 known isolates were repeatedly tested on the Bruker Sirius.

Results:
300 isolates were analyzed and accurate identifications were obtained for 28 taxonomic groups. Overall, the Bruker Sirius accurately identified over 99% of all isolates tested. Ten taxonomic groups successfully met clinical verification standards, encompassing over 30 species associated with human disease. In this context, the Bruker Sirius was able to provide a valid identification for 72% of all clinical isolates received for testing in Alberta over the course of this study.

Conclusions:
The Bruker Sirius demonstrates excellent accuracy when identifying diverse groups of mycobacteria. Results generated from the instrument can be used to identify patient samples, but only for verified organisms. With continued testing, more species can be verified to further enhance the utility of the Bruker Sirius. Ultimately, ProvLab is well on its way to achieving a significantly more robust pipeline for the rapid diagnosis of mycobacterial infections.

Figure 1: Successful Identifications by the Bruker Sirius in Comparison to the Reference MALDI-TOF Instrument.

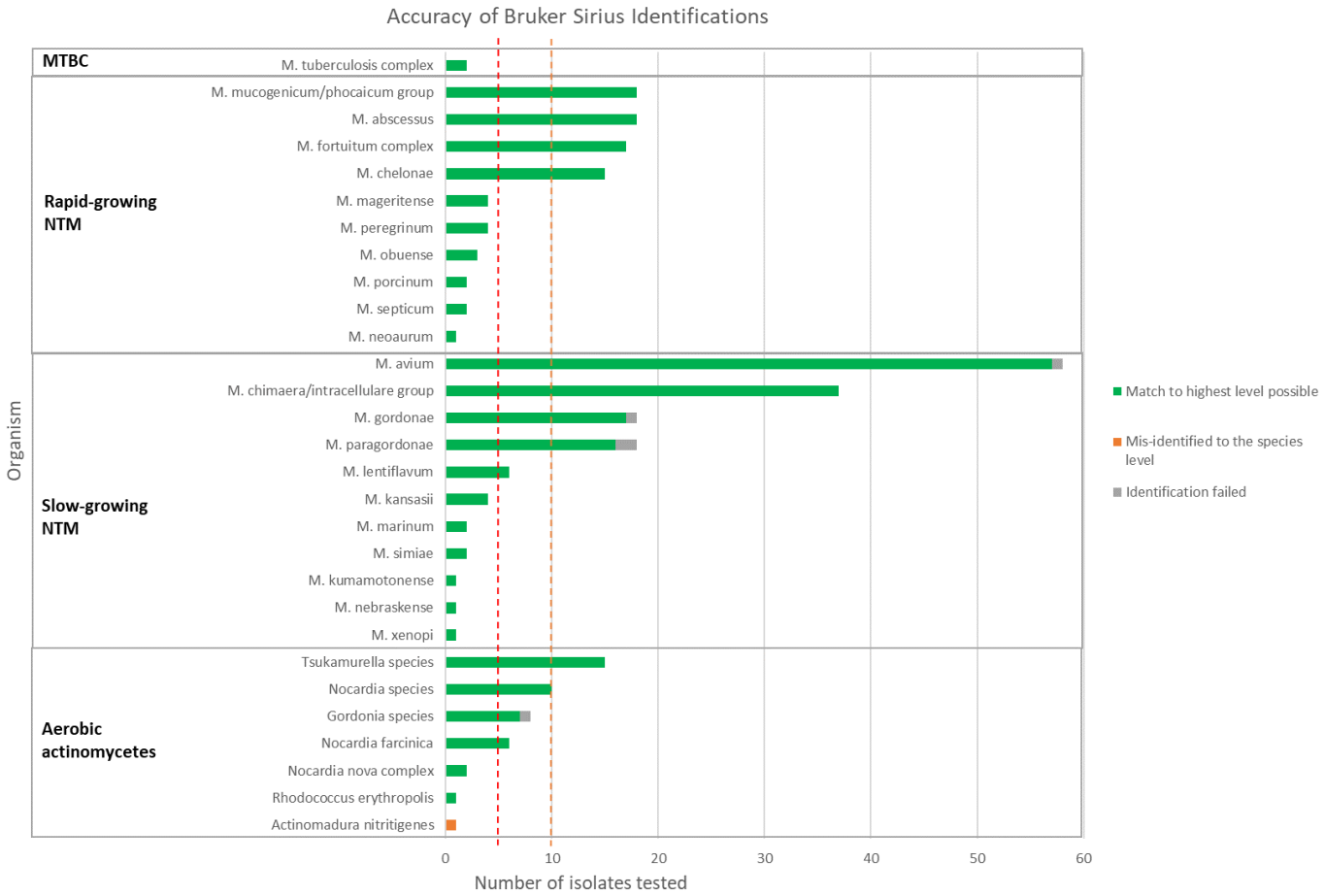


Figure 1. MTBC – *Mycobacterium tuberculosis* complex, NTM – non-tuberculous mycobacteria. The red dotted line indicates the minimum number of successful identifications required for a species to meet clinical verification standards. The orange dotted line indicates the minimum number of successful identifications required for a complex or group to meet clinical verification standards.

Table 1: Summary of Successful Identifications by the Bruker Sirius in Comparison to the Reference MALDI-TOF Instrument.

Category	n (isolates)	Match to highest level possible		Match to genus level only		Identification failed	
		Percentage	Number of isolates	Percentage	Number of isolates	Percentage	Number of isolates
MTBC	2	100%	2	0	0	0	0
Rapid-growing NTM	84	100%	84	0	0	0	0
Slow-growing NTM	148	97%	144	0	0	3%	4
Aerobic actinomycetes	45	96%	43	2%	1	2%	1
Total	**269**	**98%**	**263**	**0%**	**1**	**2%**	**5**

MTBC – *Mycobacterium tuberculosis* complex, NTM – non-tuberculous mycobacteria

Name: Stephanie Long – MLS Student

Title:
Investigation of new methods of cold agglutinin titre testing for Alberta Precision Laboratories

Authors:
Stephanie Long, Bruce W Lyon, Rosalyn Doepker, Dr. Hanan Gerges, and Dr. Bryan Tordon

Background and Aim:
Cold agglutinin titre testing is a diagnostic test for cold agglutinin disease. Currently, Alberta Precision Laboratories (APL) uses a tube method to quantitate cold agglutinin titres, however, this method is time-consuming and produces large variability. Limited research has been performed on improving the cold agglutinin titre method. The objective of this study is to determine whether a gel method of titre testing produces equivalent results to the current tube method.

Material and Methods:
This study was performed at the University of Alberta Hospital. 13 EDTA samples with positive cold agglutinin screen results were selected to test against the current tube method and a gel method using ORTHO buffered gel cards incubated at 4°C and 20°C. Titre results from the tube method and gel methods were compared using concordance, Cohen's Kappa, and the Wilcoxon signed-rank test to establish equivalency of the methods.

Results:
The gel method incubated at 4°C and 20°C both produced significantly different titre results from the tube method (4°C, $P < .0001$; 20°C, $P < .0001$). The overall agreement between the tube and the gel method incubated at 4°C was fair ($k = 0.21$) and concordance between methods was 13%. The overall agreement between the tube and the gel method incubated at 20°C was none to slight ($k = 0.11$) and concordance between methods was 10%.

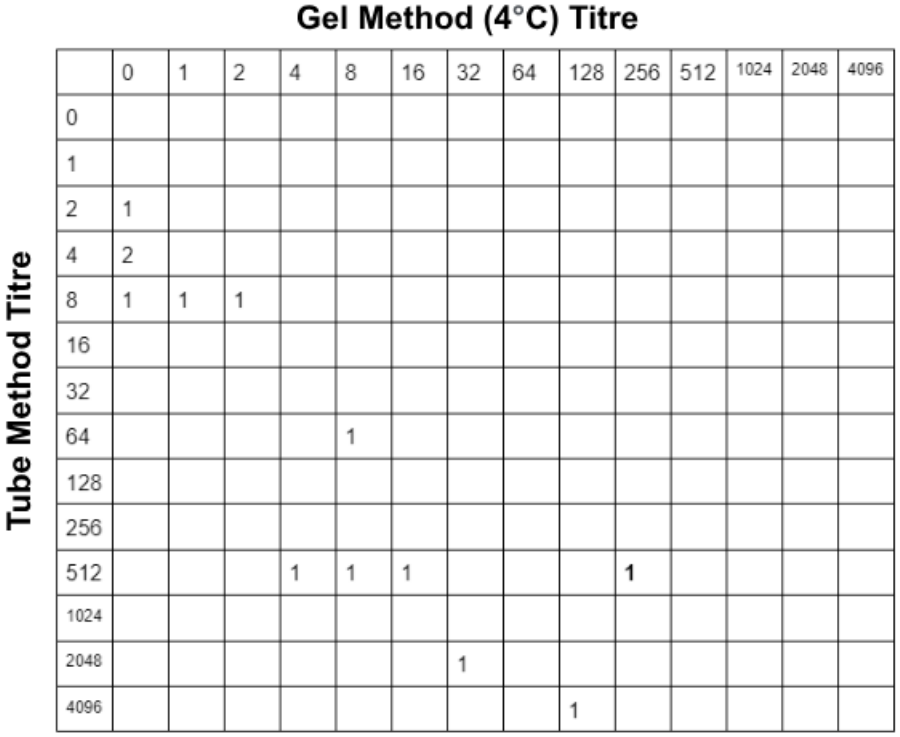
Conclusions: This study's data suggests that gel method results are not equivalent to the current tube method for cold agglutinin titre testing.

Table 1. Comparison of cold agglutinin titres between the tube method and the gel method incubated at 4°C

	Concordance, n (%)				Discordance, n (%)		
Antibody (n)	Total	TM=GM	TM>GM	TM<GM	TM>GM	TM<GM	Kappa (95% CI)
Anti-I Auto (13)	1(0.08)	0(0.00)	1(0.08)	0(0.00)	12(0.92)	0(0.00)	0.24 (0.08-0.40)
Anti-I Group O (13)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	13(1.00)	0(0.00)	0.36 (0.20-0.52)
Anti-i Cord (13)	4(0.31)	1(0.08)	2(0.15)	1(0.08)	8(0.61)	1(0.08)	0.02 (-0.14-0.18)

Concordance is considered ±1 titre difference between methods. The overall concordance rate between TM and GM was 13% and the overall agreement was fair (k = 0.21). Cohen's Kappa is interpreted as follows: ≤ 0, no agreement; 0.01-0.20, none to slight; 0.21-0.40, fair; 0.41-0.60, moderate; 0.61-0.80, substantial; 0.81-1.00, almost perfect.
Abbreviations: TM, tube method; GM, gel method; CI, confidence interval; n, number

Figure 1. Comparison of auto anti-I cold agglutinin titres between the tube method and gel method incubated at 4°C



The numbers indicate the total number of samples with the corresponding titre results for each method. Values with concordant titres (±1 titre step difference between methods) are bolded. A significant difference is observed for the titre results between the tube method and gel method incubated at 4°C using the Wilcoxon signed-rank test (P = .001, Z = -3.19).

Name: Wei Feng - Post Doctoral Research Fellow

Title:
Understanding the reaction kinetics of CRISPR-Cas systems for sensitivity RNA detection

Authors:

Wei Feng, Hongquan Zhang, and X. Chris Le

Background and Aim:
The discovery and characterization of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems with *trans*-cleavage activity led to the rise of CRISPR-based diagnostics. The performance of CRISPR-based assays depends on the reaction kinetics of the CRISPR-associated (Cas) systems. CRISPR-Cas13a is very useful for RNA manipulation and detection due to its function of RNA targeting and cleavage. Cas homologs, crRNA sequences, targets, reporters, co-factors, and reaction temperature affect the performance of CRISPR-Cas13a system, but the detailed contribution of such factors has not been fully studied. Our research aims at understanding the kinetics of reactions involving CRISPR nuclease, and developing CRISPR techniques for analytical applications.

Material and Methods:
We systemically studied the kinetics of different reaction steps of CRISPR-Cas13a system. We studied the contribution of different factors to the reaction kinetics of CRISPR-Cas13a. We also studied the compatibility of the CRISPR-Cas system with isothermal amplification techniques and the stability of crRNA-Cas ribonucleoproteins.

Results:
We established a method for kinetics measurement, and unraveled the reaction kinetics of CRISPR-Cas13a operation. The improved understanding contributed to the development of a sensitive, homogenous, fluorescence assay for the detection of microRNA using Cas13a alone, without nucleic acid amplification. We have also overcome the problem of insufficient sensitivity of stand-alone CRISPR-Cas assays for pathogen detection. We developed a strategy to integrate reverse transcription (RT), recombinase polymerase amplification (RPA), and CRISPR-Cas12a nuclease reactions into a single tube, resulting in the sensitive detection of SARS-CoV-2.

Conclusions:
We demonstrated the importance of kinetics understanding in CRISPR-based assay development.

Name: Emeka Enwere - Resident

Title:
Using Machine Learning to Predict Oncotype DX Risk-of-recurrence Categories in Early-Stage Breast Cancer

Authors:
Emeka K. Enwere, Gilbert Bigras, and David Beyer

Background and Aim:
Gene expression assays such as Oncotype DX are useful clinical decision-making tools which help identify patients with early-stage, hormone-positive breast cancer who are at high risk of disease recurrence, and who are thus most likely to benefit from adjuvant chemotherapy. Given that these assays are typically time-consuming and very expensive, there remains an unmet need for fast, low-cost methods to reliably risk-stratify at least a subset of patients without need for expensive gene expression testing. We hypothesized that a machine-learning model, trained on H&E slide images and Oncotype DX risk-of-recurrence (ROR) categories, could determine ROR categories when provided with images alone.

Material and Methods:
The study cohort consisted of 137 patients with breast cancer who received Oncotype DX testing. Ten H&E image tiles from each of 115 patients, as well as their matched Oncotype DX high- or low-risk categories, were used to train and validate an image-classification model using Google VertexAI. The model was tested on 22 cases; a case was scored as “high” or “low” if the model categorized more than half of the 10 tiles per case as high or low ROR, respectively.

Results:
The model correctly assigned 100% of low ROR cases, and 44% of high ROR ($p = 0.015$, chi-squared test). One case was indeterminate and was not scored. The overall accuracy was 76%. A review of the image tiles as marked-up by the model indicated that tumor nuclear features were most significant in categorization decisions.

Conclusions:
Our model demonstrated good ability to identify ROR categories in breast cancer based on H&E image data alone, even in a small dataset. Future studies would incorporate other clinicopathological characteristics into the ROR category prediction.

Name: Charles Yin - Resident

Title:
CAR T Cell Exhaustion But Not *Ex Vivo* Cytotoxicity Is Predictive of Patient Clinical Response: An Interim Analysis of ACIT001/EXC002, a Phase Ib/II Trial of Decentralized Production of CAR T Cells for Treatment of Relapsed/Refractory Aggressive NHL and ALL

Authors:
Charles Yin[1,2], Bindu Thapa[1], Carina Debes-Marun[1], Zackariah Breckenridge[3], Irwindeep Sandhu[1], Faisal M. Khan[4,5] and Michael P. Chu[1]

[1]Department of Oncology, University of Alberta, Edmonton, AB, Canada; [2]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada; [3]Clinical Trials Program, University of Calgary, Calgary, AB, Canada; [4]Department of Pediatrics, University of Calgary, Calgary, AB, Canada; [5]Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, AB, Canada

Background and Aim:
Chimeric antigen receptor (CAR) T cell therapy is a next-generation immunotherapy used in both hematologic and solid tumor malignancies. Multiple CAR T products have received regulatory approval for use as treatment in relapsed and refractory leukemias and lymphomas. However, approximately half of these patients do not respond to therapy, and we are still unable to reliably predict which patients will be responders.

Material and Methods:
In this study, we analyzed the CAR T cells from patients enrolled in ACIT001/EXC002, a Canadian phase Ib/II single-arm clinical trial of decentralized production of second-generation CD19/41BB/CD3z CAR T cells for treatment of multiply relapsed/refractory non-Hodgkin lymphoma (NHL) and acute lymphoblastic leukemia (ALL). CD3-positive T cells and CAR T products were comprehensively immunophenotyped by flow cytometry. The capacity for manufactured CAR T cells to kill tumor cells was assessed using an *ex vivo* cytotoxicity assay. CAR T cell immunophenotype and cytotoxic potential were correlated with clinical outcomes including response to therapy, progress-free survival (PFS) and overall survival (OS).

Results:
To date, 29 patients have been enrolled, of which 20 had an aggressive NHL while the remaining 9 had been diagnosed with B cell ALL. Mean age of participants at time of enrollment was 56 (range: 26-77) and 65.5% were male. A majority of patients achieved a complete response as evaluated by PET scan or bone marrow biopsy on day 28 post-CAR T cell infusion with only 3 patients showing disease progression, 2 patients achieving only partial response, and 3 patients being unevaluable. We were able to evaluate PFS and OS at 6 months in 19 out of 29 patients, with 42.1% of patients experiencing progression and/or mortality within this period. Surprisingly, while CAR T cell cytotoxicity *ex vivo* trended higher in patients that achieved a complete metabolic response compared to those with partial response or disease progression, this did not reach statistical significance ($p = 0.280$). However, expression of T cell exhaustion markers such as PD-1 CAR T products was found to be significantly increased in non-responders ($p = 0.010$).

Conclusions:
In this analysis, we found that CAR T cell exhaustion rather than cytotoxic activity is correlated with response to treatment. Our data indicate that CAR T cell exhaustion and persistence following infusion rather than cytotoxic potential is a key predictor for patient response to treatment.

Table 1. Patient demographics.

Demographic		DLBCL (n=20)	B-ALL (n=9)	
Age (range)		58.8 (41-77)	51.0 (26-66)	p = 0.355
Sex (%)	Male	13 (63.2 %)	6 (75.0 %)	p = 0.551
ECOG status	0	3	0	
	1	12	8	p = 0.137
	2	5	1	
Relapse status	Primary refractory	12	1	
	Relapsed (<12 months)	3	3	p = 0.881
	Relapsed (>12 months)	5	5	
Number of prior therapies	2	14	5	
	3	4	4	p = 0.482
	4+	2	0	
Prior SCT	Yes	9	5	p = 0.901
	No	11	4	
Stage	2	1	n/a	
	3	4	n/a	
	4	16	n/a	
R-IPI score	1	2	n/a	
	2	6	n/a	
	3	9	n/a	
	4	3	n/a	
Pathology	GCB	10	n/a	
	Non-GCB	7	n/a	
	Unknown	3	n/a	
MYC status	Positive	3	n/a	
	Negative	13	n/a	
	Unknown	4	n/a	
Ph status	Positive	n/a	1	
	Negative	n/a	8	
MRD status	Positive	n/a	4	
	Negative	n/a	3	
	Equivocal	n/a	2	
Extramedullary disease	Yes	n/a	5	
	No	n/a	4	

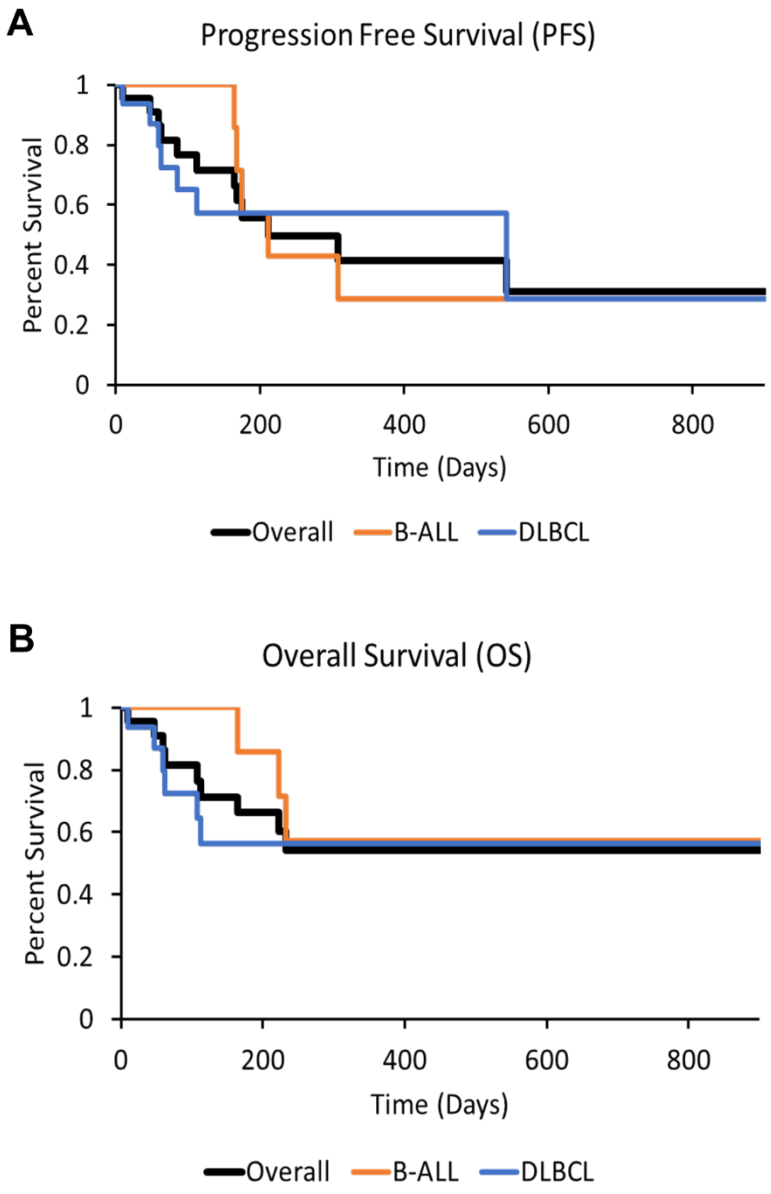


Figure 1. Long-term patient outcomes following CAR T infusion. Kaplan-Meier curves showing that approximately one-third to one-half of patients experience long-term disease remission as measured by both **A**) progression-free survival (PFS) and **B**) overall survival (OS). There is no statistically significant difference in PFS or OS between the B-ALL and DLBCL sub-groups using a log rank test.

Name: JJ Mráček - Resident

Title:
Perceptions of Death in Autopsy Providers: A Qualitative Sociological Study

Authors:
Dr. J.J. Mráček, Dr. Steven Kleinknecht, Dr. Khaldoon Aljerian, J.P. Armstrong

Background and Aim:
This study analyzes the experiences of pathologists, pathology residents, pathologists' assistants, and autopsy techs who perform autopsies. Major points of interest include views about death, feelings about death and how this impacts sense of self, and the ways that performing autopsies shapes the experiences of the living.

Material and Methods:
To explore these ideas, I have been performing in-depth qualitative interviews of people who perform autopsies to hear their perspectives. The interviews are semi-structured and involve a list of questions pertaining to the participants' experiences with death, such as, "Can you tell me about how you coped with the most recent death in your personal life?", "What does thinking about the inevitable cessation of your own consciousness bring up for you?", "What components to conducting autopsies lead to negative emotional responses for you"", and "What do you think matters most at the end of life?". The interviews are transcribed and coded for common themes.

Results:
Thus far major themes surrounding death include the following: acceptance vs. rejection of the body as an object, radical acceptance of death, lack of control over life, consideration of good vs. bad deaths, coping with egregious medical errors, and lack of systemic emotional supports for those performing autopsies.

Conclusions:
Autopsies bring up complex multifaceted emotional, intellectual, and psychological experiences for those who perform them. A discussion of major themes will take place and a strategy for maintaining wellness will be delineated.

DRIvE 2024
Research Poster Presentations

Poster Number	Name	Title	Category
1	Aranas, Abigail	Identifying alloantibodies in the setting of panreactive autoantibodies: challenges in perinatal and pretransfusion testing	MLS Student
2	Asares, Ella	Battle for Alberta: Strongyloides stercoralis EIA Commercial Kits Go Head to Head.	MLS Student
3	Bach, Annie	Utilization Evaluation of Group O Red Blood Cells to Non-ABO Identical Patients and the Resulting Impact on Inventory Management in Edmonton and Calgary Hospitals	MLS Student
4	Basig, Richen	Bridging Gaps with Simulation: What can Future Doctors and Nurses Learn about the Role of Medical Laboratory Professionals?	MLS Student
5	Brown, Erykah	Clear as Mud: How Confusion About Stem Cell and Blood Donation Criteria Hinders Racialized Youth	MLS Student
6	Ferrer, Celeste	Improving Hormone Testing in Alberta by Optimizing Hormone Stability Limits	MLS Student
7	Hoang, Anthony	Method Evaluation of the Vancomycin Assay on the Roche Cobas c503	MLS Student
8	Lei, Michelle	Analytical evaluation of second trimester maternal QUAD Screen on Brahms Kryptor immunoassay analyzer	MLS Student
9	Reyes, Janika	Developing an audio podcast for an undergraduate Medical Laboratory Science course to evaluate the efficacy of the learning tool in healthcare education	MLS Student
10	Salaveria, Ana	Examining phase change material configurations for transporting platelet and plasma protein products at room temperature	MLS Student
11	Salaveria, Priscilla	Can MALDI-TOF mass spectrometry detect the diphtheria toxin from clinical samples?	MLS Student
12	Vasilache, Adela	Is being Greedy actually a bad thing? A review of rule in and rule out criteria for	MLS Student

DRIvE 2024
Research Poster Presentations

13	Wasylenko, Jacob	Method Evaluation of a Novel High Sensitivity Troponin I Assay Suitable in Rural Laboratories	MLS Student
14	Chau, Jenny	Effect of Spring Runoff on 2,6-dichloro-1,4-benzoquinone Formation during Water Treatment	MSc
15	Huang, Camille	Improving the Specificity of CRISPR-Cas Systems for the Detection of Single Nucleotide Polymorphisms.	MSc
16	Lau, Chester	Arsenic Speciation in Freshwater Fish from Alberta Waterbodies: Long-Term Data Analysis of >1400 Fish Samples	MSc
17	Osmani, Rafay	Ice Recrystallization Inhibitors Permeate Zebrafish Embryos and Minimize Ice Recrystallization	MSc
18	Turner, Zoe	Quantification and Characterization of the Endogenous Polyclonal Antibodies by ImmunoAffinity-Mass Spectrometry	MSc
19	Walsh, Taylor	Universally screening for chlamydia and gonorrhea in a post-natal population at a single hospital site in Edmonton, Alberta	MSc
20	Davydiuk, Tetiana	Characterization of arsenic speciation profiles using simultaneous elemental and molecular mass spectrometry (HPLC-ICPMS/ESIMS)	PhD – Post candidacy
21	Xiao, Huyan	Construction of a DNAzyme motor system enabling cancer cell imaging without the need for washing	PhD – Post candidacy
22	Xu, JingYang	Extraction, concentration, and preservation of viral RNA from saliva and gargle samples	PhD – Post candidacy
23	Yazdanbakhsh, Mahsa	Young RBC Subpopulation from Frequent Senior Blood Donors Has Lower Hemoglobin-Oxygen affinity	PhD – Post candidacy

DRIvE 2024
Research Poster Presentations

24	Shen, Qiming	Advanced Nontarget Characterization of Amine-containing Compounds in Suwannee River Standard Reference Materials	Post Doctoral Research Fellow
25	Thilakarathna, Surangi	The optimization of a viability real-time PCR assay to detect viable Salmonella spp. in diarrheal stools: a spiked-stool study	Post Doctoral Research Fellow
26	Anderson, Danielle	Process Improvement Recommendations to Optimize Detection of Microfilariae	Resident
27	Esfandiari, Nina	Validation of a Virtual Reality Headset Display for Digital Pathology Sign-Out	Resident
28	Hua, QingYun	Isohemagglutinin Titration in Pooled and Apheresis Platelets	Resident
29	Niu, Candy	Comparison of Automated Solid Phase vs Manual Saline Indirect Antiglobulin Test Methodology for non-ABO Antibody Titration: Implications for Perinatal Antibody Monitoring	Resident
30	Shan, Shubham	Electron Microscopy for evaluation of ultrastructural cellular features from differentially perfused myocardial samples	Resident
31	Umar, Saadiya	SARS-CoV-2 seropositivity in Alberta: The Omicron era	Resident
32	Zolfaghari, Sima	VEXAS syndrome complicated by disseminated histoplasmosis: a case report	Resident
33	Bigras, Gilbert	Towards Accurate Deep Learning-Based Prediction of Ki67, ER, PR, and HER2 Status from H&E-stained Breast Cancer Images	Academic/Clinical Faculty
34	Han, Jian	Bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate Enhances foxo1-Mediated Lipophagy to Remodel Lipid Metabolism in Zebrafish Liver	Exchange/Visiting Professor
35	Wang, Hui and Wang, Honghong	CRISPR/Cas13a-responsive and RNA-bridged DNA hydrogel capillary sensor for point-of-care detection of RNA	Laboratory Scientist

Name: Abigail Aranas - MLS Student

Title:
Identifying alloantibodies in the setting of panreactive autoantibodies: challenges in perinatal and pretransfusion testing

Authors:
Abigail Aranas, Gerri Barr, Brenda Caruk, Melanie Bodnar

Background and Aim:
Panreactive autoantibodies (PAA) pose a challenge in perinatal and pretransfusion serological testing as they interfere with the identification of clinically significant alloantibodies which can cross the placenta and cause Hemolytic Disease of the Fetus and Newborn (HDFN) or put transfused patients at risk of hemolytic transfusion reactions. This study evaluated the efficacy of laboratory techniques for identifying underlying alloantibodies in the presence of PAA.

Material and Methods:
A retrospective review of laboratory testing records for perinatal (PN) and pretransfusion (PT) samples with PAA (Oct 1 2021-Dec 31 2023) was performed to assess DAT strength, results of standard antibody identification procedures, and efficacy of WARM autoadsorption (WARM) to identify underlying alloantibodies.

Results:
Of 52 cases (31 PN/21 PT), 26 WARM were performed with 16 successfully removing PAA. WARM failed in 10 (38%) of cases. Differential reactivity between antibody identification techniques enabled resolution in an additional 8 cases and in 6 PN cases the PAA disappeared on a subsequent sample. Overall, clinically significant alloantibodies could not be excluded in 22 (42%) of cases and 3 alloantibodies were identified (all in PN patients). Lack of sufficient sample precluded further evaluation in 9 (17%) cases.

Conclusions:
In a significant proportion of patients with panreactive autoantibodies, underlying clinically significant alloantibodies could not be excluded. Inadequacy of existing techniques and lack of sufficient sample were the most common reasons. Additional work is underway to evaluate the utility of a dilute SIAT method which has been promoted in a recent best practice guideline.

Name: Ella Asares – MLS Student

Title:
Battle for Alberta: Strongyloides stercoralis EIA Commercial Kits Go Head to Head.

Authors:
Ella Asares, Kinga Kowalewska-Grochowska, Ruwandi Kariyawasam

Background and Aim:
Strongyloides stercoralis is the causative agent of strongyloidiasis. Strongyloides has the ability to auto-infect its host, leading to persistent chronic infections, often asymptomatic and unnoticed. However, when the immune system is compromised, the infection can become fatal, due to uncontrolled multiplication and severe strongyloidiasis. Currently, Alberta does not perform serological testing for Strongyloides stercoralis. Our objective is to conduct a validation study to determine the most suitable commercially available serological kit for the diagnosis of strongyloidiasis.

Material and Methods:
Retrospective, prospective, and cross-reactivity testing were conducted using the Euroimmun EIA and Phoenix Airmid EIA kits. Each kit was then assessed based on accuracy, precision, specificity, and sensitivity.

Results:
During retrospective testing (n = 88), Euroimmun exhibited an accuracy of 71%, and Phoenix Airmid exhibited an accuracy of 77%. Euroimmun had a specificity and sensitivity of 46% and 100%, respectively, while Phoenix Airmid had a specificity and sensitivity of 75% and 94%, respectively. During cross-reactivity testing (n = 12), Euroimmun exhibited an accuracy of 83%, and Phoenix Airmid had an accuracy of 75%. Prospective testing (n = 73) resulted in an accuracy of 100% and 96% for Euroimmun and Phoenix Airmid, respectively. On average, Phoenix Airmid exhibited less variability (CV = 13.26) than Euroimmun (CV = 15.81) during precision testing.

Conclusions:
In conclusion, Phoenix Airmid is better suited to meet the demands of the laboratory in Alberta. The Phoenix Airmid EIA kit showed consistent performance across different testing parameters. Only marginal differences were observed when Euroimmun outperformed Phoenix Airmid.

Name: Annie Bach – MLS Student

Title:
Utilization Evaluation of Group O Red Blood Cells to Non-ABO Identical Patients and the Resulting Impact on Inventory Management in Edmonton and Calgary Hospitals

Authors:
Annie Bach[1], Heather Blain[2], Dr. Gwen Clarke[1], Nicole Gettle[2], Karen Hobbs[2], Bruce Lyon[2], Dr. Susan Nahirniak [1,2]

Background and Aim:
Due to its universal compatibility and insufficient inventory, there have been recurrent shortages of group O blood. To determine if policies and interventions are required to guarantee optimal use and optimize inventory, this study examined the utilization patterns of group O blood to non-ABO identical patients.

Material and Methods:
A retrospective study was conducted to examine transfusion data over an 8-month period from May 2023 – December 2023. Data was extracted from WellSky, a web-based transfusion software used for inventory management and transfusion records. Extracted data includes patient demographics, transfusion date, product type, ABO/Rh, unit number, storage facility, issue status, and expiration date. Units were categorized and assessed to determine the clinical indication and whether the use of group O was appropriate or inappropriate.

Results:
There were 2976 and 3482 RBC transfusions of group O to non-O patients in Calgary and Edmonton, respectively. Of the units transfused, 9.1% (n = 272 RBCs) of the transfusion in Calgary and 5.4% (n = 187 RBCs) of the transfusions in Edmonton were deemed inappropriate.

Conclusions:
The majority of the group O units transfused to non-O patients were considered appropriate. Though many of the units met the qualifications to be considered appropriate, it does not mean it was the most efficient use of the inventory available. The findings from this study suggest that additional measures may be required to lower the use of group O to non-O patients to allow for optimal utilization and to maintain an adequate supply of group O units.

[1] University of Alberta
[2] Alberta Precision Laboratories

Name: Richen Basig – MLS Student

Title:
Bridging Gaps with Simulation: What can Future Doctors and Nurses Learn about the Role of Medical Laboratory Professionals?

Authors:
Richen Basig, Lisa Purdy, and Amanda Van Spronsen

Background and Aim:
The key role of medical laboratory professionals (MLPs) in patient care is often misunderstood by other healthcare professionals. This pilot study aims to bridge the interprofessional 'role clarification' gap by delivering two virtual simulations to pre-clinical nursing and medical students to showcase MLP roles and responsibilities. The findings from this pilot will be used to validate the simulations and develop a formal survey for further study. The results can inform educators about virtual simulations' effectiveness in role clarification during interprofessional education.

Material and Methods:
The immersive simulations are designed as branching scenarios set in a clinical laboratory. Participants engage in clinical situations as MLPs, completing tasks that require collaboration with nurses and physicians. Structured interviews before and after the simulation explored participants' understanding of MLP roles and responsibilities.

Results:
First-year nursing (3) and medical (3) students from the University of Alberta participated. All recommend the simulations to enhance understanding of the laboratory's role in patient care. Some participants were surprised by the breadth of the laboratory's responsibilities, extending beyond sample collection and machine operation. The absence of formal interprofessional training surprised participants given the collaboration required with the laboratory. Following the simulations, the participants reported increased knowledge about the laboratory.

Conclusions:
Preliminary analysis suggests that the participants enjoyed the simulation and found it valuable. Participant responses affirm the need for more interprofessional education opportunities. Future simulation projects will encompass a broader range of MLPs' scope of practice to further enhance understanding. Overall, the results suggest value in expanding the pilot to a larger sample.

Name: Erykah Brown – MLS Student

Title:
Clear as Mud: How Confusion About Stem Cell and Blood Donation Criteria Hinders Racialized Youth

Authors:
Erykah Brown

Background and Aim:
The BIPOC community makes up less than 1/3 of our stem cell registry, reducing their chances of finding a donor match on the unrelated hematopoietic stem cell registry (UHCSR). Effective recruitment of ethnically diverse individuals is hindered by unaddressed barriers to registration and donation. Misinformation and/or a lack of information has been identified as a key driver of ambivalence and attrition among prospective and current UHCSR registrants. This research explored the extent to which confusion of criteria to join the UHCSR with the criteria to donate blood affects young, racialized adults' decision to join the registry.

Material and Methods:
Racialized youth (18-35 years old) were recruited using a combination of snowball and purposive recruiting strategies, completed a pre-screening survey, and participated in qualitative focus-groups and interviews. All data collected was analyzed using a thematic analysis and grounded theory framework.

Results:
While the majority of participants had moderate knowledge of blood donation processes and criteria, knowledge about the UHCSR and donation criteria was very limited with only 15% of survey respondents having joined the registry. Though ⅓ of focus group participants were on the UHCSR, they all expressed a lack of awareness of the eligibility criteria they had to meet. This unawareness extended to non-UHCSR registered participants. Transposition of ineligibility for blood donation to SCR eligibility was also identified.

Conclusions:
Preliminary findings suggest that before blood operators can address ***confusion*** of criteria as a barrier, there is a desperate need to bolster general awareness of the SCR across the target demographic for improved diversity of the stem cell registry.

Name: Celeste Ferrer – MLS Student

Title:
Improving Hormone Testing in Alberta by Optimizing Hormone Stability Limits

Authors:
Celeste Ferrer, Josh Raizman and Yury Butorin

Background and Aim:
Proper handling and specimen processing is crucial for delivering high-quality patient care. The aim of this study was to determine the stability limits of Adrenocorticotropic hormone (ACTH), Total Testosterone (TT) and Sex Hormone Binding Globulin (SHBG) and whether ACTH requires strict handling and processing conditions.

Material and Methods:
Four different ACTH handling and processing conditions were compared against each other. The current protocol being implemented across the province was used as the control (Condition A). Plasma stability of ACTH as well as TT and SHBG were evaluated by storing 200 uL aliquots into Room Temperature (RT), 4°C and -20°C across 24, 28 and 72 hours.

Results:
After analysis, Condition B (pre-chilled tube-ice pack-RT spin) had a 0.3±3.1 % difference, Condition C (non-pre-chilled tube-no ice pack-RT spin) had -4.1±2.3% and 1.8±2.9% for Condition D (pre-chill-ice pack-cold insert but RT spin). ACTH in plasma is stable up to 72 hours when stored in fridge (4°C) and freezer (-20°C) but not when stored in RT for more than 24 hours as it exceeds the total allowable error (TAE) of -27%. On the other hand, TT and SHBG is stable up to 72 hours regardless of the temperature, not exceeding the TAE of -17% including the freeze-thaw cycles.

Conclusions:
This study found that ACTH does not need strict handling and processing conditions wherein a RT spin with or without a cold insert will suffice. ACTH levels in plasma remain consistent for a period of 72 hours when stored in a refrigerator (4°C) or freezer (-20°C), and for up to 24 hours at RT. In addition to ACTH, TT and SHBG exhibited reliable stability for up to 72 hours regardless of the storage temperature.

Table 1. Four different handling and processing conditions in which ACTH was subjected to.

Condition/ Protocol	Specimen collected in	Blood tube handled in	Centrifugation including temperature requirements
A (control)	Pre-chilled 6mL EDTA	Place in an ice pack immediately after collection	Spin in refrigerated centrifuge (4°C), 1300g (rcf*) for 10 minutes
B	Pre-chilled 6mL EDTA	Place in an ice pack immediately after collection	Centrifuge in RT (20°C), 1300g (rcf*) for 10 minutes
C	Room temperature (RT) 6mL EDTA	RT (do not place in an ice pack)	Centrifuge in RT (20°C), 1300g (rcf*) for 10 minutes
D	Pre-chilled 6mL EDTA	Place in an ice pack immediately after collection	Centrifuge in RT (20°C) but use a pre-chilled cold insert (-20°C), 1300g (rcf*) for 10 minutes

*rcf, relative centrifugal force

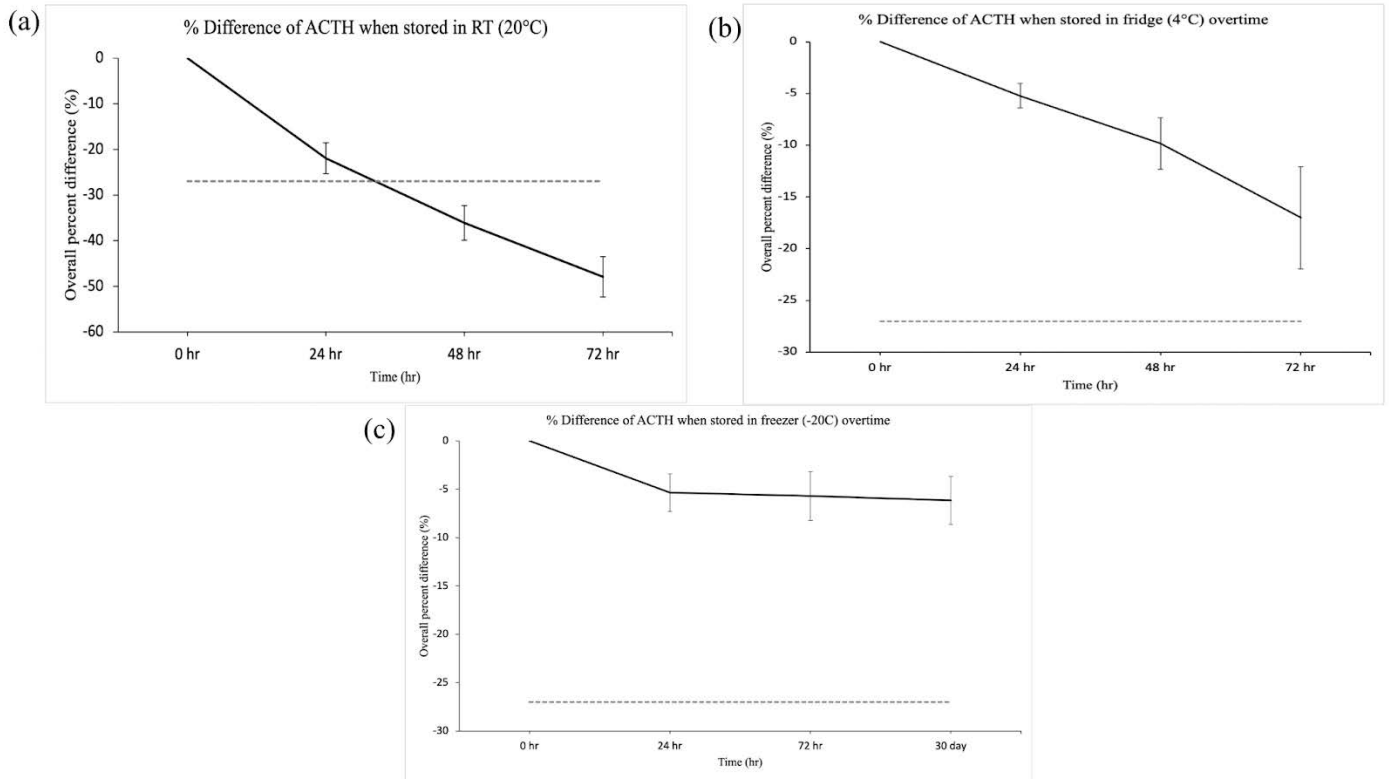


Figure 4. Stability of ACTH in plasma across different time points. 0hr acts as a baseline and broken line represents the TAE of -27%. (a) error bar illustrating the decreasing overall % difference in RT, (b) displays overall % difference in fridge (4°C), and (c) overall % difference in freezer storage (-20°C).

Name: Anthony Hoang – MLS Student

Title:
Method Evaluation of the Vancomycin Assay on the Roche Cobas c503

Authors:
Anthony Hoang, Shelby Sissons, and Josh Raizman

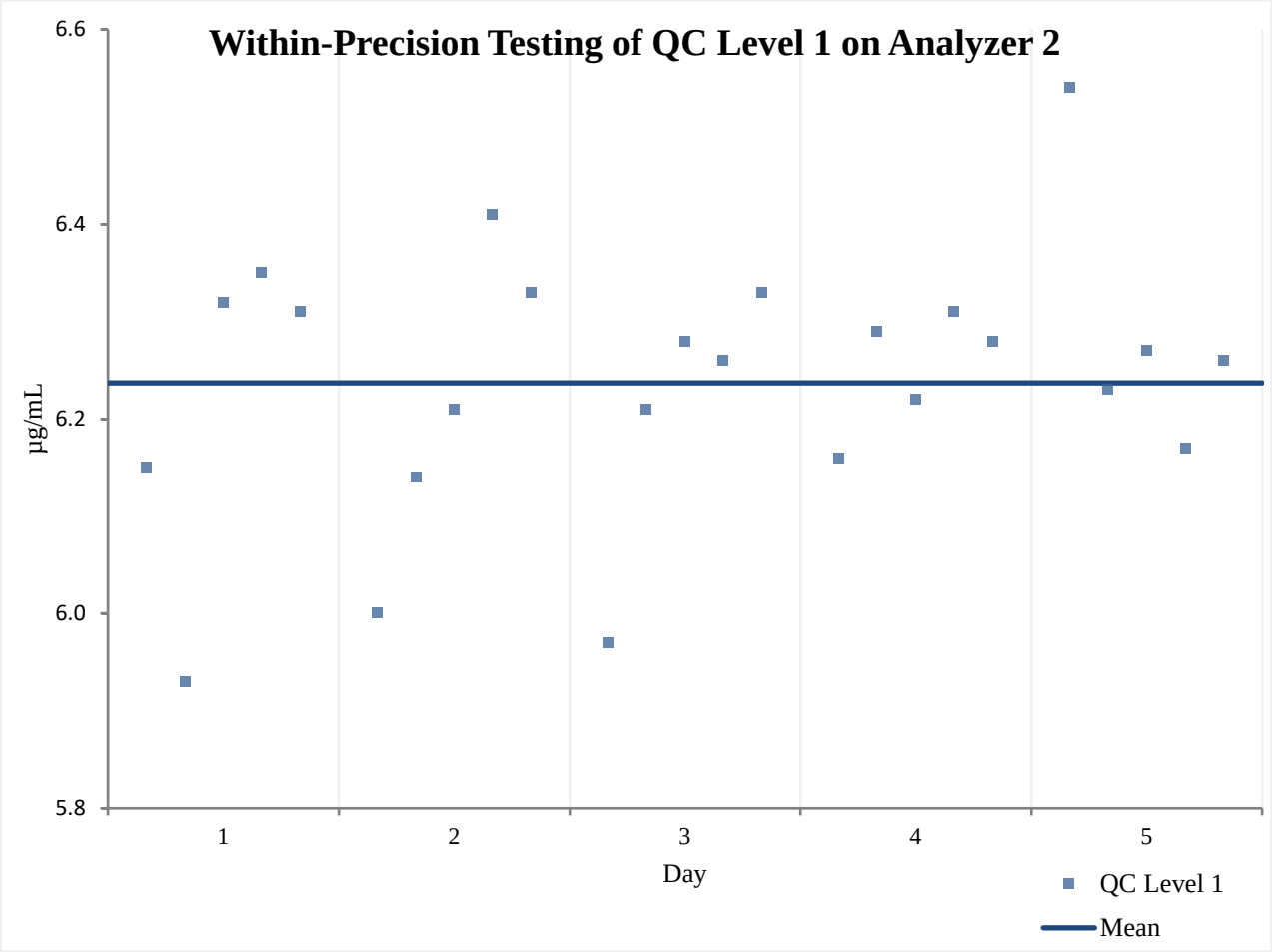
Background and Aim:
The Sturgeon Community Hospital (SCH) routes vancomycin testing to the University of Alberta Hospital (UAH). This delay prevents timely decisions on dose adjustment in hospitalized patients. To determine if vancomycin can be added to the SCH test menu, a method evaluation was performed on the Roche Cobas vancomycin assay.

Material and Methods:
Vancomycin was measured on two Roche Cobas c503 instruments. Quality control (QC) material was used to evaluate total precision. Discarded heparinized plasma samples with vancomycin levels across the analytical measuring range (AMR) were used for inter-instrument and reference site comparisons. Linearity was evaluated using commercial standards. Proficiency testing (PT) material was also run to evaluate accuracy. Analytical performance was assessed using a total allowable error of 20%.

Results:
Both analyzers showed good precision with a coefficient of variation (CV) of 1.5-2.5% across two QC levels. Both analyzers were linear across the AMR, with acceptable comparability at each level to target concentrations of 0-7.8%. Inter-instrument comparison showed close agreement ($y=1.0327x - 0.7805$, $R=0.9982$) with an average bias of 0.28%. Comparison to a reference site was also acceptable ($y=0.9568x - 0.0926$, $R=0.9964$) with an average bias of -6.19%. Comparison of PT results to the peer group had an average standard deviation index of -1.1.

Conclusions:
The Roche Cobas c503 vancomycin assay demonstrated acceptable analytical performance. Implementation onto the SCH test menu will improve TAT and allow faster dose adjustment decisions.

Figure 1 Within-Lab Precision Study of Biorad Liquicheck Vancomycin QC Lot 85351 on Analyzer 2 over Five Days.



Note: Replicates of 5 QC samples ran each day for 5 days.

Name: Michelle Lei – MLS Student

Title:
Analytical evaluation of second trimester maternal QUAD Screen on Brahms Kryptor immunoassay analyzer

Authors:
Michelle Lei, Miranda M Brun

Background and Aim:
Second trimester maternal serum screening can be used to screen for common fetal aneuploidies (Trisomy 21, Trisomy 18), and open neural tube defects in pregnancy. At the University of Alberta Hospital, second-trimester maternal serum screening uses markers alpha-fetoprotein (AFP), unconjugated estriol (uE3), human chorionic gonadotropin (hCG), and dimeric inhibin A (DIA) in combination with patient demographic data to calculate risk for Trisomy 21, Trisomy 18, and open neural tube defects. This study compares the current immunoassay analyzer and Brahms Kryptor for second trimester maternal serum screening.

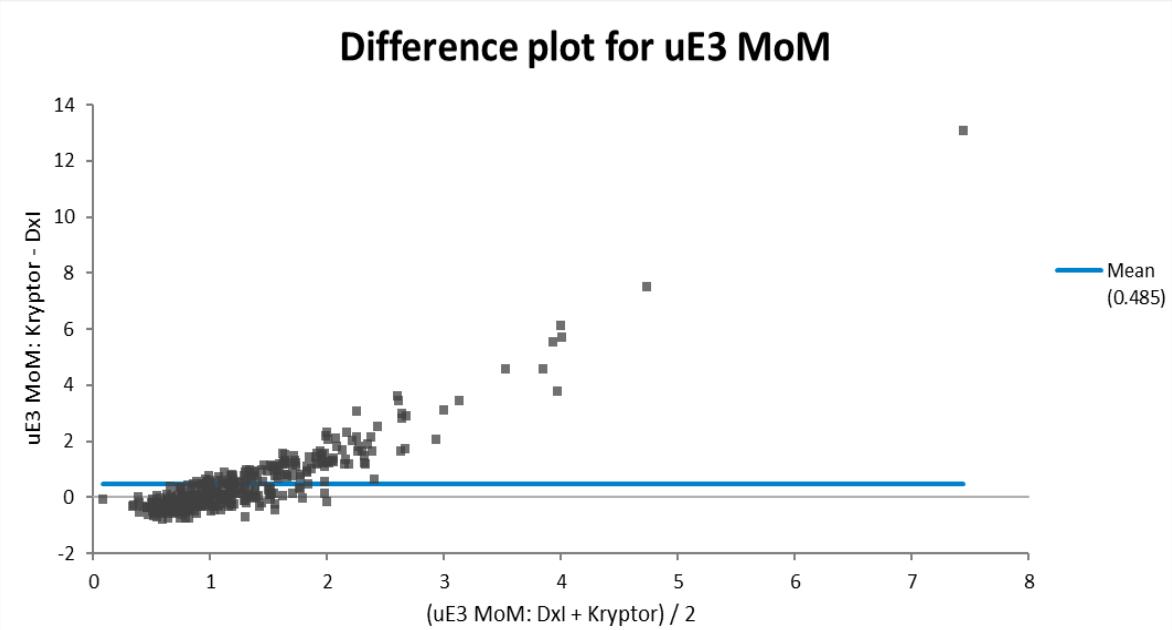
Materials and Methods:
We analyzed 379 patients but 366 were included in the data analysis. Precision, interference, linearity, and detection limits of the Kryptor were evaluated. For method comparison, we computed risk assessments on Benetech Prenatal Risk Assessment software and employed Analyse-it statistical software to compare biomarker concentrations, and multiples of the median using Passing-Bablok regression and Bland-Altman difference plots. Concordance between the two platforms was assessed.

Results:
The Brahms Kryptor demonstrated acceptable within-run and between-run precision for method validation. Detection limits for AFP, uE3, and DIA verified detection limits in instructions for use. UE3 linearity could not be verified. Method comparison identified bias in concentrations of DIA, uE3, and hCG between Brahms Kryptor and Beckman Dxl. Concentration of AFP compared well. Concordance for Trisomy 21, Trisomy 19 and open neural tube defects risk assessments exceeded 90%.

Table 1: Concordance for Down Syndrome risk assessment

		Beckman DxI	
Brahms Kryptor		Positive	Negative
	Positive	20	24
	Negative	12	310
% Concordance	90.16%		

Graph 1: Bland-Altman Difference plots for uE3 MoM of DxI and Kryptor



Conclusions:

Overall, the Brahms Kryptor satisfies method validation standards for precision, interference, and detection limit. Method comparison between the two instruments suggests that there are possible biases in DIA, hCG and UE3.

Name: Janika Reyes – MLS Student

Title:
Developing an audio podcast for an undergraduate Medical Laboratory Science course to evaluate the efficacy of the learning tool in healthcare education

Authors:
Janika Reyes, Lisa Purdy, Roberta Martindale, Anna Nevesinjac

Background:
Podcasts have gained popularity in healthcare education as a supplementary learning tool to traditional teaching methods but require further research in Medical Laboratory Science (MLS). This study presents a novel approach by developing and evaluating the utility and impact of audio podcasting in an undergraduate MLS course. The aim of this study is to enhance students' academic performance and integrate this innovative tool into a curriculum, presenting a fresh perspective on MLS education.

Methods:
A total of 23 episodes, each 10 minutes or less, were created. Each episode focussed on the learning objectives of the MLSCI 242 (Pathogenic Microbiology) course at the University of Alberta. Episodes were posted on the course eClass page. This mixed-methods study included pre- and post-podcast quizzes to measure academic performance, alongside analysis of podcast engagement statistics and a post-podcast administration survey to evaluate efficacy and curriculum integration. There were 6 participants in total, 5 of whom participated in the post-podcast survey.

Results:
Scores between the pre- and post-podcast quiz were statistically significant, with students scoring lower after podcast administration (Lab 4 quiz, $n = 5$, $p<0.05$). The YuJa analytics show that not all those consented to participate in this study listened to the Winter 2024 podcast episodes. All participants who listened to the Winter 2024 podcast episodes listened on a computer, instead of a mobile device. Most survey respondents (3/5, 60%) found the audio podcasts beneficial in preparing for their assessments. Significantly, all survey respondents (5/5, 100%) responded favourably to incorporating audio podcasting across the MLS disciplines, indicating a high potential for the integration of this tool. Most survey respondents (75%) found the podcast to be a good review of the topics. A challenge for most respondents (60%) was the incompatibility of the podcast with their learning style, and some respondents (67%) suggested increasing the length of podcast episodes to more than 10 minutes.
Conclusion: Audio podcasting is a promising educational tool for MLS students. It offers flexibility in reviewing course learning objectives for assessments. However, this study presents several limitations, such the limited sample size and the challenge of establishing a clear correlation between the use of podcasts and academic performance. Further evidence-based studies are needed to examine its efficacy.

Name: Ana Simonette Salaveria – MLS Student

Title:
Examining phase change material configurations for transporting platelet and plasma protein products at room temperature

Authors:
Ana Simonette Salaveria, Heather Blain, Anne Burry, Bruce Lyon, Ryan Hollman, Heather Malcolm, and Dr. Susan Nahirniak

Background and Aim:
Platelets (PLTs) and plasma protein products (PPP) transported at room temperature (RT) have been poorly studied. This experiment aims to examine phase change material (PCM) configurations for transporting products at room RT.

Material and Methods:
Configurations of either two 22C Akku 2L DeltaT (large PCMs) or two stacks of 22C TempShell Frames/Elements (ring PCMs) inside 20L BlueLine DeltaT transport containers. Bubble wrap or RT-incubated Cryopak Ice Blankets were used to remove dead space and offer cushioning. Minimum (one PLT unit or one 100mL albumin) or maximum (25 boxes for large PCMs, 12 boxes for rings, or three PLTs for both) were loaded. These were tested for ~24 hours at four external temperatures: -28°C, 4°C, 20-24°C (RT), and 37°C. Data loggers were used to record the temperature within the transport container. Data was compared using linear regression.

Results:
Table 1 illustrates the durations of all incubations using the different configurations and external temperatures. 20°C-charged large PCMs kept stock within 20-24°C at RT and ≤4 hours at 4°C. 18-20°C-charged rings kept stock within 20-24°C for ≤24 hours at RT and <2 hours at 4°C. Large PCMs charged consistently and displayed temperature more accurately. Temperature variations within the laboratory prevented consistent 20°C charging of PCMs and pushed 50% of RT incubations below 20°C.

Table 1. Incubation Duration In Different Configurations, External Temperature, and Load

	Large PCM, RT, Min	**Large PCM, RT, Max**	**Large PCM 4°C, Min**	**Large PCM 4°C, Max**	**Large PCM -28°C, min**	**Large PCM 37°C, min**	**Rings RT, min**	**Rings RT, max**	**Rings 4°C, min**	**Rings 4°C, max**	**Rings -28°C, min**	**Rings 37°C, min**
Max (Hrs)	24	24	2.5	4	0.17	19	24	2.17	0.67	2	0.17	10.67
Min (Hrs)	23.33	0.83	0.17	0.33	0.17	17	0.17	0.50	0.17	2	0.17	10.67
Mean (Hrs)	23.83	16.64	1.73	1.75	0.17	18	12.08	1.34	0.42	2	0.17	10.67

Conclusions: Large PCMs showed promise for RT transport requirements. However, charging PCMs at 20°C and maintaining appropriate ambient temperatures in our laboratories and courier vehicles require tight controls.

Name: Priscilla Laetitia Salaveria – MLS Student

Title:
Can MALDI-TOF mass spectrometry detect the diphtheria toxin from clinical samples?

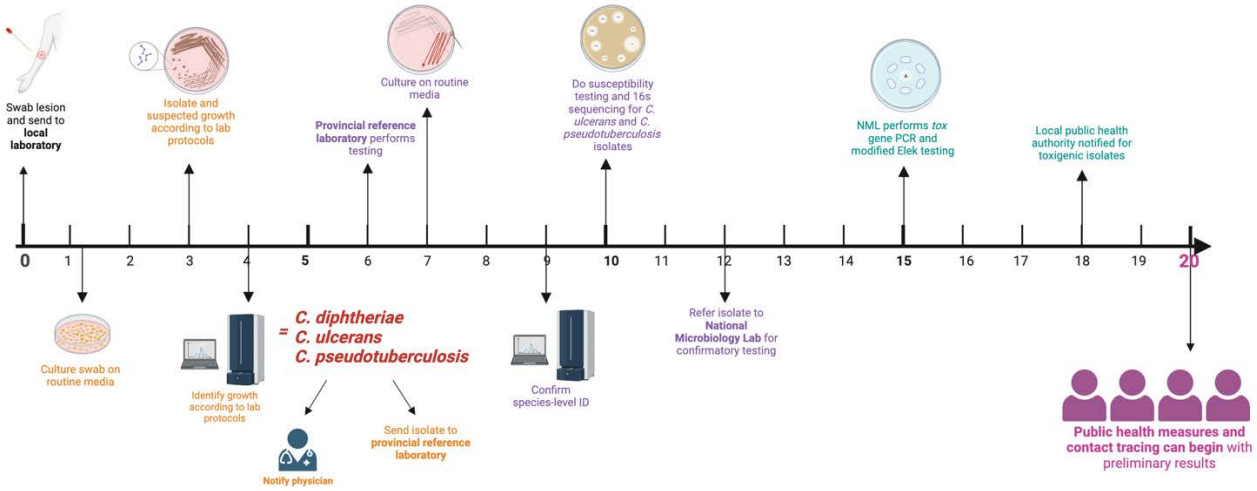
Authors:
Priscilla Laetitia M Salaveria[1], Natalie Marshall[1,2], Tanis Dingle[2]

Background and Aim:
Toxigenic diphtheria infections are a public health emergency that require rapid diagnosis and laboratory testing for prompt patient treatment and contact tracing. Current diagnostic practices involve testing in multiple reference laboratories to determine whether a clinical isolate of *Corynebacterium diphtheriae* is toxigenic or not, which slows down the public health response. This study assessed the utility of MALDI-TOF mass spectrometry in predicting toxigenicity from pure isolates of *C. diphtheriae* without requiring out-of-province testing, and whether this proposed method could be used for diphtheria cases in Alberta.

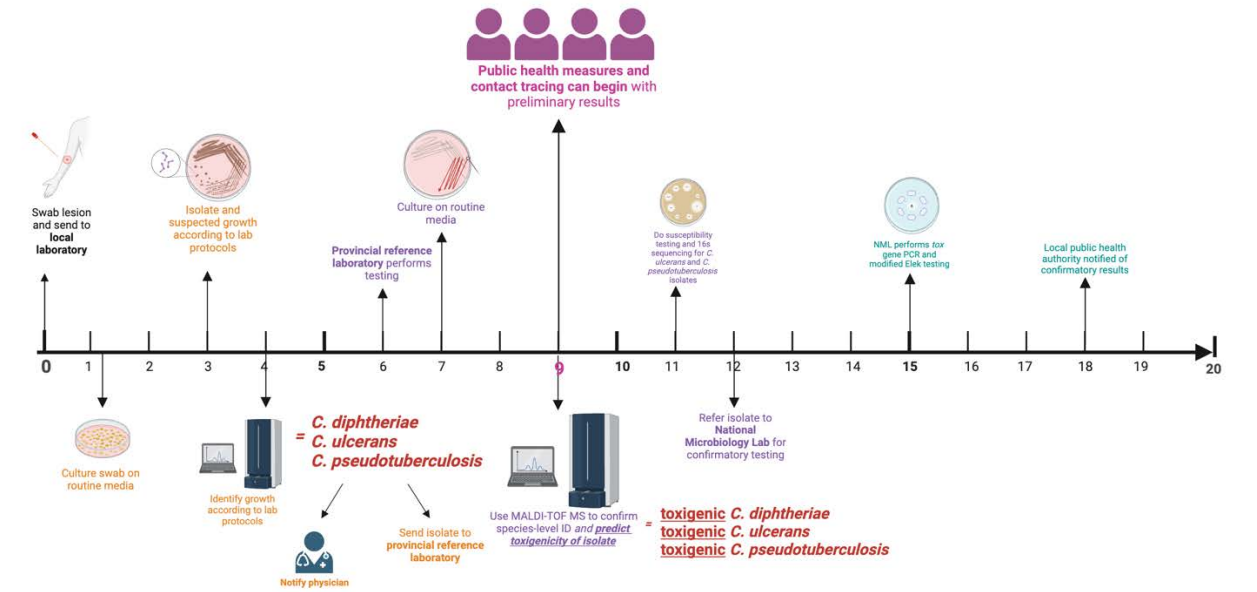
Material and Methods:
Clinical isolates of *C. diphtheriae* and *C. ulcerans* were obtained from Alberta's public health lab (ProvLab) and the National Microbiology Laboratory (NML). 20 toxigenic and 32 non-toxigenic isolates were analyzed by MALDI-TOF MS. Mass spectra obtained from the software were visually examined to identify significant and differential peaks that might predict toxigenicity according to NML's reference method.

Results:
A review of the current diagnostic pathways for diphtheria testing suggested that MALDI-TOF MS, if reliable, would reduce the total turnaround time for toxigenicity testing by several days. Mass spectral analysis of the clinical isolates showed at least ten potential biomarkers of toxigenicity. 4 peaks (m/z = 5460, 6462, 7156, 7172) were exclusively found in toxigenic strains and 1 peak (m/z = 7201) was identified in non-toxigenic isolates only.

Conclusions:
This method is a promising way that clinical laboratories could provide important information earlier on. This study showed that MALDI-TOF MS could be used by clinical laboratories for rapid preliminary diphtheria toxin testing. However, further research using statistical models are needed to build a robust toxigenic biomarker profile before it could be used for diphtheria cases in Alberta.



a



b

Created with BioRender.com

Fig. 1. Difference in total turnaround times (TAT) between current and proposed workflows for diphtheria cases in Alberta. Color change signifies transport between multiple reference laboratories. (a) The current clinical protocol for diphtheria cases in Alberta, depicted in days taken (0-20) until public health measures can begin (TAT = ~20 days). (b) Proposed method using MALDI-TOF MS for prediction of toxigenicity showing a reduced TAT and improved public health response (TAT = ~9 days).

Name: Adela Vasilache – MLS Student

Title:
Is being Greedy actually a bad thing? A review of rule in and rule out criteria for transfusion medicine antibody investigations

Authors
Adela Vasilache[1], Bruce Lyon[2], Jennifer Duke[2], Kristy Schmitt[2], Penelope Harder[2], Dr. Susan Nahirniak [1,2], Dr. Gwen Clarke[1]
[1]Department of Laboratory Medicine and Pathology, University of Alberta
[2]Alberta Precision Laboratories

Introduction and Purpose:
Rule-in and rule-out criteria are critical to confidently identify blood group antibodies to avoid a potentially fatal transfusion reaction. Sources disagree on the minimal criteria needed to confirm specificity. We compared different rule-in and rule-out criteria sets, comparing accuracy, testing time, and reagent resources.

Methods:
The two rule-out criteria differed in the use of the patient's phenotype. Three rule-in criteria differed by the number of exclusion cells used—one, two, or three. These were further subdivided into two categories each; a) required the set amount of needed inclusion cells per suspected antibody and b) allowed the same inclusion cells to rule-in more than one antibody (i.e. Greedy method). Rule-in and rule-out criteria were mixed and matched into sets. For accuracy comparisons, the probabilities of the identified antibodies were calculated for each set of criteria. For comparisons of time and resources needed, the number of additional exclusion and inclusion cells required were recorded. The number of donor units screened for crossmatch compatibility was calculated.

Results:
75 antibody investigations were re-evaluated. Ten had single specificities so were not applicable for any criteria sets that included the Greedy method. Rule-in criteria results are seen in **Table 1** and rule-out criteria results are seen in **Table 2**.

	Average number of additional exclusion cells	**Average testing time (min)**	**Average number of donor units screened**
Without phenotype	2.2	33	39.8
With phenotype	2.04	30.6	30.6

Table 1: Summary of rule-in criteria results.

	Average number of additional inclusion cells	**Average testing time (min)**	**Average antibody probabilities**
One inclusion cell	0.23	3.4	0.0569

One inclusion cell + Greedy	0	0	0.163
Two inclusion cells	0.92	13.8	0.0306
Two inclusion cells + Greedy	0.12	1.8	0.154
Three inclusion cells	1.64	24.6	0.0142
Three inclusion cells + Greedy	0.43	6.5	0.142

Table 2: Summary of rule-out criteria results.

Discussion:

The number of inclusion cells needed per antibody has the greatest impact on antibody probability. Use of the same inclusion cells for two or more antibodies was not justified due to the poor probability values ($p>0.05$). Use of two inclusion cells yielded sufficient probability values for accuracy without utilizing as many resources as using more. Addition of the phenotype for rule-out saved time and reagents while maintaining accurate results.

Name: Jacob Wasylenko – MLS Student

Title:
Method Evaluation of a Novel High Sensitivity Troponin I Assay Suitable in Rural Laboratories

Authors:
Jacob Wasylenko, Miranda Brun, Poonam Reddy, Mireille Kattar, Sean van Diepen, Isolde Seiden-Long, Albert KY Tsui

Background and Aim:
The TriageTrue® hs-cTnI assay is a recently Health Canada approved assay capable of performing hs-cTn in small rural centers. The goal of this study is to evaluate the analytical performance of this new assay for use in accelerated chest pain pathways.

Material and Methods:
Quality control (QC) materials and patient EDTA plasma pools were assayed for 5 replicates/day over 5-6 days. Fresh EDTA, lithium heparin and barricor samples were collected from the coronary care unit at the University of Alberta Hospital following informed consent. Fresh EDTA whole blood and plasma were measured using TriageTrue® hs-cTnI assay. All plasma sample types were measured using Roche hs-cTnT and Beckman hs-cTnI assays. Analytical concordance was assessed based on the manufacturer's suggested 99 th percentile cutoffs (Roche 14 ng/L, Beckman 18 ng/L and Quidel Ortho TriageTrue® 21 ng/L). Linearity and limit of detection were performed according to CLSI guidelines.

Results:
QC materials had a coefficient of variation (CV) of 7.1% at = 26 ng/L and 12.9% at = 572 ng/L. Patient pools at = 4 ng/L had a CV of 12.9%. Preliminary comparison results (n=30) demonstrated an analytical concordance of 93-97% with Beckman hs-cTnI and 67-69% with Roche hs-cTnT.

Conclusions:
Precision studies using quality control materials and patient pools confirm the TriageTrue® hs-cTnI assay demonstrates adequate precision. Additional validation studies are required to demonstrate the clinical accuracy of this method for ruling in and ruling out myocardial infarction.

Name: Khac Ngoc Minh (Jenny) Chau - MSc

Title: Effect of Spring Runoff on 2,6-dichloro-1,4-benzoquinone Formation during Water Treatment

Authors: Nicholas J. P. Wawryk, K. N. Minh Chau, Caley B. Craven, Kristin Carroll, Xing-Fang Li

Background and Aim:
High stream flows during spring runoff events can carry more organic matter into source water than normal. The elevated organic matter during spring runoff may increase the formation of disinfection byproducts (DBPs) formed during water treatment temporarily. Among the vast amount of known DBPs, halobenzoquinones (HBQs), have become an important class of unregulated DBPs due to their high cyto- and genotoxicity. It is well known that the aromatic component of organic matter in source water is a precursor to HBQs. Aromatic amino acids (AAs) are a group of small aromatic compounds in source water that could act as precursors to HBQ formation. Thus, AAs were monitored along with other water quality parameters in source water to determine if they were predictors of HBQ formation.

Material and Methods:
The concentration of HBQs in finished drinking water was determined using SPE-HPLC-MS/MS and was compared to common water quality parameters and AAs in source water.

Results:
The results indicated that the concentration of HBQs correlated well with common water quality parameters and showed a similar trend with the aromatic AAs. Additionally, the concentration of HBQs determined immediately following the addition of chlorine and the presence of its transformation product, hydroxylated HBQs (OH-HBQs), in finished drinking water were correlated.

Conclusions:
These results indicate that the formation of HBQs during water treatment is affected by changes in organic matter observed during spring runoff. Future research on potential HBQ precursors in urban runoff can reduce the gap of knowledge in HBQ formation, benefiting water treatment utilities, policymakers, and public health.

Name: Camille Huang – MSc

Title:
Improving the Specificity of CRISPR-Cas Systems for the Detection of Single Nucleotide Polymorphisms.

Authors:
Camille Huang, Jianyu Hu, Hongquan Zhang, X. Chris Le.

Background and Aim:
Single nucleotide polymorphisms (SNPs) can result from mutations and become significant in phenotype. A T G mutation causes vancomycin intermediate resistant Staphylococcus aureus (VISA). Therefore, accurately discriminating between SNP-containing targets is crucial. CRISPR associated proteins (Cas) have recently been used for the detection of nucleic acids. However, higher specificity is needed for differentiating SNPs. We hypothesize that modifications to the CRISPR-RNA (crRNA), such as the addition of a hairpin (hp) forming extension to the crRNA, can modulate the energetics involved in DNA target binding so that only the perfect on-target strand can activate the CRISPR-Cas system.

Material and Methods:
Two dsDNA sequences from VISA were chosen as the on-target and off-target. The hp-crRNAs designed contained differing stem lengths of 8, 10, 12, 13, and 14 nucleotides. LbCas12a was incubated with hp-crRNA to form LbCas12a-hp-crRNA ribonucleoproteins (RNP). Binding of the target by the RNP activates its trans-cleavage activity to collaterally cleave fluorophore-quencher reporter probes and generate fluorescence for detection. Increased ratios of off-target to on-target were tested.

Results:
Hp-crRNA8, Hp-crRNA10, and Hp-crRNA12 showed higher specificity for the on-target. Hp-crRNA13 and Hp-crRNA14 showed low signal intensity and poor discrimination. After optimization, Hp-crRNA10 gave the greatest discrimination factor (DF) of 13.1. Even at 250x more off-target a higher DF was obtained compared to 1x using crRNA

Conclusions:
Modifying the crRNA with an extended hairpin region significantly improves the specificity of the CRISPR assay for the target, enabling discrimination between SNPs.

Name: Chester Lau - MSc

Title: Arsenic Speciation in Freshwater Fish from Alberta Waterbodies: Long-Term Data Analysis of >1400 Fish Samples

Authors: Chester Lau[1]*, Xiufen Lu[2], Jennifer A. Graydon[3], Megan Reichert[3], Ruth Mitchell[3], and X. Chris Le[1,2]

[1]Department of Chemistry, Faculty of Science, University of Alberta, Edmonton, AB, T6G 2G2, Canada
[2]Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta, Alberta T6G 2G3, Canada
[3]Alberta Health, Health Protection Branch, Edmonton, Alberta, Canada

Background and Aim: The International Agency for Research on Cancer (IARC) classifies arsenic as a group 1 human carcinogen. Arsenic toxicity varies with its chemical form and oxidation state. While chronic exposure to inorganic arsenic is highly toxic and associated with various cancers, exposure to arsenobetaine, commonly found in seafood, is considered nonharmful. Therefore, determining and quantifying individual arsenic species in fish is necessary for meaningful risk assessment of fish consumption. Many Albertans consume freshwater fish due to its abundance of vitamins, proteins, essential trace minerals, and omega-3 polyunsaturated fats. We aim to determine arsenic species concentrations in freshwater fish and conduct statistical analysis to evaluate differences between fish species, and waterbodies/years that the fishes were caught.

Material and Methods: Over 1400 freshwater fish samples representing 14 most commonly caught species were collected from Alberta waterbodies over ten years. After undergoing many treatment steps, fish extracts were analyzed for arsenic species using high-performance liquid chromatography (HPLC) separation and inductively coupled plasma mass spectrometry (ICPMS) detection. Statistical analyses of arsenic speciation data were completed using RStudio.

Results: Arsenobetaine was detected in most samples. Arsenic species concentrations varied with fish species and waterbodies. Individual fish samples from the same species and waterbodies showed differences in arsenic species concentrations. Variations in arsenic speciation patterns between fish species may suggest differences in methylation (metabolic) efficiency or exposure to unique arsenicals.

Conclusions: Our research findings are used for assessing daily intake and potential consumption risk and contributing to the ultimate goal of monitoring local ecosystems and protecting the public from excess arsenic exposure.

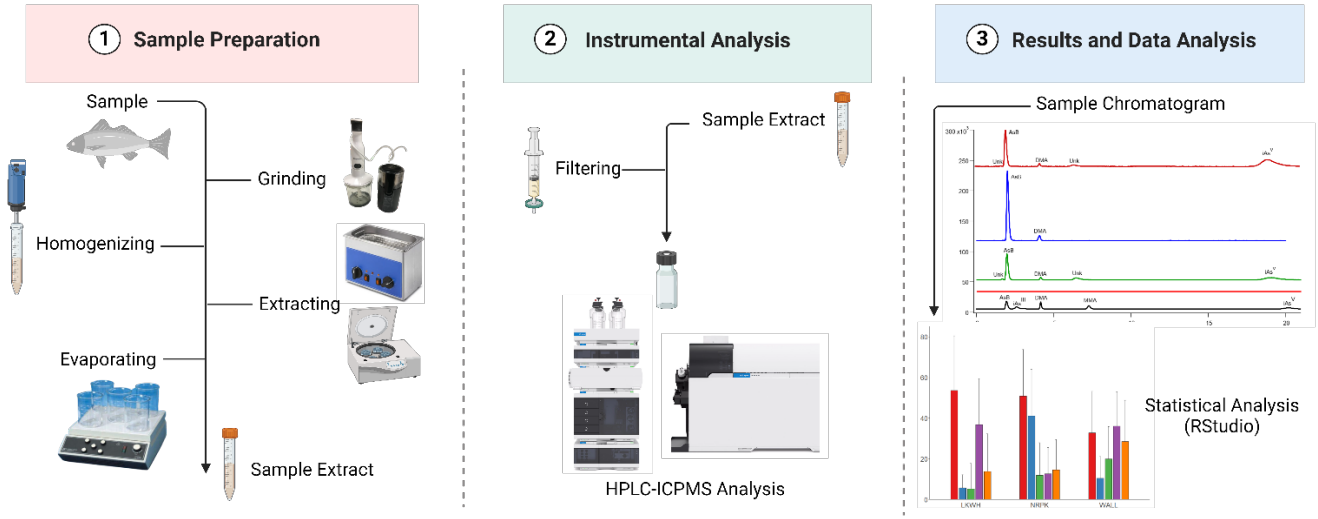


Figure 1. Preparation of freshwater fish samples for high-performance liquid chromatography (HPLC) separation and inductively coupled plasma mass spectrometry (ICPMS) detection of arsenic species. Statistical analysis was performed using data acquired from instrument analysis.

Name: Rafay Osmani - MSc

Title:
Ice Recrystallization Inhibitors Permeate Zebrafish Embryos and Minimize Ice Recrystallization

Authors:
Rafay Osmani, Nishaka William, Jason Acker

Background and Aim:
Over 44,000 genotypes of Zebrafish are used in research. However, fish lines circulate in and out of use. By cryopreserving zebrafish embryos (ZFEs), we can more efficiently and safely archive their genetic material. ZFEs are highly impermeable and sensitive to ice formation. Conversely, many traditional cryoprotective agents (CPAs) are toxic at the required concentrations. Using ice recrystallization inhibitors (IRIs) has not been explored in ZFEs but can lead to new and improved cryopreservation protocols.

Material and Methods:
Toxicity of 9mM of *N*-2-flurophenyl-D-gluconamide (2FA) is evaluated by exposing various development stages of ZFEs with and without their chorion. Survival is defined by ability to survive to subsequent development stages. Toxicity trials were run in triplicates across three batches. ZFEs incubated with 2FA for a maximum of 70 hours are frozen using liquid nitrogen, transiently warmed to induce ice recrystallization, stained hematoxylin and eosin (H&E). Surface area of crystals in the eosin-dense regions are measured using Nikon NIS-Elements software. Significance is calculated using only the 10 largest crystals between experimental and control groups.

Results:
No significant toxic affects of 2FA are observed in intact or dechorionated embryos, regardless of the development stage at time of exposure (Table 1). The eosin-dense regions of the myotome cells show significantly smaller crystals ($p<0.5$).

Table 1: **Survival after 2FA exposure of intact and dechorionated ZFEs.** Each development stage has a specific time frame and corresponding exposure duration: the blastula stage spans from 2.25 to 5.25 hours post-fertilization (hpf) with an exposure time of 3 hours; the gastrula stage from 5.25 to 10.33 hpf with 5 hours of exposure; the segmentation stage from 10.33 to 24 hpf with 14 hours of exposure; the pharyngula stage from 24 to 48 hpf with 24 hours of exposure; and finally, the hatching stage from 48 to 72 hpf, also with 24 hours of exposure.

Intact Embryos							
	Pos. Ctrl (n=80)	Neg. Ctrl (n=88)	Blastula (n=90)	Gastrula (n=90)	Segmentation (n=90)	Pharyngula (n=80)	Hatching (n=60)
Survival at 72hpf	90.9 ± 9.3%	94.3 ± 10.2%	96.7 ± 5.0%	88.9 ± 11.7%	97.8 ± 4.5	97.5 ± 4.6%	98.3 ± 4.1%
Dechorionated							
	Pos. Ctrl (n=70)	Neg. Ctrl (n=70)	Pharyngula (n=70)		Hatching (n=70)		
Survival at 72hpf	94.3 ± 5.3 %	90 ± 14.1%	95.7 ± 7.9%		87.1 ± 18.9%		

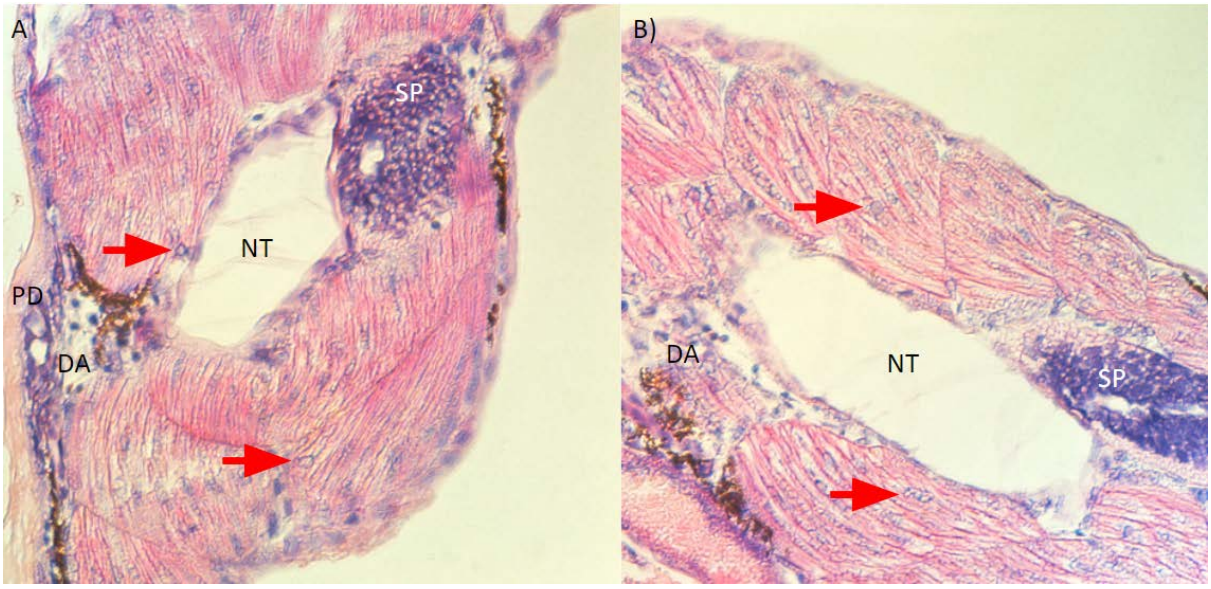


Figure 1: Sagittal perspective of cryopreserved H&E-stained ZFEs at 72 hours post-fertilization. Visible structures include: the notochord (NT), spine (SP), dorsal artery (DA), and pronephric duct (PD). Ice crystals are indicated by arrows. Image A shows embryos treated with 2FA, while image B displays those without any treatment. 40x magnification.

Conclusions:

2FA successfully permeates the developing body of the ZFEs and inhibits ice recrystallization significantly. Unlike traditional CPAs, it is also not toxic to the development of the embryo. This technology may lower the required concentrations of CPAs and reduce lethal ice formation.

Name: Zoe Turner – MSc

Title:
Quantification and Characterization of the Endogenous Polyclonal Antibodies by ImmunoAffinity-Mass Spectrometry

Authors:
Zoe Turner, Weize Tang, Yasmine Rais and Andrei P. Drabovich

Background and Aim:
Indirect immunoassays are currently the only method to quantify the endogenous antibodies. They are simple and high throughput but suffer from semi-quantitative measurements, cross-reactivity, and inability to characterize antibodies. Mass spectrometry and proteomics have enabled the development of a proteomic toolbox for specific, sensitive and reproducible quantification and profiling of immunoglobins.

Material and Methods:
The proteomic toolbox can be utilized for enrichment by IA-MS, characterization and quantification of antibodies. We utilize NISTmAb8671 antibody and its interaction with the RSV F antigen (RSV-F) to evaluate our toolbox. In the toolbox is antigen affinity enrichment, complementary proteases digestion, and Mass Spectrometry measurements. Heavy-labelled internal standards enabled 'absolute' quantification of all isotypes and subclasses of human immunoglobins. Simple experimental design, fast liquid chromatography and mass spectrometry will provide robust, reproducible, sensitive, and high throughput method.

Results:
Cleavage and reduction of NISTmAb by IgdE protease generated antibody fragments. Using LC-MS fragment masses were accurately determined enabling paring of light and heavy chains. Furthermore, digestion of NISTmAb revealed proalanase and trypsin as complementary proteases. High-affinity interaction between NISTmAb and RSV-F protein was utilized to validate IA-MS assays and characterization polyclonal antibodies enriched from serum. Quantification of anti-RSV-F antibodies revealed IgG1, IgA1, and IgM as the most abundant isotypes. Interestingly, we also detected IgG2, IgG4 and IgA2 isotypes, which may indicate differences in the immune response to RSV.

Conclusions:
Further developments of IA-MS assays will aid the development of serological assays, provide a platform for precision serology, and uncover mechanisms of immune response.

Name: Taylor Walsh – MSc

Title:
Universally screening for chlamydia and gonorrhea in a post-natal population at a single hospital site in Edmonton, Alberta

Authors: **Taylor M. Walsh[1,2]**, Sabrina S. Plitt[3,], Carmen L. Charlton[1,2,4,5]

[1]Deparment of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB
[2]Women and Children's Health Research Institute (WCHRI), University of Alberta, Edmonton, AB
[3]School of Public Health, University of Alberta, Edmonton, Alberta, Canada
[4]Alberta Precision Laboratories, Edmonton, AB
[5]Li Ka Shing Institute of Virology, University of Alberta, Edmonton, AB
School of Public Health

Background:
In 2018, Alberta implemented universal first-trimester and risk-based third-trimester screening for chlamydia and gonorrhea (CTNG). The Public Health Agency of Canada recommends universal third-trimester screening. Here we examine a universal third-trimester screening program in a single hospital site in Edmonton to determine implementation success and positivity outcomes.

Material/Methods:
Between March 20th and October 17th 2023, labour and delivery and post-partum units at the Royal Alexandrea Hospital (RAH) obtained opt-out consent from individuals participating in the study. Urine specimens were analyzed at ProvLab and data outputs were extracted (ProvLab LIS). STATA v.17 was used to analyze corresponding outcomes. Provincial risk-based (third trimester/at birth) CTNG screening data from March 20 to October 17, 2022 was analyzed as a comparator group.

Results:
A total of 3,761 individuals were admitted to the participating RAH units. 600 individuals participated (16.0% participation rate). The majority (51.0%) of participating individuals were from the lowest income quintiles and the majority (85.9%) resided in metropolitan areas. A positivity rate of 2.3% was identified (14 positive results (10 CT, 2 NG, 2 CTNG)). In comparison provincially, out of 27,548 prenatal women, 3,927 were screened, and 92 were positive (14.3% participation and 2.3% positivity rate).

Conclusions:
Due to low participation levels, study outcomes are not reflective of a universal program. Results show positivity rates are approximately equal to current provincial risk-based screening outcomes. Low participation rates and potential screening bias require that additional research be performed to determine if universal third trimester screening is superior to current testing.

Name: Tetiana Davydiuk - PhD – Post candidacy

Title:
Characterization of arsenic speciation profiles using simultaneous elemental and molecular mass spectrometry (HPLC-ICPMS/ESIMS).

Authors:
Davydiuk T., Uppal J. S., Lu X., Ahsan H., Pierce L. B., Graydon J. A., Reichert M., Le X. C.

Background and Aim:
Inorganic arsenic (iAs) is one of the contaminants of the highest priority with more than 90 million people worldwide estimated to be exposed to elevated iAs concentrations. To estimate exposure magnitude and metabolic efficiency of iAs, most studies focus on measurement of five arsenic species in urine: arsenite (iAs^{III}), arsenate (iAs^{V}), monomethylarsonic (MMA), dimethylarsinic (DMA) acids, and arsenobetaine (AsB). Exposure to complex organic arsenicals may alter arsenic metabolic profiles. Therefore, comprehensive characterization of arsenic metabolites would allow more accurate interpretation of the arsenic metabolism and types of exposure.

Material and Methods:
Urine samples (n = 1873) were provided by the volunteers of Health Effects of Arsenic Longitudinal Study based in Araihazar, Bangladesh. We analyzed iAs metabolites (iAs^{III}, iAs^{V}, DMA, MMA) and arsenobetaine (AsB) in the samples using anion exchange high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICPMS). To achieve more detailed characterization, simultaneous elemental and molecular characterization was performed for a wider range of arsenic species using HPLC-ICPMS/ESIQTRAP.

Results:
Total arsenic concentrations in urine ranged from 0.9 up to 2633.6 µg/L. Arsenic species had high detection rates (≥ 98%), except for iAs^{III} (detected in 71% of the samples). Unknown arsenic peaks were detected in 40.1% of the samples. Simultaneous elemental and molecular characterization was successfully performed for a range of arsenic species achieving low detection limits in both ICPMS and ESIQTRAP.

Conclusions:
Detailed characterization of urinary arsenic metabolites can be achieved by simultaneous elemental and molecular characterization. This approach has the potential to be applied to other types of samples.

Name: Huyan Xiao - PhD – Post candidacy

Title:
Construction of a DNAzyme motor system enabling cancer cell imaging without the need for washing

Authors:
Huyan Xiao, Jeffery Tao, Jingyang Xu, Wei Feng, X. Chris Le, Hongquan Zhang

Background and Aim:
Cancer imaging and immunohistochemical staining (IHC) are commonly used for identifying specific antigens in cells and tissues for diagnosis and prognosis. However, the operation of IHC is laborious and time-consuming. It requires extensive blocking to decrease the non-specific adsorption of antibodies and tedious washing to remove unbound antibodies. This research aims to construct a DNAzyme motor system that generates localized, amplified fluorescence signals upon the binding of two affinity ligands to the same cell surface protein molecule without the need for washing. The need for two binding events for signal generation significantly reduces the background from non-specific adsorption.

Material and Methods:
We constructed the DNAzyme motor system by using a magnetic nanoparticle (MNP) as the scaffold onto which hundreds of track strands and tens of inactivated DNAzyme motors were conjugated. We designed two probes enabling the binding of specific proteins to trigger autonomous walking of the DNAzyme motor along the MNP. Each walking step restores the fluorescence of a pre-quenched dye molecule localized on MNP. Therefore, multiple dye molecules can be lighted up in response to a single protein molecule, achieving amplified detection.

Results:
We constructed a DNAzyme motor system and tested it for imaging cancer cells. The motor system was able to image HER-2 on the breast cancer cell without the need for washing or separation.

Conclusions:
We have successfully constructed the DNAzyme motor system responsive to a specific protein and achieved cancer imaging. We will further apply the motor system to IHC staining.

Name: JingYang Xu - PhD – Post candidacy

Title:
Extraction, concentration, and preservation of viral RNA from saliva and gargle samples

Authors:
JingYang Xu[1], Huyan Xiao[1], Yanming Liu[1], Bo Pang[1], Jeff Tao[1], Wei Feng[1], Teresa Kumblathan[1], Xiaojian Chen[1], Michael A. Joyce[2], D. Lorne Tyrrell[2], X. Chris Le*[1], and Hongquan Zhang*[1]

Background and Aim:
Robust and reliable methods for specimen collection and treatment are essential for clinical diagnosis and surveillance of infectious diseases. Saliva and gargle are alternative sampling options to the conventional nasopharyngeal swabs for diagnosis of respiratory infections, such as COVID-19. The collection of saliva and gargle is minimally invasive, and the specimens are readily available. However, due to the dilution of viral particles in a large sample volume and the complexity of saliva and gargle matrices, there is a need for an efficient and simple method to extract, concentrate, and preserve viral RNA from saliva and gargle samples. We report a method for extracting viral RNA containing a 3' poly-A tail, found in positive-sense RNA viruses, from biological samples.

Material and Methods:
We applied poly-T conjugated magnetic beads for capturing viral RNA from gargle and saliva. We developed an extraction buffer that enabled efficient lysis of viral particles and preservation of the released viral RNA.

Results:
RNA extraction can be completed within 10 minutes at room temperature. The released viral RNA remained stable at ambient temperature for more than 2 weeks. The extraction buffers were compatible with the subsequent nucleic acid amplification and analysis. We achieved near 100% recovery of viral RNA and were able to detect as few as 10 SARS-CoV-2 viral particles in 100 µL of saliva or gargle samples using RT-qPCR.

Conclusions:
We demonstrated an application of the method for the detection of viral RNA in saliva and gargle. We also coupled this method to reverse transcription loop-mediated isothermal amplification (RT-LAMP) for point-of-care detection.

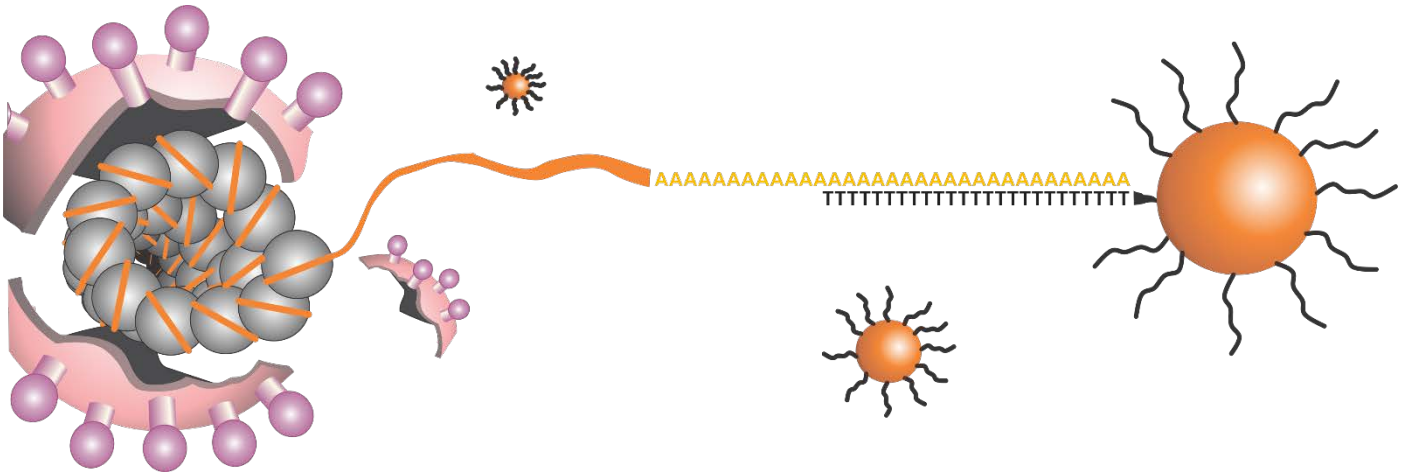


Figure 1: Diagram of viral RNA extraction vial poly-T magnetic beads.

Name: Mahsa Yazdanbakhsh - PhD – Pre candidacy

Title:
Young RBC Subpopulation from Frequent Senior Blood Donors Has Lower Hemoglobin-Oxygen affinity

Authors:
Mahsa Yazdanbakhsh, Jason Acker

Background and Aim:
Red Cell Concentrates (RCCs) contain cells at different stages of their life cycle. p50 refers to the partial pressure of oxygen (pO2) in blood at which hemoglobin is 50% saturated with oxygen. Frequent blood donations may alter the quality of blood components by modulating RBC characteristics.
Aims: Evaluate hemoglobin-oxygen affinity of the subpopulation of “young” and “old” RBCs during hypothermic storage as a function of the blood donor age and frequency of blood donation.

Material and Methods:
RCCs were collected from healthy frequent teenagers (n=5), non-frequent teenagers (n=5), frequent seniors (n=5) and non-frequent seniors (n=5). Samples were percoll-density separated into Y-RBCs and O-RBCs. Changes in MCV, MCHC, and p50 were assessed on days 5,14, 28, and 42 of storage using established methods in our group.

Results:
The lowest Hb-Oxygen affinity was related to the Y-RBCs from frequent senior blood donors over all storage periods (21.72 ± 3.01 mmHg). Also, there was a significant difference between Y-RBCs non-frequent seniors and non-frequent teenage donors on day 42 of storage in all RBC age groups (p= 0.0038). The highest Hb-oxygen affinity (lowest p50) was related to the O-RBCs from non-frequent teenage blood donors compared to all other blood groups (p=0.0013).

Conclusions:
The frequency of blood donations might impact the distribution of Y-RBCs, by inducing Y-RBCs release to blood circulation and influencing the hemoglobin-oxygen affinity of red blood cells. This, in turn, could affect the effectiveness of blood transfusions, leading to higher oxygen release in recipients receiving blood from frequent donors.

Name: Qiming Shen - Post Doctoral Research Fellow

Title:
Advanced Nontarget Characterization of Amine-containing Compounds in Suwannee River Standard Reference Materials

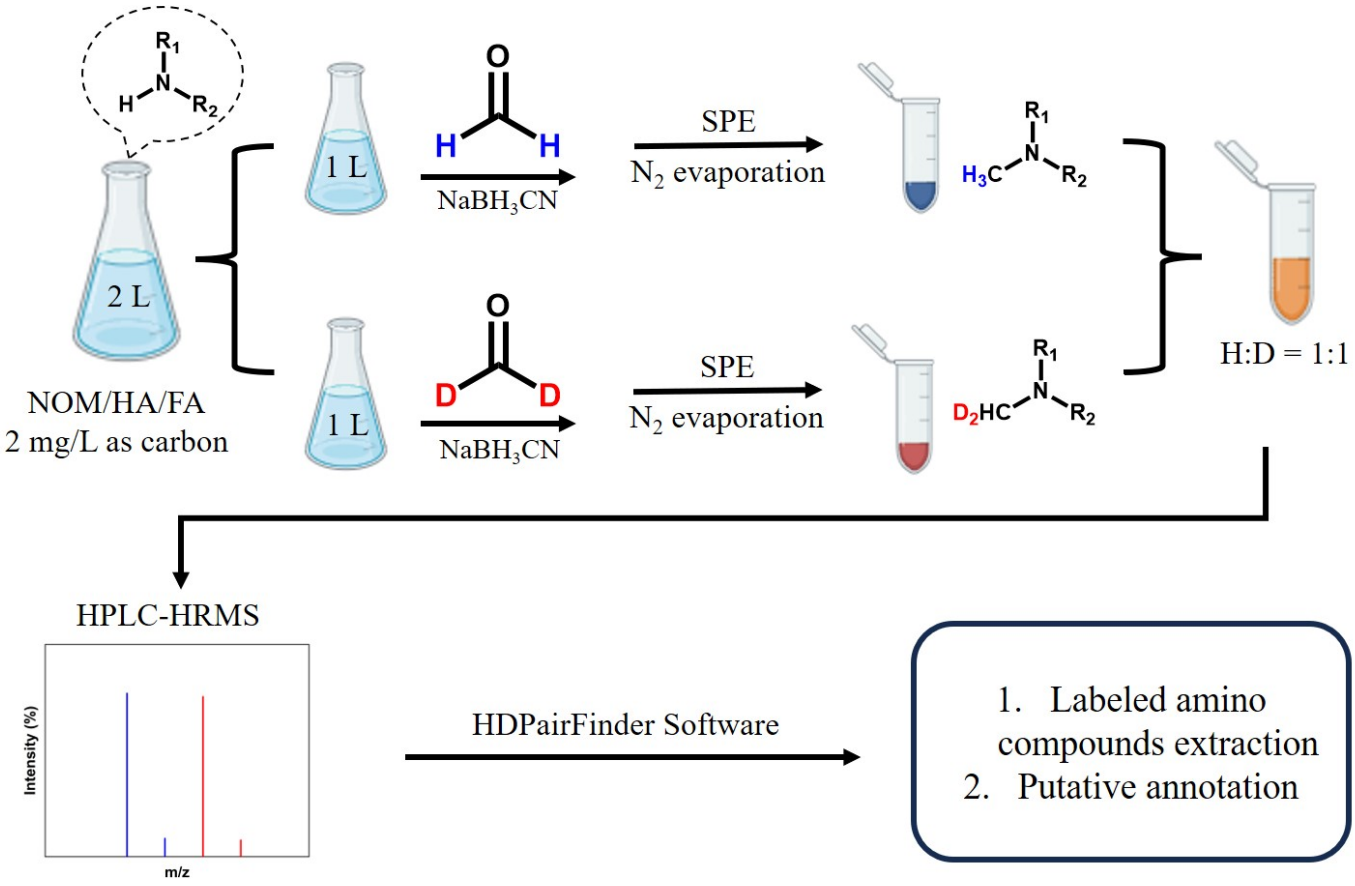
Authors:
Qiming Shen; Nicholas J. P. Wawryk; Tingting Zhao; Di Zhang; Kristin Carroll; Tao Huan; Xingfang Li

Background and Aim:
Concerns arise about nitrogenous disinfection byproducts (N-DBPs) due to their higher toxicity than regulated DBPs. Studies indicate that a substantial proportion of N-DBP precursors consists of amine groups. Comprehensive characterization of amine-containing precursors in source water is necessary for N-DBPs control. The Suwannee River standard reference materials (SRSRMs) have been widely used as a precursor material to study DBP formation. However, the chemical composition of these standard reference materials remains unknown. Our study aims to fill this knowledge gap by investigating the amine-containing chemicals in SRSRMs.

Material and Methods:
Stable HD isotopic labeling targeted amine-containing species by labeling amino groups with H/D methyl groups. After isotopic labeling and sample concentration, the water samples were analyzed using HPLC-HRMS. The generated HPLC-HRMS datasets were then processed using HDPairFinder to extract the labeled amine-containing compounds, which were matched against the AMINES library to find potential candidates.

Results:
After HDPairFinder processing, 7695 amino-labeled features were extracted from three SRSRMs (SRNOM, SRHA, SRFA), with 2707 high-quality features. 61.5% were putatively annotated as amine-containing compounds. SRNOM, SRHA, and SRFA had 1709, 1642, and 751 qualified features. They shared 327 features, each retaining unique ones. Most features had one or two methyl group tags, indicating primary and secondary amines. In NSR water samples, 6449 features were found, with 118 frequently detected, and 40.7% matched qualified SRSRMs features.

Conclusions:
The innovative methods provided insights into the amino-containing chemicals in Suwanee River Standard Reference Materials, enabling advanced water research.


2 L
NOM/HA/FA
2 mg/L as carbon
1 L
1 L
NaBH3CN
NaBH3CN
SPE
N2 evaporation
SPE
N2 evaporation
H:D = 1:1
HPLC-HRMS
Intensity (%)
m/z
HDPairFinder Software
1. Labeled amino compounds extraction
2. Putative annotation

Name: Surangi Thilakarathna - Post Doctoral Research Fellow

Title:
The optimization of a viability real-time PCR assay to detect viable *Salmonella* spp. in diarrheal stools: a spiked-stool study

Authors:
Surangi H. Thilakarathna and Linda Chui

Background and Aim:
Viability PCR (vPCR) is a real-time PCR (qPCR) assay where, a viability dye such as PMAxx™ is used to remove dead-cell DNA to selectively amplify live-cell DNA. In a pilot study, we reported an application of the vPCR on patients' diarrheal stools. As our next phase, we investigated the effects of stool concentration and PMAxx™ treatment conditions on stool-vPCR.

Material and Methods:
The optimum PMAxx™ treatment conditions (concentration: 100 vs 200 μM and dark incubation time: 10 vs 30 min) were determined by maximum heat-killed (HK)-cell removal in 5% stool suspensions. The effect of stool concentration on stool-vPCR was assessed by applying the PMAxx™ treatment on 5,10 and 20% *Salmonella*-negative pooled liquid and semi-solid stool suspensions spiked with live and HK-*Salmonella* dilutions. In parallel, qPCR and vPCR were performed on 5, 10, and 20% laboratory culture-confirmed *Salmonella*-positive stool suspensions.

Results:
A 100 μM PMAxx™ and a 10 min dark incubation time performed better than the other treatment conditions. The best stool-vPCR result was observed with the 5% spiked stool suspension for both stool types in which 10^7 CFU/mL HK-cell DNA was completely removed. Similarly, 5% stool suspensions provided better vPCR results when spiked with live cells. Stool concentrations showed no effect on the vPCR results of *Salmonella*-positive clinical stools ($P>0.05$).

Conclusions:
Here, we investigated the various factors affecting the stool-vPCR. With assay validation using a larger number of patient-submitted stools across multiple gastroenteric bacterial pathogens, vPCR assay has a great potential as a viability assessment tool in a clinical setting.

Name: Danielle Anderson – Resident

Title:
Process Improvement Recommendations to Optimize Detection of Microfilariae

Author:
Danielle Anderson, MD

Background and Aim:
Loiasis is caused by the migratory filarial nematode Loa loa, colloquially known as the African eye worm. Transmitted by deer fly (*Chrysops*) vectors, microfilariae circulate in the peripheral blood of the human host with diurnal periodicity. This case describes an incidental diagnosis of loiasis following manual slide review of a CBCD flagged by the hematology analyzer for microcytosis. It highlights the deficiency in current automated systems to detect microfilariae and suggests lower thresholds for manual slide review are needed.

Material and Methods:
CBCD request on a 71-year-old female ran on the Beckman-Coulter DxH-900 failed autoverification due to "RBC/PLT overlap." This prompted manual slide review and incidental detection of microfilariae. Subsequent review of thick and thin Giemsa-stained blood smears allowed speciation and quantitation. Review of CBCDs, physical exam and subsequent history provided supportive findings.

Results:
The microfilariae were characterized by a short head space, head-to-tail nuclei, colourless sheath, 240µm length; speciation consistent with Loa loa, with a parasitemia of 3337/mL. Review of available CBCDs revealed longstanding microcytic anemia with variable eosinophilia (Table 1). History was not available at diagnosis, though subsequently revealed intermittent limb swelling and recent emigration from a rural village in Cameroon, West Africa.

Conclusions:
Hematology analyzers flag eosinophilia exceeding $3.0x10^9$/L, though patients with loiasis may present with only mild eosinophilia, leading to missed diagnoses by current automated systems. To optimize microfilaria detection, any eosinophil count above the upper reference limit should prompt manual slide review with focused assessment of the feathered edge.

Table 1. Comparison of Relevant Hematologic Parameters

Date	Hb (g/L)	MCV (fL)	EOS (x10^9/L)	PBS (parasitemia)	Treatment Status
2015-Jul-15	82	67	5.1	Not performed	Pre-treatment
2021-Dec-22	75	59	0.7	Initial microfilaria detection (3337/mL)	Pre-treatment
2021-Dec-24	76	58	1.0	Not performed	Pre-treatment

Table 1. Comparison of Relevant Hematologic Parameters

Date	Hb (g/L)	MCV (fL)	EOS (x10^9/L)	PBS (parasitemia)	Treatment Status
2022-Sep-02	124	88	0.2	Microfilariae detected (4050/mL)	Post-treatment with albendazole
2023-Nov-03	130	88	0.2	Very scant and degenerated microfilariae seen	Post repeat treatment with albendazole

Name: Nina Esfandiari - Resident

Title:
Validation of a Virtual Reality Headset Display for Digital Pathology Sign-Out

Authors:
Nina Esfandiari, David Beyer

Background and Aim:
The virtual reality (VR) environment offers a new platform through which digital slides can be viewed. The College of American Pathologists (CAP) released revised validation guidelines for digital pathology and whole slide imaging (WSI) for primary diagnosis. Research on the use of VR technology for digital pathology sign-out is sparse. The aim of this study is to determine whether VR technology can provide similar results to other WSI viewer modalities for digital pathology sign-out.

Material and Methods:
Forty cases originally reviewed by the resident over one year were scanned (AperioGT450 scanner). Cases included adult participants with varying pathologies, half requiring ancillary studies. A blinded retrospective review was done by two independent observers (Pathologist and Pathology resident) using an Oculus Quest 2 headset and VR technology. The recorded endpoint was the concordance rate of the diagnosis made using VR versus other modalities.

Results:
Forty cases were reviewed (72 slides/reads). The major diagnostic concordance rate was 100% and minor discordance rate was 97%, demonstrating appropriate grading for malignant cases and interpretation of ancillary studies. The VR headset could be easily operated. Subjective feedback on image quality on the VR headset was felt to be inferior to a monitor, but still acceptable.

Conclusions:
The Oculus Quest 2 VR headset is a satisfactory alternative platform to review digital slides for pathology sign-out. Image quality is a limitation. As this technology continues to improve, there is great potential to adapt the VR environment to create an optimal digital pathology sign-out system tailored to each Pathologist.

Name: QingYun Hua – Resident

Title:
Isohemagglutinin Titration in Pooled and Apheresis Platelets

Authors:
QingYun Hua, Bruce W Lyon, Jennifer Duke, Amanda Felske, Karen Hobbs, Ryan Holman, Ghazala Radwi, Davinder Sidhu, Gwen Clarke, Susan Nahirniak

Background and Aim:
Platelet inventory constraints necessitate ABO-incompatible platelet transfusion. Many labs minimize the hemolytic impact by confirming low titre (LT) donor isohemagglutinins. This process is costly.

Pathogen-reduced platelets (PRP) and platelet additive solutions (PAS) will dilute plasma and decrease high titer isohemagglutinins (HT). We determined the proportion of HT platelets and incompatible transfusions and reassessed the need for titres following introduction of PRP/PAS.

Material and Methods:
Our titre method is manual tube (1:50) dilution of platelet supernatant tested with A1/B red cells. Testing included 49,058 pooled and 11,738 apheresis platelets over 4 years. The HT proportion was determined along with the rate of out-of-group transfusions and hemolytic reactions. The impact of PAS dilution was estimated.

Results:
60,796 platelet units were tested. Group O pooled and group B apheresis platelets had HT in 6.6% and 5.7% respectively. Group A pooled and apheresis platelets included 2% with HT. Approximately 25% of platelets transfused were ABO incompatible and no hemolytic reactions were reported. Based on the proportions of PAS-E and plasma for PRP platelets, plasma from each donor comprises 11ml (6% of total volume) vs 20-257ml in untreated pools. PAS-E will replace and therefore dilute residual plasma by at least 50%. Preliminary testing of 20 PRP components did not identify HT units.

Conclusions:
Rare platelet pools may demonstrate HT. PRP platelets with PAS will reduce titres and may abrogate the need for titration. A strategy of group specific transfusion or transfusion of group A PRP platelet transfusions may be a safe alternative.

Name: Shuang (Candy) Niu – Resident

Title:
Comparison of Automated Solid Phase vs Manual Saline Indirect Antiglobulin Test Methodology for non-ABO Antibody Titration: Implications for Perinatal Antibody Monitoring

Authors:
Shuang Niu, Megan Vetsch, Lynnette Beaudin, Melanie Bodnar, Gwen Clarke

Background and Aim:
Accurate antibody titration is crucial in prenatal evaluations to identify patients who need clinical monitoring for hemolytic disease of the fetus and newborn (HDFN) causing fetal anemia. This study compares the established gold standard method of manual tube saline indirect antiglobulin testing (SIAT) with the newer automated solid-phase (ASP) method of antibody titration and aims to establish the critical titre threshold for ASP that corresponds to the previously established SIAT critical threshold of ≥16 used in our lab.

Material and Methods:
157 prenatal and donor plasma samples with known antibodies were tested using both SIAT and ASP methodologies and results were compared.

Results:
The study found that ASP titres were, on average, 1.33 dilutions higher than SIAT titres. The critical titre cut-off for ASP was determined to be ≥32, which is one tube higher than the SIAT cut-off of ≥16.

Conclusions:
The ASP method for antibody titration offers greater reproducibility and efficiency compared to manual SIAT titration. This study suggests that a titre cut-off of ≥32 is appropriate for most clinically significant antibodies using ASP. However, further research is needed to determine the comparability of ASP with SIAT in samples with multiple antibodies, anti-M antibodies, and other less common antibodies. Validation of the ASP titre cut-off against HDFN clinical outcomes is required before implementing this test for routine use in perinatal antibody titration.

Name: Shubham Shan - Resident

Title:
Electron Microscopy for evaluation of ultrastructural cellular features from differentially perfused myocardial samples

Authors:
Shan S.D., Adam B.A., and Hatami S.

Background and Aim:
Electron microscopy (EM) can be used to assess ultrastructural cellular features in various clinical, laboratory and investigational settings. Standardization of assessment of experimental myocardial samples from porcine transplant specimens using EM is necessary for appropriate evaluation.

Material and Methods:
EM photographs from porcine myocardial specimens from three different groups (in-vivo, heart only, and heart and liver (H+L) groups) were evaluated for the following: myofibril degeneration, myofibril contraction bands, and interstitial edema (Grade 1-4), mitochondrial count and mean size (μm^2) (Figure 1). P-values were calculated using the Mann-Whitney U Test.

Results:
The results from electron micrography assessment between the three groups are reported in Table 1. Myofibril degeneration was grade 1 for the in-vivo, 2.8 for the H+L and 3.3 for the heart only (p-value of 0.018 for when IV was compared to both heart only and H+L ESHP group. Myofibril contraction bands were graded as 1.8 for the in-vivo, 2.3 for the H+L ESHP, and 3.0 for the heart only groups. Interstitial edema was graded as 2.0 for the in-vivo, 2.3 for the H+L ESHP, and 3.0 for the heart only groups. Mitochondrial count (at 1000x) was 314 for the in-vivo group, 293 for the H+L, 296 for the heart only groups. Mitochondrial mean size (μm2) was 1.7 for the in-vivo, 1.3 for both the H+L and heart only groups.

Conclusions:
Both ESHP groups showed a trend towards more injury compared with the in vivo controls, but only myofibril degeneration reaches statistical significance. The H+L group shows a trend towards less injury compared with heart-only, but the differences are not statistically significant. Nuclear size and shape were assessed but too few cardiomyocyte nuclei were present to provide a confident analysis.

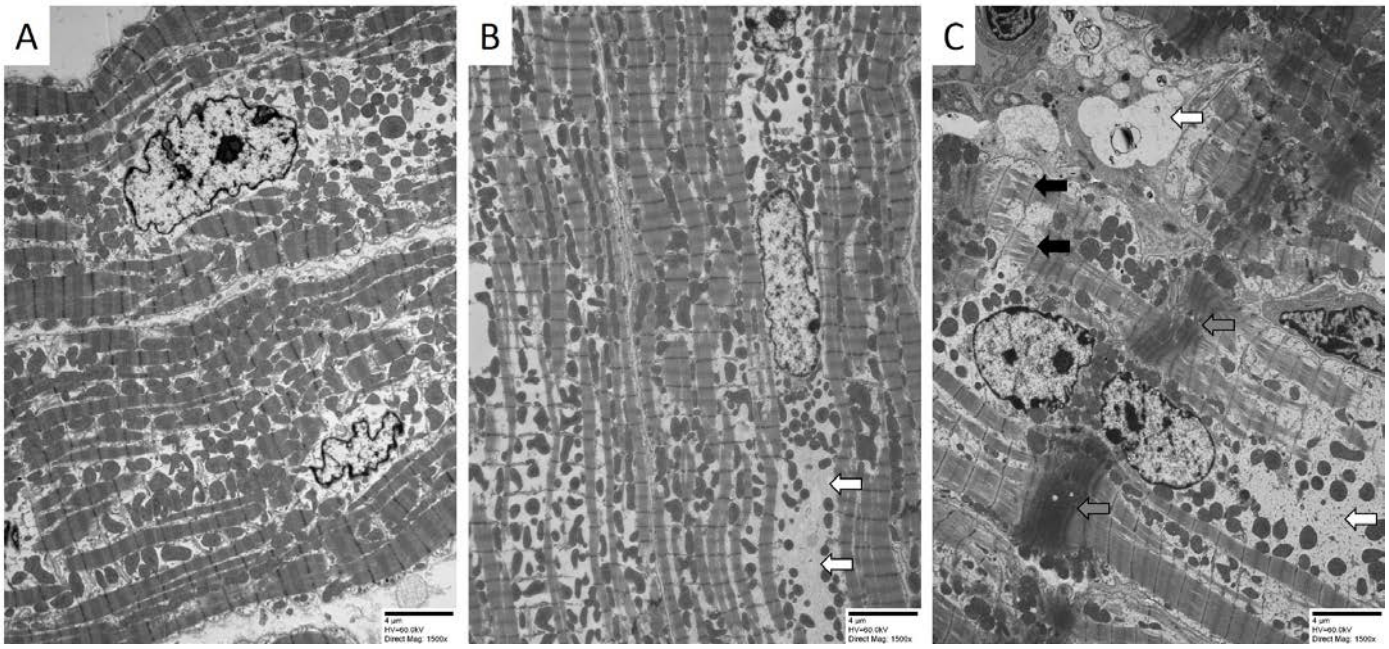


Figure 1: Electron micrographs of myocardial samples from the three groups: A is in-vivo, B is H+L, and C is heart only ESHP groups. Examples of EM features: white, grey and black arrows highlight edema, contraction bands and myofibril degeneration, respectively.

Table 1: Summary for EM features evaluated amongst the three different groups: in-vivo, heart only, and H+L ESHP groups. Five features were assessed and reported. The only statistically significant parameter was myofibril degeneration: grade 1 for the in-vivo, 2.8 for the H+L and 3.3 for the heart only ESHP groups with a statistically significant p-value of 0.018 for when IV was compared to both heart only and H+L ESHP group. There was no difference in between the heart only and H+L ESHP groups. All of the other four parameters were not statistically significantly amongst the three groups and are reported above.

EM Feature	In vivo (n=4)	H+L ESHP (n=4)	Heart only ESHP (n=4)	p-value (IV vs. H+L)*	p-value (IV vs. H only)*	p-value (H+L vs. H only)*
Myofibril degeneration (Grade 1-4)	1.0 (1-1)	2.8 (2-3)	3.3 (3-4)	**0.018**	**0.018**	0.257
Myofibril contraction bands (Grade 1-4)	1.8 (1-3)	2.3 (2-3)	3.0 (2-3)	0.436	0.134	0.206
Interstitial edema (Grade 1-4)	2.0 (2-2)	2.3 (2-3)	3.0 (2-4)	0.453	0.067	0.206
Mitochondria count at 1000x (#)	314 (305-334)	293 (230-353)	296 (200-372)	0.343	1.000	1.000
Mitochondria mean size (μm^2)	1.7 (1.3-2.3)	1.3 (0.8-1.7)	1.3 (1.0-1.6)	0.343	0.200	1.000

*Mann-Whitney U test (Wilcoxon rank sum exact test for continuous data, Wilcoxon rank sum test with continuity correction for ordinal data)

Name: Saadiya Umar – Resident

Title:
SARS-CoV-2 seropositivity in Alberta: The Omicron era

Authors:
Sa'adiya K Umar , Jamil N Kanji, Carmen Charlton, Leonard T Nguyen, Sabrina S Plitt, Sheila Braun, Candace Betiku, Carol Marohn, Cheryl Lau, Mark A Joffe, Christie Lutsiak, Nathan Zelyas, Michael Mengal, Graham Tipples

Universities of Alberta and Calgary, Alberta Precision Laboratories, Public Health, Alberta and Department of Laboratory Medicine and Pathology.

Background and Aim:
We systematically evaluated SARS-CoV-2 seropositivity in Alberta pre-/post-introduction of the Omicron variant to review local COVID-19 epidemiology for public health decision making.

Material and Methods:
Residual blood samples across the province underwent SARS-CoV-2 antibody testing over 1-week periods every 3 months, September 2021 – October 2022 (**Figure).** Anti-nucleocapsid-IgG was done on all samples, and anti-receptor binding domain (RBD)-IgG done on specimens from unvaccinated individuals. The seropositivity for each sampling period evaluated, and linked to provincial administrative, laboratory, and vaccine databases.

Results:
50,187 samples from 47,282 individuals were surveyed (56.2% female; median age 55.6 [IQR 37-70] yrs). 6,637 (14.0%) were unvaccinated (57.2% female, median age 44 [IQR 27-63] yrs), **(Table**). Among the unvaccinated, RBD-IgG seropositivity was 37.5% (95% CI 34.5-40.5%) in October 2021 with a rise to 74.8% (95% CI 72.5-77.1%) in October 2022. Anti-nucleocapsid IgG increased from 6.8% (95% CI 6.3-7.4%) to 27.3% (95% CI 26.2-28.3%) from January 2022-October 2022. Between July and October 2022, the change in seropositivity of anti-RBD among unvaccinated was significant (5.6%; p=0.002) whereas the rise in anti-N was not (0.1%; p=0.17). In all periods, anti-nucleocapsid seropositivity was significantly higher among the unvaccinated group compared to those who were immunized (2-doses) or boosted (3+ doses) (all comparisons $p<0.05$).

Conclusions:
Despite high volume of infections with the Omicron variant, vaccination led to a reduced risk of infection. The initial surge of Omicron was observed in younger age groups. By July 2022, the population infection rate was constant. Longitudinal SARS-CoV-2 serosurveillance with data linkage provides valuable information for public-health policy decision making.

Figure:

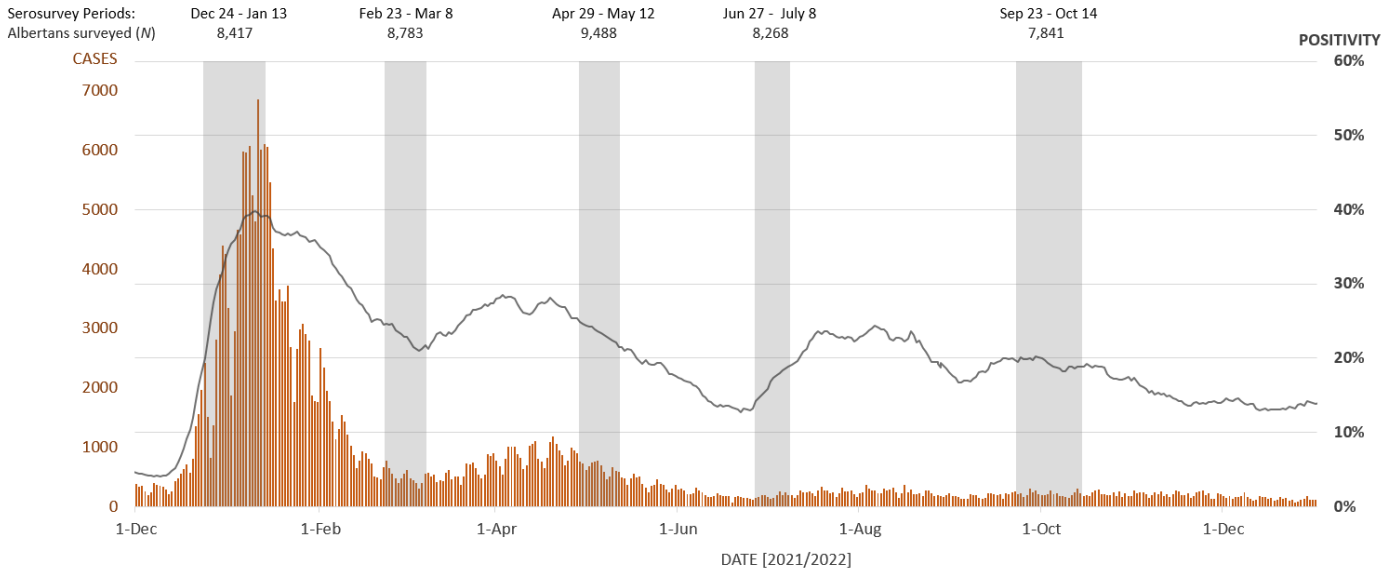


Table:

Table 1. Demographics of individuals surveyed stratified by vaccination status

	Vaccinated Albertans			Unvaccinated Albertans		
Survey period	Number tested (*n*)	Number female (%)	Median Age (years, IQR)	Number tested	Number female (%)	Median Age (years, IQR)
October 2021	5,556	3,067 (55.2)	60 (43.1-72.1)	995	550 (55.3)	46 (30-63)
January 2022	7,115	3,850 (54.1)	56 (38.7-70.2)	1,003	568 (56.6)	39 (24-60)
March 2022	7,566	4,320 (57.1)	56 (39.5-69.6)	1,215	724 (59.6)	44 (27-61)
May 2022	7,625	4,259 (55.9)	56 (37.7-69.7)	1,009	594 (58.9)	41 (25-61)
July 2022	7,187	4,045 (56.3)	57 (39.8-70.5)	1,079	633 (58.7)	43 (25-65)
October 2022	5,596	3,214 (57.4)	57 (38.9-71.4)	1,336	729 (54.6)	48 (31-66)
Total	40,645	22,755 (56.0)	57 (39.4-70.5)	6,637	3,798 (57.2)	44 (27-63)

Abbreviations: IQR – interquartile range

Comparison of ages between vaccinated and unvaccinated Albertans for all survey periods and overall is $p<0.0001$.

***Note, some had no healthcare number, therefore vaccination status unknown and are excluded in this Table**

Name: Sima Zolfaghari – Resident

Title:
VEXAS syndrome complicated by disseminated histoplasmosis: a case report

Authors:
Sima Zolfaghari, Elona Turley

Background and Aim:
VEXAS syndrome, a 2020-identified hemato-inflammatory disorder, challenges diagnosis due to limited awareness and UBA1 gene testing availability. This study presents a complex case, emphasizing morphology's role in diagnosis.

Material and Methods:
A 62-year-old male with unexplained multiorgan inflammation underwent PET-CT, bone marrow pathology, karyotyping, and UBA1 gene testing. Interventions included repeat bone marrow collection, splenectomy, antifungal therapy, and allogeneic HSCT.

Results:
PET-CT revealed abnormalities, and bone marrow pathology exhibited characteristic features. Histoplasmosis was confirmed despite initial negative findings. UBA1 testing identified an M41V mutation. Post-HSCT, assessments showed normalization and absence of the UBA1 mutation.

Conclusions:
VEXAS syndrome poses diagnostic challenges, especially when coupled with opportunistic infections. Morphologic evaluation is crucial due to limited UBA1 testing. The case underscores the intricate interplay of VEXAS and opportunistic infections, necessitating a comprehensive diagnostic approach.

Name: Gilbert Bigras - Academic/Clinical Faculty

Title:
Towards Accurate Deep Learning-Based Prediction of Ki67, ER, PR, and HER2 Status from H&E-stained Breast Cancer Images

Authors:
Amir Akbarnejad (1), Nilanjan Ray (2), Penny J Barnes (3), Gilbert Bigras (4)
1 PhD Candidate in Computing Science, Department of Computing Sciences, University of Alberta.
2 Professor, Department of Computing Sciences, University of Alberta.
3 Professor, Faculty of Medicine, Dalhousie University, Halifax, Canada
4 Associate Professor Department of Laboratory Medicine and Pathology, University of Alberta

Background and Aim:
Machine learning algorithms achieve moderate accuracy to predict molecular information from histology alone. One of the obstacles is the lack of large datasets to properly train machine learning models.

Material and Methods:
A dataset was built including 185538 breast cancer (BC) H&E and associated immunohistochemistry (IHC) images with reliable measurements for the routinely used IHC BC biomarkers: Ki67, ER, PR and HER2. IHC measurements for Ki67, ER and PR were obtained by image analysis. HER2 measurement was based on a binary label: positive if (3+) regions are present and otherwise negative. Equivocal score (2+) were excluded.

Results:
A Vision Transformer (ViT) based pipeline achieved unprecedented performances around 90% (Fig. 1) Area Under the Curve (AUC) of the receiver operating characteristic (ROC) curves. ViT outperformed the weakly supervised Clustering-constrained Attention Multiple Instance Learning (CLAM). As a first step to "explain" artificial intelligence (AI), we evaluated the ability of both classifiers to localize these high diagnostic value sub-regions by inspecting their respective "attention" heat-maps. Despite high ViT AUCROC results, heat-maps don't obviously match areas of high diagnostic value sub-regions; it might however provide direction for future work to improve AI attention within whole slide images.

Conclusions:
This work demonstrates that larger datasets combined with high-quality labels can improve the accuracy to predict molecular status of the classical BCX 4 IHCs. Current machine learning models show obvious discrepancy between human and machine learning models "attention". AI explainability might be the key to make significant progresses in the field. Our dataset was made public to trigger new proposals to improve prediction.

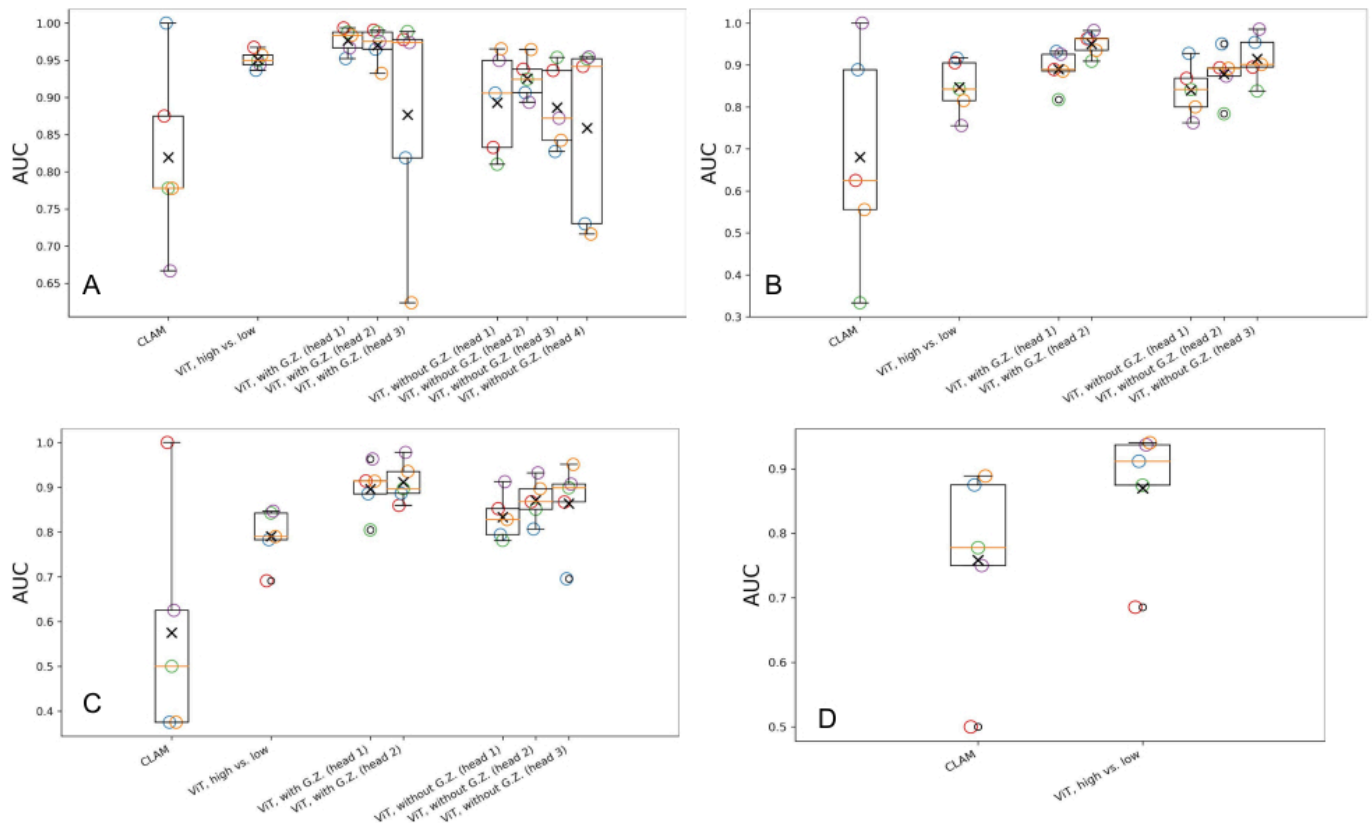


Fig. 1: Results for Ki67 (a), ER (b), PR (c), and HER2 (d).

(a) Results for predicting Ki67 status. From left to right, 1st box plot: CLAM when predicting WSI-level Ki67-percentage below 3.82 versus above 3.82. 2nd box-plot: patch-level Ki67- percentage below 3.82 versus above 3.82. 3rd-5th box-plots: patch-level Ki67-percentage (head 1: below 5 versus above 10, head 2: below 10 versus above 15, head 3: below 15 versus above 20). 6th-9th box-plots: patch-level Ki67-percentage (head 1: below 5 versus above 5, head 2: below 10 versus above 10, head 3: below 15 versus above 15, and head 4: below 20 versus above 20).

(b) Results for predicting ER status. From left to right, 1st box-plot: CLAM when predicting WSI-level ER H-score below 42.61 versus above 42.61. 2nd box-plot: patch-level ER H-score below 42.61 versus above 42.61. 3rd-4th box-plots: patch-level ER H-score (head 1: below 30 versus above 60, head 2: below 60 versus above 90). 5th-7th box-plots: patch level ER-percentage (head 1: below 30 versus above 30, head 2: below 60 versus above 60, head 3: below 90 versus above 90).

(c) Results for predicting PR status. From left to right, 1st box plot: CLAM when predicting WSI-level PR H-score below 7.373 versus above 7.373. 2nd box-plot: patch-level PR H-score below 7.373 versus above 7.373. 3rd-4th box-plots: patch-level PR H-score (head 1: below 30 versus above 60, head 2: below 60 versus above 90). 5th-7th box plots: patch-level PR percentage (head 1: below 30 versus above 30, head 2: below 60 versus above 60, head 3: below 90 versus above 90)

(d) Results for predicting HER2 status. From left to right, 1st box-plot: CLAM when predicting WSI level HER2 status positive versus negative. 2nd box plot: predicting whether 3+ patterns exist in a patch (positive/high) or not (negative/low)

Name: Jian Han - Exchange/Visiting Professor

Title:

Bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate Enhances *foxo1*-Mediated Lipophagy to Remodel Lipid Metabolism in Zebrafish Liver

Authors:

Yuxi Zhou, Fan Li, Kaiyu Fu, Yindan Zhang, Na Zheng, Huijia Tang, Zhixiang Xu, Lijun Luo, **Jian Han**, Lihua Yang, and Bingsheng Zhou.

Background and Aim:

An emerging environmental contaminant, bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH), can bioaccumulate in the liver and affect hepatic lipid metabolism. However, the in-depth mechanism has yet to be comprehensively explored.

Material and Methods:

In this study, we utilized transgenic zebrafish Tg (*Apo14: GFP*) to image the interference of TBPH on zebrafish liver development and lipid metabolism at the early development stage. Using integrated lipidomic and transcriptomic analyses to profile the lipid remodeling effect, we uncovered the potential effects of TBPH on lipophagy-related signaling pathways in zebrafish larvae.

Results:

Decreased lipid contents accompanied by enhanced lipophagy were confirmed by the measurements of Oil Red O staining and transmission electron microscopy in liver tissues. Particularly, the regulatory role of the *foxo1* factor was validated via its transcriptional inhibitor. Double immunofluorescence staining integrated with biochemical analysis indicated that the enhanced lipophagy and mitochondrial fatty acid oxidation induced by TBPH were reversed by the *foxo1* inhibitor.

Conclusions:

To summarize, our study reveals, for the first time, the essential role of *foxo1*-mediated lipophagy in TBPH-induced lipid metabolic disorders and hepatoxicity, providing new insights for metabolic disease studies and ecological health risk assessment of TBPH.

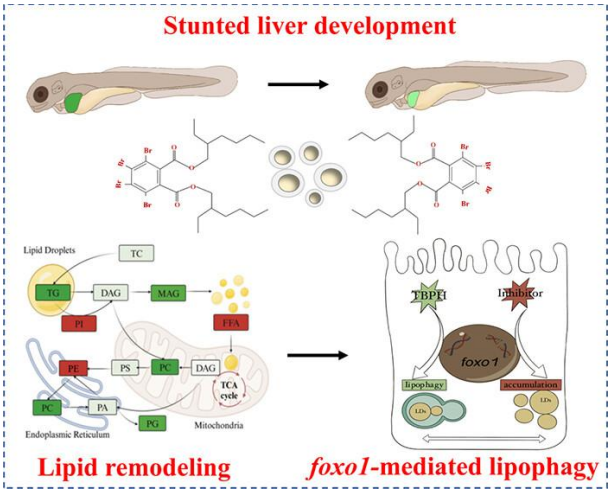


Fig.1 Graphical abstract.

Name: Hui Wang and Honghong Wang – Laboratory Scientists

Title:
CRISPR/Cas13a-responsive and RNA-bridged DNA hydrogel capillary sensor for point-of-care detection of RNA

Authors:
Hui Wang, Honghong Wang, Zhenping Li, and X. Chris Le

Background and Aim:
Disease diagnostics and monitoring increasingly highlight the importance of portable, cost-effective, and sensitive point-of-care (POC) detection of nucleic acids. Here, we report a CRISPR/Cas13a-responsive and RNA-bridged DNA hydrogel capillary sensor for the direct and visual detection of specific RNA with high sensitivity.

Material and Methods:
The CRISPR/Cas13a-responsive hydrogel capillary sensor was simply prepared by loading an ultra-thin RNA-crosslinking DNA hydrogel film (~0.2 mm ± 0.02 mm) at the end of a capillary tube. Then, the heat-treated saliva samples form SARS-CoV-2-infected and health adult volunteers were directly detected by the capillary sensor and the diagnosis results were further confirmed by RT-PCR.

Results:
All positive samples from SARS-CoV-2-infected patients produced obvious flow distance of sample solutions in the capillary tubes compared to that of the negative samples from healthy individuals as well as the blank control. In addition, quantification results are in good agreement with RT-PCR.

Conclusions:
By reading the travel distance of sample solution in the capillary tube, the capillary sensor can provide quantitative measurements using the naked eye without relying on any optical or electrical equipment. These features suggest promising potential for point-of-care and resource-limited settings.

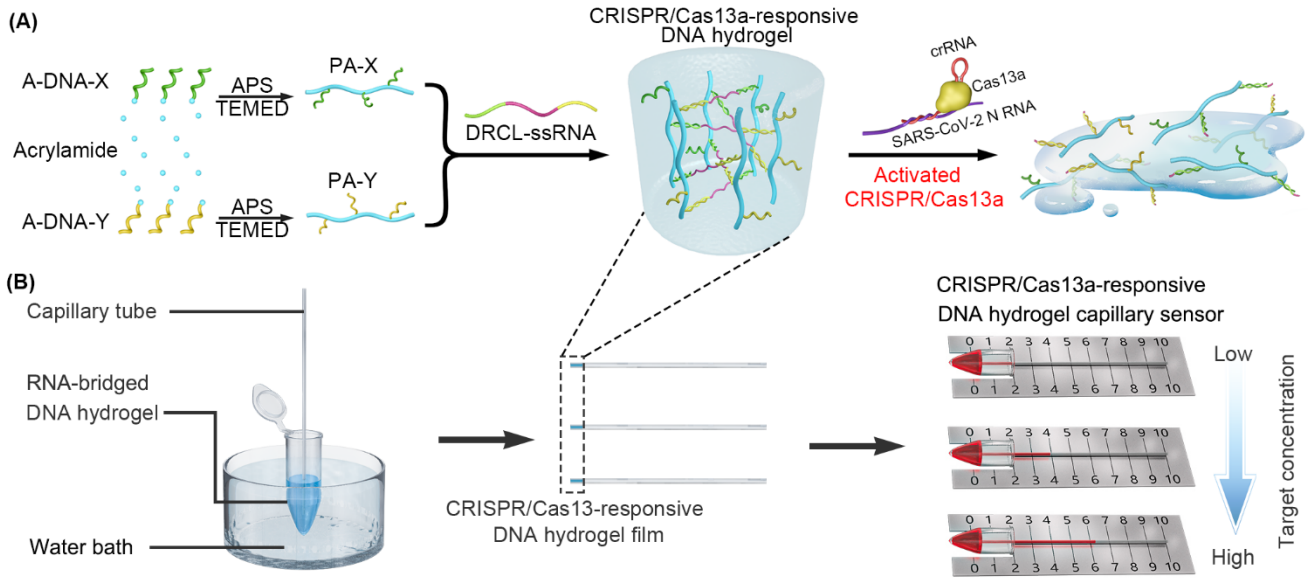


Figure 1. (A) Illustration of the design and working principle of the RNA-bridged DNA hydrogel capillary sensor for the detection of RNA. (B) The preparation and use of the CRISPR/Cas13a-responsive DNA hydrogel capillary sensor.