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Learning In Simulation (LIS):
Using curricular advancements to instill foundations of lab practice

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Background: A Laboratory Information System (LIS) is a software used to record, manage, and store clinical information. LIS competency is necessary for medical laboratory technologists as it is involved in nearly every step of the laboratory workflow. The Division of Medical Laboratory Science (MLS) developed and implemented several simulation scenarios for the first-year student curriculum that incorporated various functions of an LIS. Evaluation of student performance allowed insight into the effectiveness of using simulation to practice skills related to LIS.

Materials & Methods: The MLS-LIS Simulation Rubric (MLSR) was created using the LIS Standard Operating Procedures (SOPs) and general Canadian Society of Medical Laboratory Science competencies. This rubric was used to evaluate specific skills, behaviours and overall performance. LIS-specific tasks involved data entry, manual results entry, test cancelling and inquiry functions. Behaviours evaluated were general SOP use, communication, safety, professionalism and critical-thinking. The Simulation Thinking Category (STC) rubric categorized students’ cognitive approach to the scenario. Student perspectives on process and effectiveness were gathered using a post-simulation survey.

Results: 25 students participated in the simulations. On the STC rubric, the majority of students were categorized into the preoperational thinking category (60%). Category descriptions include: heavy reliance on SOPs, some important steps missed, and extended task completion period. The MLSR showed that students were able to use appropriate SOPs (72%) and demonstrate effective communication skills (84%). Students felt that they practised technical skills as indicated in the survey (96%).

Conclusions: Simulation in an MLS program is an effective way for students to practise LIS and other transferable skills. In addition, early use of evaluation tools can help monitor student progression in the program.
Analysis of donor blood characteristics on recipient quality of life and change in hemoglobin in frequently transfused patients

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Introduction/Objective: Factors contributing to blood product quality, include manufacturing method, storage length, and donor characteristics (age, race, sex). Canadian Blood Services uses two processing methods; whole blood filtration (WBF) or red cell filtered (RCF) method. Quality of life is important for chronically transfused patients and may be impacted by blood product characteristics. This study examined differences in transfusion outcomes associated with differences in red cell donors and manufacturing in chronically transfused patients.

Design and Methods: This study used 2 groups of regularly transfused patients to evaluate product quality. A retrospective data analysis took place using 106 pediatric patients on extracorporeal membrane oxygenation (ECMO), a type of life support, and the main outcome evaluated was the change in hemoglobin after transfusion. Subsequently, an n-of-1 feasibility study was conducted, in which six patients (2 female, 4 male) with myelodysplastic syndrome were enrolled. Patients were randomized to receive sex-matched RCF units or sex-mismatched WBF units for the first transfusion and the alternate for their next transfusion. The primary endpoints were the change in hemoglobin between transfusion episodes and the patient’s response to a transfusion quality of life survey (QUALMS). Secondary analysis included the patient’s vital signs (temperature, blood pressure, pulse) during the transfusion, and the hematocrit, hemolysis, and hemoglobin levels of the transfused red cell product.

Results: The ECMO study population showed a significantly greater increase in hemoglobin levels after transfusion of RCF units. For the n-of-1 study, analysis was undertaken to evaluate trends. There was no difference in reported quality of life, and there was a trend toward a greater increase in hemoglobin between episodes from sex-matched, RCF units (\(\bar{x}=0.032\ \text{g/ml}\)) compared to sex-mismatched WBF units (\(\bar{x}=0.020\ \text{g/ml}\)). There were several limitations, including limited blood product availability, patient complications, transfusion outside of the study, short notice for patient appointments, and non transfusion patient treatment affecting the quality of life survey.

Conclusions: This feasibility study identified issues and generated recommendations for conducting a larger scale study. This research is important to ensure high quality products are available to optimize outcomes and quality of life for chronically transfused patients.
Development of Donor Specific Antibody and Thymectomy Status in Pediatric Heart Transplant Patients

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De novo donor specific antibody (dnDSA) remains a significant barrier to long-term transplant success. Given the importance of T cells in dnDSA development, the crucial role of the thymus in T cell development, and the frequent occurrence of thymectomy in pediatric heart transplants, evaluation of thymectomy status against incidence of dnDSA was performed. Frequency of dnDSA was determined to be 25.9% in a cohort of 108 pediatric heart transplants with further evaluation of dnDSA development in regard to sex, age at transplant, and thymectomy status (n=86) revealing no significant associations. Age at thymectomy proved significant (n=47; p=0.026), suggesting earlier thymectomy results in decreased incidence of dnDSA. Duraclone IM immunophenotyping for 5 thymectomized patients was also completed to compare dnDSA positive against negative individuals. Age-matched controls were used to assess T cell phenotype changes from thymectomy. T cell responses were also investigated using the CellTrace Violet Proliferation Assay. Small sample size hindered the discovery of significant differences between groups, though comparison with age-matched controls revealed decreased %naïve T cells and a predominating memory phenotype in study subjects. In conclusion, the promising preliminary data collected from this pilot study prompts further investigation of dnDSA development in relation to thymectomy.
Human Factors: Are you being set up for failure?
Enhancing Pediatric Hematology Point-of-Care Testing using Human Factors Analysis

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Background and Aim: Human factors (HF) is a multidisciplinary perspective that considers the relationship between environment, design, user interface and training. It explores the causes of error stemming from human interaction. This study evaluated two Point-of-Care HbA1c instruments to identify the appropriate device for the UofA Pediatric Diabetes Clinic (PDC). Additionally, we identified shortcomings in the current process and provided quality improvement suggestions.

Material and Methods: An HF assessment was performed on the Roche Cobas b101 and Alere Afinion2 devices through interviews, observations and use of calculations. Thematic analysis of systematic observations and survey results were performed to evaluate the current process followed by an inductive approach to identify relevant themes.

Results: Deficits in the current process were identified as: lack of operator education, poor workflow design, existence of transient forms, and safety risk issues. The Cobas b101 proved superior in terms of interface HF; however, the Afinion2 is better suited for the PDC due to the speed of testing, and cartridge usability.

Conclusions: HF assessments are an important consideration when devising quality management programs. They identify factors outside of traditional analytics and highlight potential areas of error that could compromise patient safety.
The medical laboratory and Choosing Wisely Canada: What is being done?

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Background and Aim: The Choosing Wisely Canada (CWC) campaign releases recommendations targeting unnecessary medical tests and procedures. Roughly one-third of the current recommendations are related to the clinical laboratory. We hypothesize that many Canadian laboratories are engaged in projects optimizing laboratory utilization, but most remain unpublished and thus inaccessible. Little is known about the patterns of projects regarding coverage of laboratory-relevant recommendations.

Material and Methods: 107/327 recommendations were identified as laboratory-relevant. For existing initiatives directed at relevant recommendations, we performed an environmental scan using a literature search, online resources, and a questionnaire. Online resources used included webinars, abstract books and region-specific CWC websites. A questionnaire was delivered via phone or email to clinical laboratory contacts identified through public listings. Information was gathered on the utilization topic addressed, strategies and level of success.

Results: We identified 78 initiatives of varying scope and duration across Canada. Areas most frequently addressed were routine testing (21) and transfusion medicine (18). 1 or 2 initiatives targeted cancer, virology, genetics, toxicology and electrocardiography. No laboratory reported projects targeting antibiotics (respiratory & skin), marrow and transplant, Pap smears, and colorectal cancer. Most strategies measured test reduction and/or cost savings. Less commonly, initiatives measured product utilization and appropriate care. All completed projects were self-reported as successful.

Conclusions: The pattern of initiatives appears to mimic the typical volume of test orders, with most projects addressing frequently ordered tests. Simple measures such as order reduction and cost savings were used to demonstrate success. Low-attention areas present future utilization targets.
Group B streptococci (GBS) are the leading cause of invasive disease in neonatal and immunocompromised adults. Penicillin remains the antimicrobial of choice for invasive GBS (iGBS) disease; however, in penicillin-allergic patients, erythromycin or clindamycin may be used. Global rates of erythromycin and clindamycin resistance have gradually increased in the past 20 years. Different genetic mechanisms of erythromycin and clindamycin resistance have been characterized by the presence of \textit{erm}, \textit{mef} and \textit{Inu} genes. In this epidemiological study, 1017 iGBS isolates were collected from years 2014 to 2018 in Alberta. Disc diffusion antimicrobial susceptibility tests were performed to determine GBS resistance to erythromycin and clindamycin. Screening of resistance genes, \textit{erm}A, B, \textit{C} and \textit{T}, \textit{mef}A, \textit{Inu}A and \textit{B}, was performed using end-point PCR. Of 1017 isolates from years 2014 to 2018, 557 (54.7\%) isolates were erythromycin and clindamycin resistant. There was a decrease in erythromycin and clindamycin resistance from the year 2014 (79.1\%) to the year 2015 (51.6\%); however, susceptibility rates remained constant at an average of 51.7\% resistance from years 2015 to 2018. A total of 103 isolates which showed erythromycin and clindamycin resistance (constitutive or inducible) were screened for \textit{erm} alleles; 76 (73.1\%) harboured \textit{erm}B, and 23 (23.3\%) harboured \textit{erm}T. A total of 19 isolates which were erythromycin resistant and clindamycin susceptible harboured \textit{mef}A (100\%). One GBS isolate also was \textit{Inu}B+. In conclusion, erythromycin and clindamycin resistance in GBS in Alberta is driven by \textit{erm}B and \textit{erm}T. For those GBS isolates erythromycin resistant and clindamycin susceptible, all were \textit{mef}A positive.
Wine colored samples? Development of laboratory reporting guidelines for hydroxocobalamin (OHCob) interference in patients pulled from house fires

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Introduction: Hydroxocobalamin (OHCob) is used for the treatment of cyanide poisoning secondary to smoke inhalation from house fires. OHCob discolors bodily fluids red, potentially interfering with spectrophotometric-based assays. The objective was to investigate the impact of OHCob interference on chemistry, coagulation, urinalysis and blood gas analytes.

Methods: Normal and abnormal discard plasma, urine and whole blood samples were spiked with a high dose (1.5 mg/mL) of OHCob or equivalent diluent volume (control). Samples (n = 5) were run on 70 assays using Beckman Coulter DxC600/Access2/DxH800, Stago STA-Compact, Siemens Clinitek Atlas and Radiometer ABL800 analyzers. Dose-response treatments were performed on assays if interference was >10%. Samples obtained from a patient administered OHCob in the emergency department (ED) were analyzed for changes to color and chemistry measurements.

Results: Spiking studies revealed positive interference (range 26-1298%) to total bilirubin, lactate, magnesium, uric acid, creatinine-enzymatic, prothrombin time, partial prothrombin time and d-dimer. There was negative interference (range 12-63%) to alanine aminotransferase, aspartate aminotransferase, creatinine-Jaffe and creatine kinase. Urinalysis dipsticks were falsely increased on glucose, ketones, blood, nitrates and leukocytes. Co-oximetry parameters on the ABL800 were also significantly affected. Interference in samples from a patient administered a single dose of OHCob was not detected by hemolysis index (HI), but showed gradual recovery on select chemistry analytes as the drug was cleared and red colouration faded over time.

Conclusions: Among the assays tested, 37% (26/71) had varying degrees of interference. Lack of HI flagging further underscores importance of communication with ED to identify these samples.
Dietary Fibers Trigger a Pro-inflammatory Response in Pediatric Inflammatory Bowel Diseases

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Introduction: Inflammatory bowel diseases in pediatrics is a growing concern, accounting for 25% of cases diagnosed. Importantly, pediatric patients experience more severe symptoms that often require surgical intervention. As a result, dietary intervention has been studied in the treatment and management of IBD. The production of short chain fatty acids from the fermentation of dietary fibers by gut microbes, aid in the maintenance of colon homeostasis. In IBD, the diversity and abundance of gut microbes differ and therefore we hypothesized that the lack of fiber fermenting bacteria in the IBD gut leads to inefficient fermentation of dietary fibers which results in unfermented fibers binding to host cell receptors causing an inflammatory response.

Methods: Terminal ileum and ascending colon biopsies were collected from pediatric non-IBD, Crohn Disease (CD) and Ulcerative Colitis (UC) patients. Terminal ileum biopsies were formalin fixed, paraffin embedded, cut and immunohistochemically (IHC) stained with GLP1R antibody. Positively stained cells were quantified with ImageJ. Terminal ileum and ascending colon biopsies were enzyme digested and immune cells were sorted using the fluorescence activated cell sorter. RT-qPCR inflammasome profile was performed to detect the inflammatory effect of oligofructose on macrophages compared to no fiber. Enzyme-linked immunosorbent assay (ELISA) was used to quantify the amount of IL-1β secreted in monocytes and macrophages, in response to dietary fibers and dietary fibers pre-fermented by bacteria.

Results: Both macrophages and monocytes demonstrated an increased secretion of IL-1β in response to the dietary fibers, oligofructose and inulin compared to no fiber. Interestingly, pre-fermentation of dietary fibers by bacterial species such as Pseudomonas protegens and Enterococcus, significantly decreased the secretion of IL-1β, while Bifidobacterium infantis increased the secretion of IL-1β compared to no fiber. A total of 48 biopsies were taken from children without IBD (n=10), UC (n=16) and CD (n=22) and were used in the analysis of dietary fibers causing a pro-inflammatory response. Notably, oligofructose significantly increased secretions of IL-1β in inflamed pediatric IBD patient biopsy tissues cultured ex vivo. Interestingly, pre-fermentation with Pseudomonas protegens significantly decreased IL-1β secretions in non-inflamed CD patients and UC inflamed patients.

Conclusion: Unfermented dietary fibers trigger a pro-inflammatory response in macrophages and monocytes. Interestingly, dietary fibers also trigger a pro-inflammatory response in pediatric IBD patient biopsies cultured ex vivo. In addition, our results indicate that bacterial pre-fermentation of dietary fibers can decrease the immune response in IBD patient biopsies cultured ex vivo. Identification of which dietary fibers trigger a pro-inflammatory response and the identification of which bacterial species can reverse this inflammation is essential in the development of specific dietary recommendations and precision medicine in the IBD patient.
Provision of Relevant Clinical Information on Microbiology Requisitions:  
A quality metric in the microbiology laboratory

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**Background:** Dynalife Medical Laboratories is the largest privately operated laboratory in western Canada, serving about 2000 clinicians and one million patients. The Edmonton laboratories process about eighteen million tests annually. A large portion of these tests are microbiology specimens, and they depend on accurate and relevant clinical information being provided, to ensure quality, specific and timely testing to be carried out.

**Objective:** To examine microbiology specimens to check for the presence of relevant clinical information, to make a record of findings, and to present results of the findings to stakeholders, with a view to informing them about any shortfalls in filling out the information and improving compliance with filling out requisitions properly.

**Method:** A total of 967 requisitions were examined under six different categories of specimens: deep wounds, respiratory, genital, fecal, blood cultures and urines. Findings were recorded on an Excel spreadsheet.

**Results:** Only an average of about 20% overall were found to have relevant clinical information, and these results are skewed by deep wounds which had a fairly large amount of requisitions with relevant information.

**Conclusions:** A very low percentage of microbiology requisitions are filled out with relevant clinical information. The use of tick-boxes may help increase incidence. Stakeholders will be informed about the findings of this audit and encouraged to fill out requisitions properly, as this is not only critical to ensure adequate scope of testing, but also improves turn-around times and overall patient outcomes. It is also strongly supported by The College of Physicians and Surgeons of Alberta, as well as other regulatory bodies.
In search of patient-friendly screen test for gestational diabetes

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Background: Gestational diabetes (GD) is a common concern for mothers and their baby therefore early detection is essential for positive outcomes. Current recommendations are a 50 g oral glucose test, with abnormal results being confirmed by a 75 g follow up test. Oral glucose testing requires: fasting, an extended laboratory visit, consumption of an unpleasant drink and multiple pokes. This study aims to determine if GD can be detected by fructosamine and HbA1c values, with hopes of eliminating the oral glucose test. Thus, making GD testing more patient friendly.

Material/Methods: Patients were recruited at Oliver Park DynaLIFE collection centre, providing written informed consent. Fructosamine and Hb1A1c were measured in patients undergoing the 1-hour oral glucose challenge test (OGCT) or the 2-hour oral glucose tolerance test (OGTT) for GD screening. Variations in protein level were corrected by dividing fructosamine by total protein or albumin. Correlation studies and ROC curve analysis were performed in MedCalc.

Results: 26 patients were recruited (OGCT-20 and OGTT-6). HbA1c exhibited a moderate correlation with OGCT results \( (r=0.4261) \). ROC curve analysis suggests fructosamine <206 µmol/L can identify GD with a sensitivity of 100% and specificity of 78% \( (AUC=0.826, p<0.001) \). Correction for albumin or total protein yielded similar results.

Conclusion: This project highlights the promise of using fructosamine and HbA1c, in lieu of the standard oral glucose test for the detection of GD. However, further research is required, consisting of a larger sample population.
CD23 Labelling Provides a Useful Pronase Quality Control in Histocompatibility Flow Crossmatch

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Flow cytometry crossmatch (FCXM) is used by histocompatibility laboratories to assess donor/recipient compatibility in transplantation. Recipient antibody to donor human leukocyte antigens (HLA) is detected with fluorescence-tagged, anti-human-IgG secondary antibody. B-cell Fc receptors non-specifically bind to secondary antibody; to counter this, pronase is used to cleave Fc receptors. However, the influence of pronase has not been fully elucidated. In this study, we use different fluorescent stains to detect differences between pronased and non-pronased lymphocytes by flow cytometry. We show that Fc receptor markers CD23 and CD32 are drastically reduced in pronase treated cells. CD23 displays the largest shift in fluorescence with treatment which indicates that it could be a valuable marker as a quality control measurement for the effectiveness of enzymatic function. Pronase was noted to have effects on other surface molecules. HLA antigen density was increased in T cells with pronase treatment rather than remove them. This is possibly due to the change in surface landscape as immunophenotyping panels display pronase to cleave a wide range of surface markers. Understanding the nature of the enzymatic effect of pronase may allow us to use it more efficiently in the laboratory.
Evaluation of Colorex Strep A CHROMagar for detection of Group A Streptococcus in throat swabs

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Group A Streptococcus (GAS) is the leading cause of bacterial pharyngitis. Accurate and early detection is important for proper diagnosis and treatment. Rapid treatment with antibiotics minimizes risk of transmission and sequelae. Our goal was to evaluate whether detection of GAS could be automated on the Becton Dickinson Kiestra Total Laboratory Automation System (BD Kiestra TLA) using Colorex Strep A CHROMagar plates (CSA).

This study included two phases. In phase one, 35 recent positive ESwab specimens were plated to two CSA then one 5% sheep blood agar plate (BAP). One CSA was incubated aerobically, the other in 5% CO₂, and the BAP anaerobically. Phase one results determined the incubation atmosphere for phase two. In phase two, 302 ESwab specimens were plated using the current method, then were plated to CSA, incubated aerobically and read using the Kiestra TLA. In both phases, growth was graded 0-4+; orange colonies on CSA were identified by MALDI-TOF (Biomerieux Vitek MS) and sub-cultured to BAP; and beta-hemolytic colonies on BAP were identified by MALDI-TOF. Results were then compared to the current method.

Phase one demonstrated 93.9% and 100.0% sensitivity when CSA were incubated in CO₂ and aerobically, respectively. Phase two included 61 positive and 240 negative specimens resulting in 95.1% sensitivity, 100.0% specificity, and 98.3% agreement with the current method.

Using CSA plates on the BD Kiestra TLA is a viable method for the detection of GAS in throat swabs collected by ESwab. Further analysis of analytical sensitivity and precision is required.
Comparison between IVIG dosing based on ideal body weight calculations and actual body weight calculations for Edmonton Zone

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IVIG dosing is important for the effectiveness for IVIG products and was traditionally calculated using patients’ true/actual weight. This can result in overdosing in some patients and contribute to adverse reactions. A policy implemented in February 2018 requires use of patients’ ideal body weight. This method better determines the IVIG dosage, increasing the efficacy and tolerability of the products.

The IVIG dose administered, actual weight, dosing weight, adverse reactions, and ordering physician’s specialty were collected for 2017 – 2018 (pre policy) and 2018 to 2019 (post policy) from the IVIG database. Excel was used for processing the data for statistical analysis. T-test was used in the calculations.

There is a reduction in total IVIG usage after the policy implementation (108430 g) compared to the pre-policy period (123791.5g). The amount of IVIG used per patient after the implementation of policy (40.28g) was less than pre-policy group (45.39g) with p-value of 0.001.

There is almost no difference in the percentage of adverse reactions in both population 0.60% in pre-policy and 0.61% in post-policy, but there is a decrease in the hemolysis reactions. The overall requisitions submitted for dosing weight calculations was 46%.

The reduction of total and average IVIG used indicates effectiveness of the new dosing weight calculation in saving IVIG. The 0.001 p-value indicates there was a significant difference between the two averages, further support the hypothesis that dosing weight calculation reduce the IVIG usage. Although no difference in the occurrence of Adverse reactions overall, there was a decrease in hemolysis, which is a symptom that occurred frequently in excess IVIG dosage. This is important as continue using of IVIG calculator can help to potentially decrease adverse reactions in the future. The low compliance rate suggests more educations are needed in the future.
Chronically Transfused Patients: Reducing the Risk of Alloimmunization

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Background and Objectives Chronically transfused patients receive long term red cell (RBC) transfusion therapy; frequent exposure to donor RBCs can result in alloimmunization and adverse reactions. Current evidence suggests transfusing patients with phenotype matched RBC.

Materials and Methods Chronic transfusion recipients were identified from red cell usage reports from 2013, 2016, and 2018. Patient demographics, antibody screen, antibody identification, phenotyping, genotyping, and diagnostic data were found in the electronic health records and laboratory information system.

Results A decrease was noted in the alloimmunization rates over the years. There were more phenotyping events in 2018 compared to the other years. In terms of antigen matching rates, there has been a decreasing trend of non-antigen matching.

Conclusion Phenotype and genotype-matched RBC units are transfused to reduce the risk of alloimmunization. Further work will compare the phenotyping, genotyping, antigen matching, and alloimmunization trends across diagnostic groups who are on chronic transfusion therapy.
Assessing the Predictability of Cell Based, Flow Cytometric Crossmatch using Luminex Single Antigen Bead Virtual Crossmatch

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Background: Once a patient is placed on the transplant list, they wait for the day when an organ is available for them. When the time comes, they are crossmatched with the potential donor to detect HLA antigens on the surface of the donor organ cells which may cause detrimental effects once transplanted. In the HLA laboratory, cell based crossmatch methods are the most common method, however they require lengthy periods of incubation and numerous steps, which may delay transplant. This study aims to determine if the Luminex One Lambda single antigen bead (virtual) crossmatch method, which is quicker and more sensitive and specific, can accurately predict the outcome of the aforementioned cell-based, cytometric crossmatch.

Methods: This study represents a data analysis of crossmatch data from both methodologies, and a comparison of the results of each. The positive and negative predictive values, as well as contingency tables and p-values were calculated for each method.

Results: The virtual crossmatch was able to predict the outcome of the cell-based flow cytometric crossmatch with great specificity and sensitivity. When using an increased threshold for the virtual crossmatch, the negative predictive value increased versus using the standard threshold.

Conclusions: The Luminex One Lambda single antigen bead (virtual) crossmatch method could predict the result of the flow cytometric crossmatch reliably. This may hold the key to quicker histocompatibility testing for those in need of life-saving organ transplantation.
Standardization of serum indices in the Edmonton zone

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Purpose: To evaluate the effect of hemolysis, icterus and lipemia (HIL) interferences in serum indices and general chemistry testing with Beckman’s DXC 800 and Ortho Clinical Diagnostics’ Vitros 4600 to determine if HIL standardization is possible. To evaluate the reproducibility and reliability of visually grading interferences in comparison to using serum indices measurement.

Methods: Pooled, lithium heparin, plasma samples were aliquoted and spiked with known concentrations of hemoglobin, bilirubin or Intralipid. These spiked samples were run on the Vitros 4600 and the DXC 800 for serum indices and eighteen chemistry tests. All spiked samples were visually graded by a minimum of two laboratory technologists. Half of the visual gradings were provided a interference-free plasma sample as a reference. Chemistry results that deviated ten percent from the non-spiked plasma sample were found to have significant interference.

Results: Eleven of the same chemistry tests had significant interference in both the DXC 800 and Vitros 4600. Twenty-one different chemistry tests had significant interference in only one of the analysers. Visual grading was found to be less reliable compared to measuring serum indices.

Conclusions: Interference variations between the two analyzers indicate that HIL standardization could occur for some but not all analytes. Providing an expanded visual aid for manual HIL assessment allows closer alignment to HIL indices.
Evaluation of the Pathological Effects on Rat Tissues Given PTH-PEG (27)-BP as part of a Pharmaceutical GLP Study

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Osteoporosis in men and postmenopausal women can be managed using human parathyroid hormone (PTH) or other synthetic or recombinant versions such as teriparatide. However, these types of drugs have been shown to have pathological side effects in rodent models. This study was done to evaluate if the drug PTH-PEG (27)-BP, developed by the Doschak laboratory, has similar pathological effects in rodents compared to teriparatide. After being injected subcutaneously with 10 or 20 ug/kg/day of PTH-PEG (27)-BP, eight major organs from the rats underwent routine histologic processing and were stained using hematoxylin and eosin. The tissues were evaluated for pathology using light microscopy and computer based photographic documentation. Compared to the vehicle control and the teriparatide treatment group, there was no significant increase in abnormal pathology in the rodents treated with PTH-PEG (27)-BP. It was concluded that further research needs to be done on the effects of the drug on other tissues as well as relevance to human safety before this drug can be used clinically.
Effectiveness of Freezing Denim as an Alternative Method of Cleaning

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A recent trend emerging among raw denim jean wearers is reduced washing to promote unique wear patterns. As an alternative to washing, many blogs have suggested freezing jeans in order to reduce smell and bacteria on clothing, despite there being no scientific evidence that freezing successfully achieves these two desired outcomes. The aim of this study was to compare the bacterial growth after 2 different freezing conditions, as well as multiple freeze-thaw cycles to unfrozen fabrics. Denim swatches were inoculated with Staphylococcus aureus (SA) and Escherichia coli (EC) grown in a basic nutrient broth. Denim swatches were then subjected to different freezing conditions (2 different sized freezers and 2 different freeze/thaw cycles). Control swatches were left at constant temperature and humidity conditions for 24 hours. Bacteria were extracted from denim swatches: 1) immediately after each freeze treatment; and 2) 24 hours after freezing. Colony forming units (CFU) were calculated and compared to baseline measurements. The small, fridge freezer caused a greater percent reduction than the large, deep freezer. The additional freeze-thaw cycle was more effective than one freeze-thaw cycle, and the E. coli was reduced more than the S. aureus. Greater percent reduction occurred when specimens were left at 65% humidity and 20°C than those left in the freezer. Different methods of freezing do not provide a suitable alternative to laundering in regards to reducing bacteria on fabric.
To freeze or to heat? Alternative methods for disinfecting denim jeans

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Due to negative impacts frequent laundering can have on the environment and its degradative effects on denim jeans, alternative methods that can be reproduced at home could be a more environmentally way to disinfect denim. The purpose of this study was to determine the effectiveness of heat and freezer treatments for disinfection of denim. With regards to heat treatment, a 2x2x2 factorial design was carried out which included varying lengths of time in the oven (15 min, 30 min at 145°C), moisture content in fabrics (dry, moist), and length of times after heat treatment (0h, 24h). Denim fabric swatches were inoculated with Escherichia coli and Staphylococcus aureus culture broths (inoculated separately). Following the treatments, bacteria was extracted in PBS solution, plated onto nutrient agar, and incubated at 37°C for 24h. Heat treatments were compared to baseline measurements (no treatment at 0 and 24h) and bacterial counts obtained from frozen denim swatches were also compared to the baseline. No growth was observed following any of the heat treatments. This suggests that heat treatment can act as an effective alternative method of disinfection for denim fabrics.

The results also found a significant reduction in bacterial counts occurred due to freezing after 24 hours have passed. This contradicts the results of other studies which had found no change in counts after freezer treatment. Future studies are recommended to investigate the effectiveness of the freezer treatment.
Histopathological Study in Female Ovariectomized Sprague-Dawley Rats Treated with Bone-targeting Drug, PTH-PEG(27)-BP

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PTH-PEG(27)-BP, an anabolic bone-targeting drug, is under preclinical development to treat osteoporosis and other related bone disorders. The safety of PTH-PEG(27)-BP has yet to be determined. The objective of this project was to conduct the histopathology and light microscopy necessary to evaluate rat tissues for pathological signs of toxicity related to long term exposure to PTH-PEG(27)-BP. Sprague-Dawley rats were given daily subcutaneous injections of PTH-PEG(27)-BP at doses of 0, 10, 40 and 20 μg/kg or 40 μg/kg PTH(1–34)/teriparatide as a positive control for up to 85 days. Histopathological analysis showed no abnormal changes in the soft tissues of both PTH-PEG(27)-BP and PTH(1–34)/teriparatide treated rats. Long-term treatment of PTH-PEG(27)-BP at doses 10μg/kg and 20 μg/kg did not cause histopathological changes in the soft tissues of Sprague-Dawley rats. Further investigation is needed to make conclusions on the safety of PTH-PEG(27)-BP.
Assessment of alloimmunization rates and hemolytic transfusion reactions in patients receiving Rh mismatched blood in the Edmonton Zone

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Background and Objectives: Current transfusion policies permit the use of Rh positive blood in Rh negative patients under special circumstances. To ensure appropriate patient safety, our transfusion service committed to ongoing evaluation of alloimmunization rates and hemolytic transfusion reactions after implementing these policies. Consequently, our current study re-examined the risks of these adverse events, comparing our results to our initial validation and previously published literature.

Materials and Methods: Rh mismatch reports and electronic patient records were examined to identify Rh negative patients who received Rh positive red blood cells (RBCs) or platelets between January 2017 – December 2018. Captured parameters included age, sex, blood group, diagnosis, transfusion details, and antibody screen results. Patients included in the rate calculation had no prior anti-D and had an antibody screen at least 10 days after the transfusion.

Results: For Rh mismatched RBCs, one out of the 29 cases (3%) formed anti-D. In contrast, five out of the 26 cases (19%) formed anti-D in the initial validation (p-value = 0.0609). Comparison of current and published rates was significant (p-value: 0.0196). For Rh mismatched platelets, two out of the 90 cases (2%) formed anti-D. Comparison of current and published rates was not significant (p-value = 0.5845). In addition, no hemolytic transfusion reactions were reported.

Conclusions: For Rh mismatched RBCs, the alloimmunization rate of 3% points toward lower anti-D formation than previously reported and published. For Rh mismatched platelets, the alloimmunization rate of 2% aligns with previously published rates. These risks are acceptable when balanced with the benefit of conserving Rh negative blood.
GeneXpert MRSA NxG Assay Validation for detection of small colony variant methicillin-resistant Staphylococcus aureus at ProvLab

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Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is well-documented as having significant impact on the health care system. MRSA is difficult to treat with routine antibiotics such as oxacillin, methicillin and penicillin. Prompt identification of antibiotic resistance is necessary to improve patient outcomes. Methods of detection include microbial culture with cefoxitin disk, CHROMagar and *mecA* PCR. However, small colony variant (SCV) MRSA cannot reliably be identified by phenotypic methods. Current testing at ProvLab for SCV MRSA is *mecA* PCR followed by agarose gel electrophoresis for visualization of products. This method is time consuming and laborious; it may take up to 48 hours to produce results. The GeneXpert MRSA NxG assay by Cepheid is a real-time PCR (qPCR) method which can identify MRSA with *mecA* or *mecC* in less than 2 hours. Validation of the GeneXpert assay would allow testing to be moved from molecular diagnostics to bacteriology, freeing up more time for the molecular diagnostic technologist to perform other duties. The GeneXpert MRSA NxG assay correctly identified every sample (n=40), including the SCVs (n=20). This study supports the transition of *mecA* testing from *mecA* PCR to the GeneXpert MRSA NxG Assay.
Diagnostic Accuracy of Spectrophotometric Xanthochromia Detection Compared to Traditional Visual Methods

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Introduction: Diagnosis of certain clinical conditions, such as subarachnoid hemorrhage, can be based on xanthochromia presence in cerebral spinal fluid (CSF). This study aims to identify the superior method for detection of xanthochromia. The aim of this study is to determine whether a visual analysis is sufficient for testing, or if a spectrophotometric method should be employed. Bilirubinometer testing was also performed to determine clinical utility.

Methods: CSF samples of at least 850 µL, were tested at the University of Alberta Hospital after all other laboratory testing was complete. Samples were scanned on a Cary-100 spectrophotometer, between the wavelengths 350 and 600 nm, to determine the presence of bilirubin, oxyhaemoglobin and methemoglobin. The results were correlated to a diagnosis and compared to the visual xanthochromia determination. The samples that contained bilirubin or oxyhaemoglobin were also tested on a Reichert Unistat bilirubinometer.

Results: Sensitivity, specificity, positive and negative predictive value were compared for spectrophotometric and visual methods. The visual method had a sensitivity of 75%, specificity of 98.6%, positive predictive value of 75%, and negative predictive value of 98.6%. Bilirubinometer testing was deemed to be ineffective and did not provide results. LOQ and LOD were 0.85 µmol/L and 0.28 µmol/L respectively.

Conclusions: The visual method is a reliable and accurate method when it comes to determination of negative samples. The sensitivity of the visual method is lacking, as interfering substances can complicate the determination of true xanthochromia (bilirubin). The use of the bilirubinometer is impractical for the detection of xanthochromia.
Investigation of the Direct Antiglobulin Test (DAT) Strength in Determining the Degree of Hemolysis in Hemolytic Disease of the Newborn (HDN)

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**Background and Aim:** The direct antiglobulin test (DAT) is one of the tests used to diagnose immune-mediated hemolysis in neonates. However, a positive DAT result does not conclusively mean that the neonate is hemolyzing. This study evaluates predictive value of the cord blood DAT. This is done by examining the association of the DAT strength to hemolytic markers, and DAT positivity to antibody specificity.

**Materials and Methods:** The ARECCI guidelines determined this to be Quality Improvement research. Retrospective analysis was done on the DAT-positive cord blood samples and maternal antibody titer results from January 2017 to July 2018 from the SunQuest Laboratory Information System (LIS) and Netcare. Neonatal hemoglobin and bilirubin results were collected for two groups, DAT ≥2+ and DAT <2+. The maternal antibody titer results were separated into DAT-negative and DAT-positive results to determine significance.

**Results:** Binary Logistic Regression was done for DAT strength and markers of hemolysis, and the Fisher-Freeman-Halton test was done for DAT result and antibody specificity using IBM SPSS V25 software. For the binary logistics regressions test, the full model containing all variables was statistically significant $\chi^2 (5, N = 129) = 19.88, p < 0.05$. Only hemoglobin was statistically significant in the model ($p = 0.009$). For the Fisher-Freeman-Halton test, there was no statistical significance ($p = 0.527$).

**Conclusions:** There is further research required to determine the significance in the correlation of DAT strengths to hemolytic markers and antibody specificity. A major limitation was that most DAT positive neonates lacked lactate dehydrogenase and haptoglobin levels.
Morphological Changes Associated with Extracorporeal Lung Support Modalities in Explanted Lungs from Lung Transplant Recipients

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Objectives: Extracorporeal lung support (ECLS) is used to assist a patient’s heart and/or lungs in the event of severe dysfunction, and it can be implemented as a bridge to lung transplantation. Our objective was to separate which histopathological changes in the lung tissue can be attributed to the ECLS therapy, and which changes arise from the primary lung disease. We hypothesized that the superimposed ECLS related changes would impact our ability to recognize the underlying disease pathology.

Methods: We performed a blinded, retrospective histomorphological review of explanted lungs from adult patients (age > 16) undergoing lung transplant from 2004-2018, including an ECLS group (n=25) and a non-ECLS control group (n=25). Data for various morphological characteristics was compared using Fisher’s exact or Cochran-Armitage trend tests, in addition to summary statistics.

Results: There was a higher incidence of fresh hemorrhage (p=0.0011) and organizing pneumonia (p=0.0085) in the ECLS group, and by using these characteristics as indications of ECMO, we were able to predict ECLS use in 10/25 (40%) of cases. Of these cases, 7/10 received VV ECMO. Additionally, there was also increased occurrence of smooth muscle hyperplasia in PAH patients (p=0.0198).

Conclusions: ECLS use leads to an increased occurrence of acute lung injury, which is more recognizable in VV ECMO. These ECLS related changes do not impact the ability to identify the pathology of the primary lung disease.
Analytical Evaluation of Two Hemoglobin A1c Point-of-Care Testing Devices in a Pediatric Diabetes Clinic

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Background and Aim: Point-of-Care Testing (POCT) for Hemoglobin A1c (HbA1c) has shown it leads to improved glycemic control in diabetic patients. This study evaluated the analytical performance of the two POCT devices: the Roche Cobas b101 and the new to Canada, Alere Afinion2.

Methods: Within-Laboratory Precision, Repeatability and Method Comparison studies were performed on EDTA whole blood patient samples following Clinical Laboratory Standards Institute (CLSI) Protocols. Repeatability consisted of 20 replicates in a single run on one reagent lot for each device. Within-Laboratory Precision followed CLSI EP15-A3 protocol on two reagent lots. Method Comparison (n=40) followed CLSI EP9-A3 protocol comparing two reagent lots against a Roche Tina-quant Gen 3 central laboratory assay.

Results: Repeatability for both systems obtained a coefficient of variance (CV) <2% at both tested concentrations (6.6% and 8.5%). The Cobas obtained a CV of 2.5% at a concentration of 6.6%, and a CV of <2% at 8.3% for the Within-Laboratory Precision; whereas the Afinion had a CV of <2% for both concentrations. Method Comparison at values ≤10% passed National Glycohemoglobin Standardization Program (NGSP) criteria for Lot 2 of the Cobas. Lot 1 of the Cobas and both Afinion lots failed NGSP criteria. Clinically significant lot-to-lot variation was observed in the Cobas.

Conclusions: The Afinion was analytically superior to the Cobas in accuracy and precision studies. Input of offsets, such as slope and y-intercept, into a device could potentially alleviate bias issues and lot-to-lot variability.
Developing approaches to reagent lot-to-lot validation: Can we standardize the procedure within the Edmonton zone?

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Background: Reagent lot-to-lot comparisons are recommended by accreditation bodies to ensure that the performance of each reagent lot is stable and meets acceptable standards. There have been guidelines to perform such comparisons, but practices in Edmonton laboratories are found to be highly variable between sites with different instruments, ranging from a simple (QC only) to a comprehensive (QC + patient sample) approach. This study aimed to determine which analytes are most impacted by lot changes and whether standardization of reagent lot-to-lot comparison practices is possible between chemistry laboratories within the Edmonton Zone.

Methods: This study consists of both a retrospective and prospective analysis of chemistry analytes. Two years of retrospective lot comparison data with QC and patient sample was obtained at 2 sites with the comprehensive approach. In the prospective stage, QC and aliquots of 10 patient pools were sent to 5 sites with a Beckman Coulter (DxC 600/800) and 9 sites with an Ortho Clinical Diagnostics VITROS (4600/350) analyzer. One-way ANOVA was used to identify any statistically significant differences between reagent lots (p<0.05). Total allowable error was used to determine clinical significance.

Results: A total of 21 analytes were evaluated on Beckman analyzers. Out of the 21 analytes, chloride and carbon dioxide acid were found to have statistically significant differences between multiple reagent lots. Of the 19 analytes evaluated by the VITROS analyzers, albumin, sodium, phosphate, and total protein showed statistically significant differences between reagent lots.

Conclusions: Findings from this study demonstrate excellent lot-to-lot variability for the majority of chemistry analytes both within and between different analyzers. For the 6 analytes that show lot shifts, our recommendations are to perform instrument-specific lot validations. Our study also shows there is potential for reagent lot comparison data be shared across sites within the Edmonton Zone.
Evaluation of software applications for detection of minor variants in Sanger sequencing data and their use in confirmation of variants identified by NGS

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For years, Sanger sequencing was the gold standard for detection of single nucleotide variants. With the advent of massively parallel sequencing, or, as it is more commonly known, next generation sequencing (NGS), Sanger sequencing is beginning to be replaced. However, first, we need to confirm variants between the orthogonal method Sanger sequencing and NGS. The problem: Next generation sequencing has a better level of detection for variants (typically 5%) than the Sanger method; therefore, it is difficult to confirm variants using Sanger sequencing, that are below the 15% level of detection. This project describes using Minor Variant Finder (MVF) software from Applied Biosystems to overcome the difference in the limit of detection between the Sanger and NGS data. MVF purportedly has the power to improve the limit of detection from Sanger sequencing from 15% to 5% which is closer to the range of NGS, by using a software derived algorithm. From previously known variant samples analyzed through MVF, we investigated whether MVF was capable of detecting the variant of interest. We also performed a dilution study to confirm if the software can detect variant into the 5% interval. Significant findings were that, in a blind analysis, we were unable to determine the variant in the MVF software. With the information of the specific variant we were able to determine which suspect variant was the true AKT1 E17K mutation. The software included many ‘so-called’ variants which are commonly identified as background. The program does not allow for editing to help remove dye blobs for interpretation. Another problem for clinical usage is that some variants include insertion/deletions however the programs are not able to identify these changes. The algorithm was not disclosed; therefore, the reason why some output electropherograms were variable from one sample to the next, is not clear. Overall, this software has not performed to the expectations as described by the vendor. This finding is important because we cannot recommend the use of this program for clinical analysis.
Investigation of BINDA-LAMP ligation reaction parameter's effects on assay background

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Here we introduce BINDA-LAMP, an upcoming ultrasensitive DNA based immuno detection method. BINDA-LAMP is composed of two methods; Binding induced DNA assembly, which induces assembly of detectable DNA upon probes' binding to their analyte, and loop-mediated isothermal amplification, a high-yield isothermal amplification method. Our preliminary data shows that BINDA-LAMP is capable of detecting analyte concentration down to femtomolar levels. Its high sensitivity leads to many potential applications in research and medical field. However, the reaction parameters in BINDA-LAMP are yet to be optimized to reduce assay background. In this project, we focus on optimizing parameters of the ligation and LAMP portion of the assay to reduce assay backgrounds while preserving assay sensitivity. We've found that reduced T4 ligase concentration, ligation reaction time, and reaction buffer composition to significantly influence on assay background, while LAMP reaction temperature is linked with signal reproducibility amongst duplicate samples.