Moving Sri Lanka Towards a 100% Volunteer Blood Donor Collection System: Lessons learned from Canada

Administrative, Poster Presentation

Pavithra Aarewatte MD, Canadian Blood Services
Mark Bigham MD, Canadian Blood Services
Gershon Growe MD, Canadian Blood Services

The lead author is a consultant transfusion physician from Sri Lanka presently undertaking a one year international professional education experience through Canadian Blood Services in Vancouver.

Sri Lanka has a nationally coordinated transfusion service which oversees 84 hospital based blood banks and is the sole agency that manages the vein to vein transfusion process in Sri Lanka. Sri Lanka collects 14 units per 1000 population, where 79% of donors are voluntary and non-remunerated. By contrast, in Canada, with 100% voluntary donation, the rate of donation is double that of Sri Lanka. The average donation rate in the countries with 100% voluntary blood donation is 31 per 1000 population, suggesting that Sri Lanka might achieve a higher national blood donation rate by moving towards a 100% voluntary blood donor system.

This report summarizes the author’s evaluation of donor recruitment and other practices at Canadian Blood Services that could be utilized by the Sri Lankan transfusion service, with the aim of increasing the proportion of volunteer donors to 100%. Key recommendations include:

1. Improving knowledge of donor counseling officers and introducing consistent, evidence-based criteria for selection and deferral of blood donors.
2. Strengthening procedures for maintaining confidentiality during donor counseling at mobile blood collection sites.
3. Establishing a computerized donor database for better analysis and information retrieval.
4. Engaging communities to increase awareness and sustain support for voluntary blood donation.
5. Continuous upgrading of blood collection skills.
6. Ensuring appropriate equipment at blood collection units.
7. Improving Quality Management, through audits and external assessment schemes.
8. Introducing Standard Operating Procedures for donor adverse reaction management.
9. Establishing a system of blood donor records and:
10. Improving management and recall of deferred and lapsed donors.

According to WHO reports blood collection system in Sri Lanka maintains satisfactory standards compared to other South Asian countries. But further improvements in its quality are required with regard to blood safety and Canadian strategies in this respect are very useful.
2. **Plasma Products Redistribution Program Pilot**

Administrative, Oral Presentation

Tracy Cameron MLT, Ontario Regional Blood Coordinating Network - Northern and Eastern Ontario Region
Wendy Owens MLT, Ontario Regional Blood Coordinating Network - Northern and Eastern Ontario Region
Sarah Crymble BA, St. Michael’s Hospital

**Background**
An effective cost-saving system for redistribution of factor concentrate products from Ontario hospital sites to Hemophilia clinics has been in place since 2007. However, this program does not support redistribution of other plasma protein products (PPP) such as Intravenous Immune Globulin (IVIG). A request was made by the Ministry of Health-Long Term Care (MOHLTC) Blood Programs Coordinating Office (BPCO), to evaluate the impact of expanding the current redistribution program to include all plasma protein products throughout Ontario, including any financial gains or losses. This project had two BPCO funded blood programs, the Factor Concentrate Redistribution Program and the Ontario Regional Blood Coordinating Network (ORBCoN) working collaboratively to develop and implement redistribution of all PPP. The Eastern Ontario Regional Lab Association (EORLA), consisting of 18 hospitals, agreed to pilot this project for 6 months.

**Method**
Based on the Canadian Blood Services (CBS) expiry report received every month by ORBCoN, product information was transferred onto a notification form and sent out to all pilot sites to complete and return the form. ORBCoN reviewed the completed forms and entered the information into a data sheet for tracking purposes. ORBCoN facilitated the transfer of the products to either another hospital in the EORLA region, a hospital in a neighbouring Local Health Integrated Network (LHIN) or with the Factor Concentrate Redistribution Program located at St. Michael’s Hospital in Toronto. Any costs for shipping and loss of the near to expired products was tracked as well as cost of product that was not wasted as a result of redistribution.

**Results**
A total of 14 transfers of 7 different products took place between sites during the pilot. Two specialty products (VZIg and IMIg) could not be redistributed as no receiving site could be identified. The total net costs savings of product redistributed was $384,380. Of this, $187,986 worth of IVIG was redistributed.

**Conclusion**
The pilot has shown that there is a cost savings benefit by redistributing all plasma products throughout Ontario. The program will be rolled out across the province in 2013.
3. Reinventing the Inventory Management Paradigm: Creation of a novel informatics-driven high-resolution red cell inventory map

Administrative, Oral Presentation

Calvino Cheng MD, FRCPC, Capital District Health Authority, Dalhousie University
Stephanie Watson MLT, Capital District Health Authority
Joan Macleod MLT, Capital District Health Authority
Andrew Kumar-Misir BSc, Capital District Health Authority
Irene Sadek MD, FRCPC, Capital District Health Authority, Dalhousie University

Background
Current global inventory management paradigms are based on mathematical models and practices developed in the 1970s and 80s, and aggregated key performance/quality indicators, such as discard/expiry rates. Practically, these methodologies are approximations and proxies for understanding the internal working of the inventory ‘black box’, which the literature still has not fully characterized since the 1970s. We present a novel data-driven high-resolution inventory mapping technique which directly visualizes the red cell unit (RBC) flow inside the ‘black box’. This method can characterise inventory flow volume and performance, confirm policy adherence, and discover policy deviations at multiple abstraction levels. The technique can be scaled and applied to any information system-enabled transfusion service.

Materials/Methods
RBC data during May 1, 2009-January 31, 2013 was queried from the laboratory information system (Cerner Millennium) for all sites at Capital District Health Authority, Halifax, Nova Scotia. This data contained processing, handling, unit, and location attributes. Data validation, cleaning, and analysis was performed using commonly available database, spreadsheet and data mining software. Ethics approval was not required by institutional policies.

Results
There were 574,245 transactional states with 745 unique location and transfusion state specific activities involving 59,367 red cell units. There were 15994 transactional variants, with the most common variant involving 2811 units (4.74%) having 9 consistent temporally connected states (receivedHI>unconfirmedHI>confirmedHI>availableHI>transferredVG>crossmatchedVG>issuedMDU>transfused MDU), corresponding to a RBC commonly transfused at an outpatient oncology ward. RBC transition times through inventory obeyed a Pareto distribution, with 75% of cases completed before 9 days and 2 hours. High resolution inventory maps performance and volume data were also generated for all transfusion sites.

Conclusion
Using a revolutionary and novel high resolution process discovery technique, the flow of RBCs through our inventory was characterized. These maps directly reflected how inventory reacted to institutional transfusion practice, and allowed us to examine business practices more closely than via conventional aggregated key performance metrics data. This will allow for evaluation of policy changes, allow reverse-engineering of policies, and enable true lab-to-lab benchmarking, ultimately enabling cost savings and improved patient care in transfusion.
4. **Transfusion Bits and Bytes: Transfusion Medicine Laboratory Technologist education videos**

Administrative, Poster Presentation

Susanna Darnel ART, BC Provincial Blood Coordinating Office (BCPBCO)
Aimee Beauchamp, BC Provincial Blood Coordinating Office (BCPBCO)
Anne Lucas RT, BC Provincial Blood Coordinating Office (BCPBCO)
Sheazin Premji MBA, BC Provincial Blood Coordinating Office (BCPBCO)
Sonia Chau, BC Provincial Blood Coordinating Office (BCPBCO)

**Background**
A common thread running through the 2010 Canadian MLT's Learning Network Survey Report, administered by the Canadian Blood Services (CBS), was the inconvenience of continuing education. Respondents indicated that busy work and family schedules and high costs prohibited them from accessing educational tools that are currently offered. As a result of this study, CBS initiated the BloodTechnNet Learning Competition, designed to find innovative learning projects for Medical Laboratory Technologists in those parts of Canada served by CBS.

**Proposal**
The BC Provincial Blood Coordinating Office (PBCO), in collaboration with the BC Technical Resource Group, submitted a proposal named "Transfusion Bits and Bytes". The goal of Transfusion Bits and Bytes is to assist Canadian Medical Technologists in keeping up to date with current knowledge and changing practice specific to the field of transfusion medicine. The key points of the proposal included: web-based education that could be accessible free of charge from any location with internet access (whether at work, at home or elsewhere), and educational units that would be kept short so they could easily fit into busy schedules.

**Results**
The proposal was awarded a $25 000 prize by a judging panel. The monetary award was used to consult with an education coordinator; to develop a script; to evaluate electronic delivery platforms and to record, format, and upload lectures. The funding was also used to consult with a computer software engineer to develop applications for smartphones.

The first presentation topics chosen were relevant to new and rotating core technologists as well as those working in Transfusion Medicine in large centres. The idea was to begin with basic Transfusion Science presentations and work up to more complex or difficult topics, and also to include presentations related to management experience.

**Conclusion**
A series of six educational videos, ranging from 13-26 minutes on Transfusion Science related topics, were recorded and can be accessed through the internet on the BC Provincial Blood Coordinating Office website www.pbco.ca. The presentations are also accessible free of charge as downloadable apps on the iPhone smartphone and iPad tablet.
5. ORBCoN’s Transfusion Committee Forum: 5 years of successful collaboration

Administrative, Poster Presentation

Denise Evanovitch MLT, Dipl. Adult Ed., ORBCoN, McMaster University
Kate Gagliardi MLT, ART, BA, ORBCoN
Deborah Lauzon MLT, ART, BHA, ORBCoN
Wendy Owens MLT, ART, BComm, ORBCoN
Stephanie Cope MLA, ORBCoN
Troy Thompson MLT, ASQ-CQA, ORBCoN
Tracy Cameron MLT, ORBCoN
Heather Nesrallan BA, ORBCoN
Laurie Young MLT, ORBCoN

Background
One mandate of the Ontario Regional Blood Coordinating Network (ORBCoN) is to provide hospital transfusion services with best practice tools that support transfusion service delivery. Standards and requirements mandate the establishment of a transfusion committee (TC) and their responsibilities at transfusion service organizations. In 2006, only 57% of the responding hospitals in Ontario (response rate of 80%) had a TC in place; 7% had no TC activities at all. The Ontario Blood Programs Coordinating Office (BPCO) recognized this deficiency and recommended in December 2007 that ORBCoN develop a Transfusion Committee Toolkit and host an accompanying forum, which has continued as a popular event for the past 5 years. It is funded by the BPCO of the Ministry of Health and Long-Term Care (MOHLTC) and is accredited by the RCPSC.

Methods
A multidisciplinary, province-wide committee plans each forum. Toronto is the host city based on audience feedback from all Ontario regions, accessibility to the rest of the province and its attractions. Funding is provided for transportation and accommodation costs for all TC chairs, or their alternates if they cannot attend, to encourage their participation in this event. This is crucial for attendees from more remote areas of the province. The first forum was held in March 2008 and Transfusion Committees and the toolkit were selected as the theme. Subsequent themes were selected based on forum feedback and environmental scanning.

Results
Participant satisfaction ratings for the past 5 years have averaged 4.2-4.6/5 in each rating category, where a ‘1’ rating indicates poor and a ‘5’ indicates excellent. An overwhelming majority of the participants indicated that they learned something that will influence their practice and that they plan on attending future ORBCoN events. Despite competition from many other transfusion-related educational events, the number of attendees remains consistently between 135 – 150 each year. In 2012, the launch of a new TC handbook occurred along with videos of veteran TC members that are posted to www.transfusionontario.org.

Conclusion
The Transfusion Committee forum is a successful and popular event as evidenced by the consistent attendance and high feedback ratings by the participants.
7. Massive Hemorrhage Protocol Utilization in Patients with Gastrointestinal bleeds

Administrative, Poster Presentation

Hanan Gerges MBChB, University of Alberta
Elona Turley MD, University of Alberta
Heather Blain MLT, Alberta Health Services
Tanya McKelvey MLT, Alberta Health Services
Gwen Clarke MD, FRCPC, University of Alberta and Alberta Health Services
Susan Nahiriak MD, FRCPC, University of Alberta and Alberta Health Services

A massive hemorrhage protocol (MHP) has been available from our transfusion service since 2008. Although the protocol involves more than just product support, an integral part of the MHP is the blood cooler of 6 units of packed red blood cells, 6 units of plasma, and one pool of platelets transportable with the patients. Given the recent literature regarding transfusion practices in patients with upper gastrointestinal (GI) bleeding, and lack of evidence for 1:1:1 transfusion, we sought to evaluate massive hemorrhage protocol (MHP) activations in GI bleeding patients in our region from January 2011 to June 2012.

Over that period, 339 MHP activations occurred in the region with 78 (23%) of these resulting from GI bleeds. Thirty-nine MHP packs were issued for GI bleeds at the University of Alberta Hospital (UAH - the local major academic hospital) and the remaining 39 among the community-based hospitals in Edmonton Zone. Increased hemoglobin level post-MHP was noted in 35 (44.9%) patients. Of those, 91.4% had hemoglobin level pre-MHP <90 g/L. Sixteen (20.5%) showed decreased levels post-MHP; of which 50% had hemoglobin level <90 g/L. Utilization of issued pack components at the community hospital sites was 34.2%, 66.4%, and 70.4% compared to 48.5%, 56.2%, and 60.3% at UAH for PRBCs, plasma, and platelets, respectively. For every pack issued, the community hospitals issued an average 3.92 units RBCs, 0.90 units plasma, and 0.05 pools of platelets prior to the initiation of the MHP. UAH issued an average of 3.07 units RBCs, 0.67 units plasma, and 0.10 pools of platelets pre-MHP per pack issued. After the MHP, on average, an additional 2.38 units of RBCs, 0.79 units of plasma, and 0.33 pools of platelets were issued for every MHP pack by the community hospitals. An additional 1.92 units RBCs, 0.897 units plasma, and 0.25 platelet pools were issued per MHP pack, on average, by UAH.

Conclusion: MHP activations for GI bleeds constitute a significant number of our total MHP use. The hemoglobin level was more likely to increase if pre-MHP level was <90 g/L. The appropriateness of this utilization and approach is currently under review.
8. Competency Tools for Health Professionals Involved in Transfusion Activities

Administrative, Poster Presentation

Deborah Lauzon MLT, ART, BHA, The Ontario Regional Blood Coordinating Network (ORBCoN)
Wendy Owens MLT, ART, BComm, The Ontario Regional Blood Coordinating Network (ORBCoN)
Kate Gagliardi MLT, ART, BA, The Ontario Regional Blood Coordinating Network (ORBCoN)
Troy Thompson MLT, The Ontario Regional Blood Coordinating Network (ORBCoN)
Denise Evanovitch MLT, The Ontario Regional Blood Coordinating Network (ORBCoN)
Laurie Young MLT, The Ontario Regional Blood Coordinating Network (ORBCoN)
Tracy Cameron MLT, The Ontario Regional Blood Coordinating Network (ORBCoN)
Yulia Lin MD, FRCPC, CTBS, Sunnybrook Health Sciences Centre
Ana Lima RN, HP(ASCP), Sunnybrook Health Sciences Centre

The Ontario Regional Blood Coordinating Network (ORBCoN) was established in 2006 by the Ontario Ministry of Health and Long-Term Care to facilitate implementation of a provincial strategy for blood utilization. Inherent in this strategy is improving patient safety related to blood transfusion. The Canadian Standards Association (CSA) and the Canadian Society for Transfusion Medicine (CSTM) publish national standards for Transfusion Medicine. In Ontario, the Ontario Laboratory Accreditation division (OLA) of the Quality Management Program for Laboratory Services (QMPLS) is the primary agent that assesses health care facilities’ compliance with these standards through participation in a mandatory accreditation process. Both national standards require ongoing education and competency assessment for all health professionals involved in transfusion activities including: technologists, nurses, nurse practitioners, physicians and perfusionists. In Ontario, meeting these competency requirements continues to be one of the top 10 reported non-conformances.

In collaboration with other health professionals, ORBCoN developed three online educational programs to assist health care facilities in meeting the competency requirements: Bloody Easy Tech Assess, primary target audience- technologists, launched in 2008; Bloody Easy for Nurses, primary target audience- nurses, and also applicable for perfusion and anesthesia, launched in 2009; Bloody Easy Lite, primary audience-prescribing physicians, launched in 2012. Each of these programs offers a registration and tracking system for health care facilities in Ontario, enabling them to track participation and performance and provide evidence of ongoing competency assessment for accreditation purposes. Registration for the Tech Assess program is performed by a site administrator assigned by each health care facility.

Assessments and learning resources to augment the tech assess program are updated annually. Participants in the other two programs self register and are able to affiliate themselves with one or more facilities. Site administrators at the affiliate facility are able to monitor and track performance electronically. Reports of participation can be generated on demand by site administrators. Current registration numbers for Ontario include: 2,000 MLT’s, 7,550 RN’s and 275 MD’s. These programs provide Ontario health care facilities with a confidential, standardized, no cost solution to meeting the accreditation requirements for ongoing education and competency assessment of health professionals.
Development and Implementation of a Procedure for RBC Syringe Aliquot Preparation for Neonatal Resuscitation

Administrative, Poster Presentation

Darlene Mueller MA, ART, Fraser Health Authority
Diana Kobes RT, Fraser Health Authority
Doug Morrison FRCP, MD, Fraser Health Authority

In response to patient safety quality reviews of neonatal resuscitation events, we recognized the need for a health authority wide approach to provision of red blood cells in these critical clinical situations. As a result, the Fraser Health Transfusion Medicine Laboratory (TML), with input from the maternal and neonatal program, developed a procedure to provide pre-filtered syringes of unmatched O Negative red blood cells for immediate transfusions.

This procedure was implemented at eleven acute care facilities in Fraser Health where newborns are delivered. Implementation included the use of a training and competency assessment document and direct observation of over 200 technologists that rotate through the transfusion medicine laboratory.

One year after implementation, a TML process audit was initiated in response another patient safety review. Internal audit results revealed the need for technologists to perform this procedure on a regular basis with a focus on the physical preparation, computer steps, and communication with the clinical unit. On the clinical side, the need to notify the TML, as early in the event as possible, using consistent, clear, and predetermined language was identified as an area in need of improvement.

A revised procedure was developed, validated, and implemented with direct observation. This included a laminated, large font job aide for posting at the bench. Pre-packaged, standardized supply kits were developed to ensure that technologists could provide the syringe within 10 minutes of receiving the request. Informal feedback from technologists during implementation of these revisions indicated that the ease in preparation of this component had been improved.

Throughout this quality improvement cycle, engagement of the laboratory leadership team and the maternal/neonatal program has resulted in increased support for this critical procedure. The laboratory leadership team has endorsed implementation of an enhanced yearly competence assessment program for this and other selected low frequency, high acuity procedures. The maternal/neonatal program has committed to include information on the availability and ordering of this component as part of their health authority wide bi-annual neonatal resuscitation certification program.
10. Implementation of Two Anti-D Reagents in a Hospital-based Transfusion Service: Detection of weakened RhD serological expression

Administrative, Poster Presentation

Chantale Pambrun MD, FRCPC, IWK Health Centre
Katherine Gough MLTII, IWK Health Centre
Catherine McAuley MLTII, IWK Health Centre
Patti Burrell MLT, BHSC, DHSA, IWK Health Centre

Approximately 0.2-1% of Caucasian individuals will react weakly with anti-D reagents. (1) The detection of a weakened serological expression, as well as a discrepant result between reagents, highlight a difference in the RhD antigen, weak D or partial D genotype. It is important to detect the serological discrepancy in order to determine the underlying genetics and the risk of alloimmunization to RhD. The risk of alloimmunization to the D antigen has implications in hemolytic disease of the fetus and newborn, as well as the decision to administer Rh immunoglobulin (RhIg). The IWK Health Centre is the prenatal reference laboratory in Nova Scotia, and has recently implemented the use of two D antisera for all blood bank samples. Seventy-five percent of the samples received are from females, which is the group of interest for detection of weak D and partial D genotype. The implementation of the new policy included technical staff education, clinical input and coordination with the National Immunohematology Reference Laboratory. The most difficult element to implement was the workflow to accommodate the automated gel methodology and the manual tube methodology. The use of standard operative procedures, flow charts, and interpretative tables were an asset. Daily checks by senior technologists, were vital to pick up process errors during early implementation. To date, the published incidence of weak D and partial D is reflected in our population. Moving forward, women with a weak D genotype will not receive RhIg unnecessarily and those with a partial D genotype will be treated as Rh negative to avoid D alloimmunization and hemolytic disease of the fetus and newborn. Could Canadian hospital-based transfusion services be doing more to avoid blood product exposure (RhIg) and alloimmunization of females with child-bearing potential?

(1) Transfusion 2005;45:1547–51.
11. Transfusion Education for BC Pathologists

Administrative, Poster Presentation

Sheazin Premji MBA, BC Provincial Blood Coordinating Office
Kate Chipperfield MD, FRCPC, Vancouver Coastal Health
Jason Doyle MD, FRCPC, Interior Health
Doug Morrison MD, FRCPC, Fraser Health
Louis Wadsworth MD, FRCPC, University of British Columbia
Shelley Feenstra RN, Vancouver Coastal Health
Donna Miller RN, Vancouver Island Health Authority
Maureen Wyatt ART, Interior Health

Background
In order to identify and respond consistently to transfusion reactions in BC, new provincial minimum standards of practice have been endorsed for transfusion reaction reporting. New standardized forms, process and procedure documents for laboratory investigation, and training materials were developed, and to complement these materials, a six module online web-based education program entitled Transfusion Reaction Education for BC Pathologists was developed and launched in October 2011.

Proposal
Three cycles of the education program were offered between October 2011 and September 2012. The course was initially targeted at BC pathologists but was subsequently opened to other clinicians in BC and other provinces, and other stakeholders including doctors, nurses and technologists. The education program was accredited and provided Section 1 Credit as defined by the Maintenance of Certification program of the Royal College of Physicians and Surgeons of Canada.

The objectives for the education program included:
• Enhancing knowledge and understanding of transfusion surveillance activities in BC.
• Presenting the new provincial minimum standards of practice for transfusion reactions and supporting process and procedure documentation.
• Reviewing types, signs and symptoms of transfusion reactions.
• Introducing standardized guidelines for pathologist conclusions for transfusion reaction reporting.
• Using case studies to enhance knowledge uptake.

Results
A total of 936 completions were recorded for all three cycles (one completion is equivalent to one module completed with quiz passed) – 199 in cycle 1, 180 in cycle 2 and 557 in cycle 3. Interest in the online course was widespread, with people participating from BC, Alberta, Ontario, Saskatchewan, Nova Scotia, Manitoba, Yukon and even outside of Canada. Participant types included Doctors, Technologists, Nurses, Clinicians, Transfusion Safety Officers, and Medical Services Coordinators.

Conclusion
Participants were asked to complete an optional online evaluation at the end of each module. Out of 936 completions, 608 (65%) participant evaluations were received. The majority of the respondents ranked the overall quality of presentations as excellent (43%) or good (49%).

Outcomes and results from before and after the education initiative will be measured and reviewed to assess the effectiveness of this initiative and to help identify further education opportunities.
Clinical Abstracts

12. A Closer Look at the Emergency Department STAT Type & Screens: When is it really an emergency?

Clinical, Poster Presentation

Salah Aboughouche Grad Student (pre-residency), University of Saskatchewan
Karen L. Dallas, Saskatoon Health Region
Linda Friesen MLT, Saskatoon Health Region

Introduction
Saskatoon's biggest Emergency Department (ED) routinely sends STAT type and screen test to the Transfusion Medicine Laboratory (TML). Some of these test are clearly emergencies and sometimes blood must be issued out prior to the completion of this pre-transfusion testing. However, at other times, testing is sent without apparent rationale. With increasing constraints on our budget and workforce in TML and with an increasing focus on patient-first care, we felt it prudent to look into possible unnecessary testing.

Objective
Objective of this study was to assess the significance and clinical utility of STAT type and screen testing sent from the ED to TML.

Method
All of the patients who had STAT type and screens sent from the ED during the months of April and May 2012 were studied. Data was manually collected on a spreadsheet and included Diagnosis, Hgb level, whether or not blood was requested, whether the patient got admitted and whether the patient had a positive antibody screen. Data was then entered into an Excel format for analysis.

Results
624 patient had data collected. Of these patients, 514 (82.4%) had Hgb levels of >100 g/dL. Additionally, 555 patients (88.9%) did not have a requisition for blood. 269 (43.1%) patients went on to be admitted to hospital while the rest were discharged. Also important for the lab, nearly 5% of patients had positive antibody screens which required a full work-up.

Conclusions
In conclusion, we have uncovered a large source of wastage when it comes to the utilization of pre-transfusion testing. Not only does this waste time and resources for the lab, it affects patient care — both for those patients we are needlessly drawing blood on and potentially for other patients who are not getting as timely testing in TML because of these unnecessary STAT specimens. Clearly there is opportunity here for improvement in our local practice and we are currently in active discussion with our clinical colleagues about this matter.
Background
Transfusion-related acute lung injury (TRALI) is a devastating transfusion-associated adverse event reported after transfusion. There is a paucity of data on the incidence and characteristics of TRALI cases that occur preoperatively. We classified suspected perioperative TRALI cases reported to Canadian Blood Services, between 2001 and 2012, and compared them to non-perioperative cases, to elucidate specific surgical factors that make them particularly at risk for developing TRALI.

Study Design and Methods
Using Canadian Consensus Conference definitions, all suspected TRALI cases reported to Canadian Blood Services (CBS) since 2001 were reviewed by two experts or, from 2006 to 2012, an expert TRALI Medical Review Group (TMRG) were classified and detailed in a database. Two additional reviewers further categorized them as occurring within 72 hours from the onset of surgery (perioperative) or not in that period (non-perioperative). Various demographic and characteristic variables of each case were collected and compared between the groups.

Results
In Canada, between 2001-2012, a total of 303 suspected TRALI cases were reported to Canadian Blood Services. Of those, 38% (112) were identified as occurring during the perioperative period. Patients who underwent cardiac surgery requiring cardiopulmonary bypass (25.0%), general surgery (18.0%) and orthopedics patients (12.5%) represented the three largest surgical groups. Perioperative TRALI cases comprised of more men (53.6 vs. 41.4, p=0.0395) than non-perioperative patients. More perioperative TRALI patients required supplemental O2 (14.3% vs. 3.1%, p=0.0003), required mechanical ventilation (18.8% vs. 3.1%), or were from the ICU (14.3% vs. 3.7%, p=0.0043), than non-perioperative TRALI patients before their operations. The surgical patients were transfused approximately 673 products and on average were transfused more products than non-perioperative patients (6.0[SD=8.3] vs. 3.6[5.2], p=0.0002). Perioperative TRALI patients were transfused more frozen and fresh frozen plasma and cryoprecipitate than non-TRALI patients. Non-perioperative patients utilized more cryosupernatant plasma. There was no difference between donor antibody test results between the groups.

Conclusion
CBS data has given more insight into the nature of TRALI cases that occur perioperatively; this group represents a large proportion of TRALI cases. Perioperative transfusion that occurs within this specific population should be carefully considered and monitored, especially in patients with at-risk characteristics. Further research will be required to delineate the exact mechanisms behind perioperative TRALI.
Intravenous immune globulin (IVIg) use for neurological conditions accounted for 36% of total IVIg use in BC at a cost of $11.9 million in 2011/12. Neurology is the second fastest-growing specialty user of IVIg; up 5.6% last fiscal year and up 6.2% in the last three fiscal years (2009/10 through 2011/12), compared to an overall provincial IVIg increase of 5.3% (1 year) and 11.7% (3 years). Neurology is also the highest dose specialty, with a mean use of 449 grams/patient in 2011/12 compared to a mean use of 293 grams/patient for all specialties.

The IVIg Neurology project entailed working with a task force of provincial Neuromuscular (NM) Neurology representatives to define the approaches and develop the systems to effectively manage patients who require IVIg for neuromuscular conditions. The project involved finalizing a provincial approach for IVIg use for approved clinical conditions including treatment, patient follow-up and reassessment for IVIg efficacy and dose reduction. The use of the IVIg dose calculator which was brought into effect in BC last year as a part of the IVIg directives was incorporated into guiding NM prescribing practices. The IVIg NM task force put together a physician toolkit for physicians across BC to use to support standardization of clinical approaches. This will help improve patient care and product utilization across the province. The IVIg NM task force also established a provincial Neurology IVIg screening panel to assess the appropriateness of using IVIg to treat rare and complicated cases through the evaluation of treatment outcomes.
15. **Loss of RhD Expression Coinciding with Relapsed Acute Myelogenous Leukemia**

Clinical, Oral Presentation

Signy Chow MD, University of Toronto
Vikas Gupta MD, FRCP, FRCPath, Princess Margaret Hospital, Toronto
Jacob Pendergrast MD, FRCP, University Health Network, Toronto
Chistine Cserti-Gazdewich MD, FRCP, FASCP, University Health Network, Toronto

**Background**
RHD expression is expected to be stable outside of hematopoietic stem cell transplantation (HSCT), although spontaneous loss due to chimerism, mosaicism or somatic mutation has been described, with such events predominantly associated with malignancy.

**Case**
We describe a case of a 50 year old acute myelogenous leukemia (AML) patient whose loss of RHD expression coincided with relapsed malignancy. The original diagnostic bone marrow suggested preceding chronic myelomonocytic leukemia (CMML) in the company of splenomegaly, while JAK2 and BCR-ABL studies were negative. Standard induction chemotherapy was followed by bone marrow evidence of morphologic remission two months later, and no cytogenetic abnormalities were noted at diagnosis or post-induction.

His presenting transfusion laboratory sample typed as O, RHD-positive with a negative red cell antibody screen, and stable repeat grouping for two months. Platelet transfusion refractoriness developed (PRA 99%), and he qualified for procurement of HLA-matched platelets. The AML relapsed, and he was then found to have mixed field reactions on RHD grouping (despite no transfusions with O- RBC or HSCT), with two distinct populations of O+ and O- cells. Cytogenetic re-analysis of marrow did not demonstrate any abnormalities by G-banding.

Analysis of a peripheral blood sample demonstrated the RH genotype ccEe with D (R2r), although the ratio of e/E was out of the usual range for heterozygosity, with e present more abundantly than E. Repeat testing with alternative sequencing primers ruled out unequal amplification of allele-specific polymorphisms at primer sites, while the absence of other out-of-range data for other antigens excluded chimerism. With the mixed field reactions indicating loss of the RHD, this suggested a specific deletion of an entire DcE (R2) allele at a clonal pre-erythroid level.

**Discussion**
Loss of heterozygosity (LOH) on chromosome 1 has been shown to be an important mechanism of RHD loss. While such loss may be benign, the role of LOH in leukemogenesis is also well known, with cases of the loss and resurgence of RHD expression paralleling the development and remission of malignancy. Alteration in RH expression may be either a surrogate for relapsed malignancy or the effect of an ongoing clonal evolutionary process.
**Adult Thalassemia Patients Are Not Disadvantaged By Matching Less Rigorously Than The Sickle Cell Standard**

Clinical, Poster Presentation

Christine Cserti-Gazdewich MD, FRCPC, University Health Network and University of Toronto
Michael Angers MLT, University Health Network
Anumithraa Niranjan BHS(cand), University of Ontario Institute of Technology
Gisele Thorpe MLT, University Health Network
Jacquie Beal MLT, University Health Network
Sally Balmer ART, University Health Network
Janice Hawes BA, MLT, University Health Network
Elana Tsiokos MLT, University Health Network
Jacob Pendergrast MD, FRCPC, University Health Network and University of Toronto
Marciano Reis MD, FRCPC, Sunnybrook Health Sciences Centre & University Health Network, University of Toronto

**Background**

Transfused patients are at risk of alloimmunization according to their “immune responder” status and the extent of their exposures to erythrocytes (RBCs) bearing unfamiliar antigen types. Sickle cell disease patients (SCD) are known to be strong responders, with transfusions occurring intermittently amid inflammatory/ischemic-hemolytic crises, while facing ancestrally more divergent (predominantly non-African) donor antigen profiles. Conversely, chronically transfused thalassemia major (TM) patients are exposed in a more tolerizing schedule since their infancy, with the quality of blood having greater incidental phenotypic similarity but the quantity of donor exposures unsurpassed. In SCD, guidelines promote prophylactic antigen matching (PAM) towards RHCE and KELL, with extensive antigen matching (EAM) towards DUFFY, KIDD, and Ss after sensitization towards any significant minor red cell antigen, therein avoiding further seroconversions and sequelae thereof.

**Problem**

At our 1000-bed acute care teaching hospital, policies since 1991 advise PAM/EAM to SCD patients by blood group profile and serostatus. TM patients are not phenotyped, but are empirically given units tagged as Kell-negative, with antigen-negative blood only as needed. Whether TM warrants the SCD standard of matching is unsettled.

**Study Design and Methods**

On-site summary alloimmunization and transfusion prevalences for SCD and thalassemia patients were compared to determine if the latter were disadvantaged by our policy. Patients registered within the Laboratory Information System (HCLL 4.6.0.2, Mediware Info Sys Inc, Oakbrook IL) were filtered by diagnosis-related instruction and dates of first-to-last transfusions within a 19y post-policy period (11/1993–11/2012).

**Results**

Twice as many SCD (538) as thalassemia (257) were registered, although only 38% (208) of the former were transfused compared with 66% (169) of the latter. RBC sensitization occurred in 83 SCD patients (40% of the transfused [95% CI: 33–47%], or 15% overall), and in 75 thalassemia patients (44% of the transfused [95% CI: 37–52%], or 29% overall); transfusees were thus not statistically different (2-tailed Z-score 0.87). Despite policy, 74 SCD RHCE/KELL antibodies (46%) and 20 thalassemia KELL antibodies (13%) occurred/pre-existed, while insignificant antibodies dominated in thalassemia (81 or 52% vs 45 or 28% in SCD).

**Conclusion**

Resource-intensive matching, although of limited efficacy, is best reserved for SCD.
17. Hospital-based Transfusion Error Tracking from 2005 to 2010: Identifying the key errors threatening patient transfusion safety

Clinical, Poster Presentation

Helen Downie MLT, Sunnybrook Health Sciences Centre
Carolyn Maskens BSc, University of British Columbia
Alison Wendt MLT, Sunnybrook Health Sciences Centre
Lisa Merkley MLT, Sunnybrook Health Sciences Centre
Ana Lima RN, Sunnybrook Health Sciences Centre
Yulia Lin MD, Sunnybrook Health Sciences Centre
Jeannie Callum MD, Sunnybrook Health Sciences Centre

Background
This report provides a comprehensive analysis of transfusion errors occurring at a large teaching hospital and aims to determine key errors that are threatening transfusion safety, despite implementation of safety measures.

Study Design and Methods
Errors were prospectively identified from 2005-2010. Error data was coded on a secure online database called the Transfusion Error Surveillance System (TESS). Errors were defined as any deviation from established standard operating procedures. Errors were identified by clinical and laboratory staff. Denominator data for volume of activity were used to calculate rates.

Results
A total of 15,134 errors were reported with a median number of 215 errors per month (range 85-334). Overall, 9083 (60%) errors occurred on the transfusion service and 6051 (40%) on the clinical services. In total, 23 errors resulted in patient harm: 21 of these errors occurred on the clinical services and 2 in the transfusion service. Of the 23 harm events, 21 involved inappropriate use of blood. Errors with no harm were 657 times more common than events that caused harm. The most common high severity clinical errors were sample labeling (37.5%) and inappropriate ordering of blood (28.8%). The most common high severity error in the transfusion service was sample accepted despite not meeting acceptance criteria (18.3%). The cost of product loss due to errors was $593,337.

Conclusion
Errors occurred at every point in the transfusion process, with the greatest potential risk of patient harm resulting from inappropriate ordering of blood products and errors in sample labeling.
Background
Chagas Disease is caused by a protozoan parasite, Trypanosoma cruzi, transmitted by triatomine bugs in Mexico, Central and South America. Increasing immigration to North America from these countries has prompted the screening of blood donors for Chagas Disease. In May 2010, Canadian Blood Services (CBS) began testing ‘at risk’ donors for Chagas antibody. A seroprevalence study was set up in September 2010 to test a subset of ‘no risk’ donors. This was done primarily to assess the efficacy of the risk questions in identifying donors for Chagas testing.

Method
A target of at least 60,000 donors from clinics in areas reflecting immigration from endemic countries was selected for Phase I and included donors from southern Ontario, Manitoba, Alberta and British Columbia. Phase II was carried out in Manitoba only. Donors who answered no to all three Chagas risk questions were tested for Chagas antibody using the Abbott PRISM Chagas assay. All repeat reactives were sent to the National Reference Centre for Parasitology (NRCP) at McGill University, Montreal, for testing by ELISA, immunoblot and Polymerase Chain Reaction (PCR). Samples were sent to Quest Laboratories (U.S.) for the Radioimmunoprecipitation assay (RIPA) and to Blood Systems Inc. laboratories for the Ortho Chagas assay used for donor screening in the U.S.

Results
From September 2010 to October 2012, 84,078 donors were tested, with 62,117 tested in Phase I and 21,961 in Phase II. Only one confirmed positive donor was identified in Phase I. Further investigation revealed that the donor was infected via vertical transmission from her mother who had been transfused, around the time of the donor’s birth. This donor would not have been detected by any risk questions. The recipient of the only product transfused from this donor, tested negative for Chagas Disease. Phase II found no additional confirmed positive donors.

Conclusions
Based on the finding of only one confirmed positive donor of more than 80,000 ‘no risk’ donors tested, CBS has maintained the strategy of testing only at risk donors, identified by questioning. This strategy represents a new paradigm in infectious disease testing at CBS.
Investigation of a Possible Case of WNV Transfusion Transmission: Summer 2012 season

Clinical, Poster Presentation

Elaine Fournier RN, Canadian Blood Services
Peter Lesley MD, Canadian Blood Services
Melanie Tokessy MLT, The Ottawa Hospital
Barbara Hannach MD, Canadian Blood Services
Kai Makowski MLT, Public Health Agency of Canada
Vito Scalia MSc, Canadian Blood Services
Margaret Fearon MD, Canadian Blood Services

Background
On August 24, 2012, a sixty five year old man presented to the Ottawa Hospital Emergency Room with pancytopenia and progressive fatigue. Five units of red cells and one unit of pooled platelets were transfused over the course of a week. A diagnosis of myelodysplastic syndrome was made and the patient was discharged home on August 30. On September 1, after being home for one full day, he returned to the Emergency Room with fever, sore throat, nausea and neurological symptoms. The patient was transfused with more platelets and red cells. On September 12, the Ottawa Hospital reported a possible transfusion transmitted West Nile Virus infection. Canadian Blood Services was advised the recipient had tested positive for WNV IgM on September 7 and was confirmed to have WNV encephalitis.

Method
As the patient was at home for less than forty eight hours and did not report any mosquito bites during that time, the source of the West Nile Virus infection was suspected to have been from a transfusion. Five units of red cells and two units of pooled platelets (four donors each) were possibly associated with the West Nile Virus infection. All thirteen associated donors were contacted by Canadian Blood Services and requested to return for follow up testing. All of the donors complied with the request. Testing was performed at Canadian Blood Services for WNV NAT and serology (IgM and IgG).

Results
All donors tested negative for WNV NAT. Serological testing for WNV IgM and IgG identified one donor positive for IgG and two donors as IgG equivocal. All donors were IgM negative. The manufacturer of the antibody test kits subsequently recalled the IgG kits being used by Canadian Blood Services due to inaccurately high readings. Consequently, Canadian Blood Services retested the three donor samples (which had tested IgG positive or IgG equivocal) using antibody test kits with new lot numbers, supplied by the manufacturer. The three initially IgG reactive donors were found to be negative on retest. The three donor samples were also tested by the PHAC, National Microbiology Lab in Winnipeg and all IgG results were confirmed negative. One donor tested WNV IgM equivocal in the Winnipeg lab but was NAT and IgG negative. The donor who initially tested IgG positive and the one who tested IgG indeterminate (on the recalled test kits) were antibody negative on follow up samples three weeks after the initial test. The third donor was unavailable to provide a second sample for follow up antibody testing.

Conclusion
Although the source of WNV infection could not be identified, it is likely that the infection occurred as a result of mosquito borne transmission, despite the narrow window of opportunity. There is no laboratory evidence to support that this was a transfusion transmitted West Nile Virus infection.
20. Use of Intravenous immunoglobulin in Neurological Conditions

Clinical, Poster Presentation

Angela Genge MD, FRCP(C), Montreal Neurological Hospital

Introduction
This abstract reviews the uses of immunoglobulin across the spectrum neurological diseases. In addition it will include the range of doses and the schedules in use as well as the practical approach to determining the ideal dose frequency. Finally the outcome measure used to determine eventual long-term scheduling and dis continuation will be discussed.

Methods/Design
This is review of all patients who have received intravenous immunoglobulin for neurological conditions at the Montreal Neurological Institute, both as inpatients and outpatients for the last 12 months ending March 15, 2013. Currently over 50 percent of the intravenous immunoglobulin used at the McGill University Health Center is prescribed for neurological indications. The doses used are significantly higher than used in immunodeficiency indications. The longest period of time that any current patient has been receiving intravenous immunoglobulin at our institution is 23 years.

Results
There are 208 outpatients and 20 inpatients who have received intravenous immunoglobulin over the last 12 months in our institution. The diagnoses include NMDA encephalitis, rasmussen’s encephalitis, guillian barre syndrome, chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy, neuromyelitis optica, myasthenia, gravis, dermatomyositis, polymyositis, inclusion body myositis, polyarteritis nodosa, diabetic neuropathy/diabetic amyotrophy and necrotizing myositis secondary to statin use.

Modes of therapy presented in this review are exclusively intravenous. Doses range from 0.4 mg per kg per day to 1 gm per kg per day. Frequency of infusions range from 3–5 consecutive days to weekly to every 6 weeks.

The initiation of IVIG for all conditions in which it is used is based on the evidence in each condition that it is autoimmune in etiology. Further evidence supporting the use of IVIG is in the form of published case series essentially over the past 25 years. Initial dosing was determined arbitrarily to be 0.4 mg/kg per day in a study in Guillian Barre Syndrome. A subsequent series of studies in multifocal motor neuropathy have led to using an induction dose of 1g/kg/day for 3 days. In the neuromuscular condition strength assessment and functional deficits are used to guide frequency. In patients who are quadriplegic dosing starts at weekly up to 1g/kg/day. As specific goals are achieved in ambulation the frequency is reduced. IVlg alone does not induce remission in autoimmune neurological disease. However it returns patients to a normal level of functioning and maintenance therapy enables return to work.

The only neurological disease for which intravenous immunoglobulin is approved is chronic inflammatory demyelinating polyneuropathy.

Conclusions
IVlg is an extremely effective and important treatment for otherwise untreatable or poorly treated neurological conditions. Using various doses and frequencies IVlg is an important therapy in neurological diseases.
Pre-Transfusion Testing: Are we wasting our time?

Chaturika Herath, Saskatoon Health Region
Karen L. Dallas, Saskatoon Health Region
Linda Frieson MLT, Saskatoon Health Region

Introduction
Pre-transfusion testing ensures ABO compatibility between donor and recipient and detects most clinically significant red cell alloantibodies that could react with donor red cells and cause a hemolytic transfusion reaction. According to current Saskatoon Health Region Guidelines, this testing must be performed ≤ 96 hours prior to the administration of red cells to determine ABO and Rh compatibility and, more importantly, whether new alloantibodies have developed. Over time, some clinical areas have adopted a practice of repeating testing every 96 hours regardless of whether or not a patient is being transfused, thus being “ready” just in case the patient does need a transfusion.

Objective
The objective of this study was to assess the significance and clinical utility of this every-96 hour group and screen practice on the Hematology-Oncology ward.

Method
All of the patients who had grouping and screening performed during the month of April 2012 were retrieved from the computer database. Data on those who had red cell transfusions during this period was also retrieved.

Patients who had grouping and screening every 96 hours (regardless of plans to transfuse) and the others (i.e. patients who had pre-transfusion testing done less frequently) were categorized into Groups A and B, respectively. Transfusion trends among Groups A and B were assessed.

Results
Total of 51 patients had grouping and screening during the month of April. Three were excluded as they were admitted towards the last day of the month. Total of 47 patients were included in the study. Among 47 patients, 28 (59.6%) had every 96 hour grouping and screening (Group A). The rest (19/47, 40.4%) had infrequent grouping and screening (Group B) 11 out of 28 (39.2%) Group A patients had no transfusions. 12 out of 19 (63.1%) Group B patients had no transfusions. 42.9% of Group A patients had just one transfusion. Only 17.9% of Group A patients had more than one transfusion.

Conclusion
The every-96 hour group and screen practice – in anticipation of possible transfusion – does not appear to hold any value. It is likely increasing patient discomfort, leading to iatrogenic anaemia, and increasing the work load of nursing and laboratory staff. Certainly, it leads to significant resource wastage within our health region. Discussions are currently underway between the laboratory and the clinical team to change this practice.
22. An In-house Method to Assess T Activation in Paediatric Patients with Pneumococcal Hemolytic Uremic Syndrome

Clinical, Poster Presentation

Wendy Lau MD, The Hospital for Sick Children
Archana Bhatt MLT, The Hospital for Sick Children

Background
Hemolytic uremic syndrome (HUS) is often associated with Escherichia coli or Shigella infection, with a small proportion caused by Streptococcus pneumoniae. Neuraminidase released by Streptococcus pneumoniae can lead to de-sialylation of red cell membrane glycoproteins exposing the cryptic Thomson-Friedenreich antigen (T-antigen), resulting in hemolytic anemia. The lectins for T activation testing are not routinely available in most transfusion laboratories, so we devised an in-house method to test for T activation when results are needed urgently.

Case Report
A four year old girl was admitted with Pneumococcal HUS. Her admission Hb was 86g/L, which dropped to 40g/L a few hours later. She was transfused with washed red cells. The Transfusion Service was asked to look for T-activation as her Partial Thromboplastin Time (PTT) was elevated and the clinician was contemplating plasma transfusion prior to line insertion for dialysis. Our transfusion service does not have the lectins to test for T activation, and this being Friday afternoon, the patient’s sample would not arrive at the reference laboratory until the following Monday.

Methods
Monoclonal antisera routinely used for ABO typing do not contain anti-T, so the patient’s red cells were tested against plasma samples from patients over 10 years of age, as well as against plasma samples from neonates.

Results
Patient is B positive, antibody screen negative, DAT negative.

<table>
<thead>
<tr>
<th>Patient cells</th>
<th>Monoclonal anti-A</th>
<th>Monoclonal anti-B</th>
<th>Plasma (Gp A)</th>
<th>Plasma (Gp B)</th>
<th>Plasma (Gp AB)</th>
<th>Plasma (Gp O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Prewarmed SIAT</td>
<td></td>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baby plasma (Gp A)</td>
<td>Baby plasma (Gp B)</td>
<td>Baby plasma (Gp AB)</td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td>0 to wk</td>
<td>wk</td>
<td>0 to wk</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusions
These results suggest the presence of an IgM antibody, present in plasma of all ABO groups, reacting with patient’s red cells. Given the clinical history, this antibody is most likely anti-T, indicating the patient’s cells are T activated. It was recommended to the clinician that plasma transfusion be avoided if possible. Thus, on a Friday afternoon, this in-house test allowed us to make informed decisions about transfusion before T activation can be confirmed by a reference laboratory.
A Case of Drug-induced Hemolytic Anemia Due to Ceftriaxone in a Paediatric Patient

Clinical, Poster Presentation

Wendy Lau MD, The Hospital for Sick Children
Archana Bhatt MLT, The Hospital for Sick Children

Case Report
A six year boy with HbSC disease was transferred from another hospital with fever and upper respiratory tract infection. He was being treated with ceftriaxone. On admission, his Hb was baseline (123g/L), showing a slow decline over a few days with evidence of intravascular hemolysis. On the fourth day, a precipitous drop to 13g/L occurred, with loss of consciousness and grand mal seizures. He was transfused urgently but Ceftriaxone was continued for 2 more days before it was stopped. A few days later, the Blood Transfusion Laboratory was asked to look for evidence of ceftriaxone antibody.

Serological Testing
Patient is O positive with no history of clinically significant antibody. On admission, his antibody screen was negative, DAT positive with complement. The initial diagnosis was paroxysmal cold hemoglobulinuria, but the Donath-Landsteiner test was negative. When ceftriaxone-induced hemolytic anemia was suspected, we tested the eluate and the patient’s plasma by adding ceftriaxone in a 1:1 ratio.

Results

<table>
<thead>
<tr>
<th>Date</th>
<th>12/25</th>
<th>12/30</th>
<th>01/01</th>
<th>01/02</th>
<th>01/04</th>
<th>01/05</th>
<th>01/09</th>
<th>01/12</th>
<th>01/15</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DAT (C3)</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>wk</td>
<td></td>
</tr>
<tr>
<td>DAT (IgG)</td>
<td>0</td>
<td>wk</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Eluate</td>
<td>wk</td>
<td>wk</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Eluate + Ceftriaxone</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Plasma + Ceftriaxone</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

The test for ceftriaxone antibody was finally negative a month after admission. During this time, the patient received a total of 20 units of red cells, and underwent plasma exchange multiple times, using 132 plasma units in total.

Conclusions
On reviewing the patient’s history, it was discovered that he had been briefly DAT positive with anti-complement a year ago, when he was treated with ceftriaxone. This second exposure to ceftriaxone resulted in much more severe hemolysis. The hypoxemia has resulted in ischemic lesions and gangrene of his fingers and toes. Ceftriaxone is a commonly prescribed antibiotic for children with sickle cell disease. This case illustrates the severity of ceftriaxone-induced hemolytic anemia and the importance of early recognition and diagnosis.
Two Episodes of Anaphylactoid/Anaphylactic Reactions in One Paediatric Patient Related to Food Allergies

Clinical, Poster Presentation

Wendy Lau MD, The Hospital for Sick Children
Kathleen McShane MLT, The Hospital for Sick Children
Mary Huang RN, Canadian Blood Services

Case Report
A nine year boy was diagnosed with medulloblastoma in March, and was treated with chemotherapy and tandem autologous stem cell transplants in Aug, Sept and Oct. On Nov 12, he ate salmon at home and 5 minutes later started coughing and complaining of foreign body in throat and swollen lip. On arrival in Emergency, he was hypotensive and tachycardic, but was conscious and complained of a bone stuck in his throat. He responded to treatment with benadryl. Immunology recommended radioallergosorbent test (RAST), avoid salmon and vertebrate fish, and carry an EpiPen. On Nov 16, while at home, he ate one Reese peanut butter cup and within 10 minutes had vomiting and swelling of his top lip. He did not have an EpiPen yet, so he went to Emergency and was treated with benadryl. On Nov 18, he was admitted for his fourth transplant. Skin tests showed strong reactions to mixed fish, salmon, peanut and tree nut mix. The patient had no known allergies to fish or nuts, and had been eating these foods without incident until his last transplant. As it is highly unusual to develop new allergies at this age, we reported the reactions to the blood centre to investigate the blood donors involved. Since Nov 1, he had received one red cell unit, 2 platelet pools and two apheresis platelet units. The red cell unit, which contained little plasma, was not a concern, so we started with the apheresis donors, who denied any allergies. Then the male donors who provided the plasma for the platelet pools were interviewed; one donor had no allergies, the other reported allergies to shellfish and fish and all nuts including peanuts. This platelet pool was transfused on Nov 3. The patient’s RAST subsequently came back positive for both salmon and peanut.

Conclusion
Two episodes of anaphylactoid/anaphylactic reactions, after eating different foods, occurred 9 and 13 days after one transfusion from a donor with allergies to the same foods. The most likely cause is passive transfer of IgE antibodies from donor to patient. This donor has been deferred from future donations.
25. **Red Cell Recycling: An Update**

Clinical, Poster Presentation

Tanya McKelvey MLT, BSc (MLS), Alberta Health Services  
Gwen Clarke MD, FRCPC, University of Alberta and Alberta Health Services  
Susan Nahiriak MD, FRCPC, University of Alberta and Alberta Health Services

**Background**

In April 2003, a red blood cell redistribution system was implemented that allowed recycling of near outdate red cell units to the University of Alberta Hospital (UAH) Transfusion Medicine laboratory. In the first year of the program, 4 rural sites from Northern Alberta recycled a total of 138 units. These four sites had success rates (defined as a unit being acceptable for inventory entry at the UAH) ranging from 59%-78%, with an overall success rate of 69%. Of the units accepted into inventory, 89% were group O. The majority of the failures were temperature failures (49%), tamper evident seal missing/broken (28%), and other (23%). This prompted re-education of participating sites with re-evaluation of packing configurations and standardization of requirements allowing an additional 2 sites to come on board in each of the third and fourth year of the program.

**Results**

By the end of 2012, a total of 9 sites participated in the program. In 2012, a total of 950 units were returned. The success rate for these sites ranged from 69%-100% with an average rate of 92%. The average age of the units on receipt was 29 days. Of the units considered acceptable for inventory 67% were group O, 98% were utilized and only 0.2% were outdated (≤ 1 day from outdate upon receipt). The majority of failures were temperature failures (28%), data logger failures (23%), transport time > 24 hours (16%), and other miscellaneous problems (35.1%).

**Conclusion**

Although we have increased the number of units recycled by 688% and more than doubled the number of participating sites, up to 8% of recycled units are still being discarded because they are not acceptable for inventory. Root cause analysis and attention must be given as to how we can improve this process further so we can maximize the utility of these resources, especially as we have recently expanded the program to accept redistribution of plasma protein products and have been approached to expand to additional sites.
Troubleshooting the Automated Blood Bank Analyzer

Clinical, Poster Presentation

Tanya McKelvey MLT, BSc (MLS), Alberta Health Services
Gwen Clarke MD, FRCPC, University of Alberta and Alberta Health Services
Susan Nahiriak MD, FRCPC, University of Alberta and Alberta Health Services

Background
In June 2009, automation was introduced in Edmonton Transfusion Medicine laboratories. Effective troubleshooting of automation breakdowns is critical to manage loss of functionality and tech time during downtime. Automated Blood Bank analyzers contain numerous interconnected hardware modules that must be running at peak performance to achieve accurate test results. Identification of components with recurring breakdowns and exploration of the root cause can highlight areas where preventative maintenance not addressed by the manufacturer would be of benefit.

Results/Case Study
Over the past 4 years, the fluidics module has been identified by “primary operators” (technologists with advanced training) as a recurring source of breakdowns. The most common breakdowns are 1) blocked probe rinse station, 2) blocked wash station, 3) sensor degradation in the saline and waste containers, and 4) corrosion of fluidics hardware including the peristaltic pump. Extensive troubleshooting of these issues has provided experience based training towards efficient troubleshooting. For example, leakage from the probe rinse station is often due overflow from a blocked filter. Increased equivocal results can be due to saline crystallization in the wash station. Quick identification of such blockages translates to downtime resolution in minutes versus > 24 hours for a service call and in the latter case, immediate result quality improvement. Efficient identification of breakdowns that require a service call, like degraded sensors or a corroded peristaltic pump, can further save valuable tech time otherwise spent on excessive and unnecessary troubleshooting. All these experiences have highlighted easy to perform preventative maintenance tasks not otherwise recommended or performed by the manufacturer which include 1) internal cleaning of the probe rinse station semi-annually, 2) checking and, if necessary, replacing the filter in the probe rinse station quarterly, and 3) in depth cleaning and dusting of the instrument monthly.

Conclusions
Identification and efficient troubleshooting of problematic hardware components, and developing preventative maintenance plans that exceed the manufacturer recommendations can reduce loss of functionality to downtime, save tech time and improve result quality. Advanced training is currently limited to “primary operators” but should be expanded to include all frontline staff to maximize troubleshooting potential in the lab.
27. Streamlining and Process Improvement in the Transfusion Medicine Laboratory

Clinical, Poster Presentation

Tanya McKelvey MLT, BSc (MLS), Alberta Health Services
Gwen Clarke MD, FRCPC, University of Alberta and Alberta Health Services
Hilda Gaal, Alberta Health Services
Angela Goulard, Alberta Health Services
Susan Nahirniak MD, FRCPC, University of Alberta and Alberta Health Services
Amanada Oleksy, Alberta Health Services

Background
With current fiscal constraints in healthcare, the need for efficiency and streamlining of processes is critical to manage operational costs. To promote staff engagement in streamlining of processes, select frontline staff were offered the opportunity to participate in workshops and training modules focused on identifying waste and streamlining processes in the workplace.

Process
A Six Sigma Yellow Belt project to reduce inefficiencies and duplication of work within the reagent entry process was performed between November 2011 and February 2012. By defining opportunities and applying the basic streamlining principles of eight wastes identification (with emphasis on duplication of work), value stream maps, and change management to the reagent entry process three “quick wins” were identified. The quick wins were: 1) better standardize the procedure for reagent entry; 2) eliminate the need for manual documentation in a circular binder; and 3) create a database to track reagent discards. These small but effective changes to the process decreased the average time for reagent entry by 19.3% within just 2 months. In tech time savings alone this translates to 7 seconds and 8 cents per vial entered or an estimated 15 hours and $600 annually. In addition, numerous other process improvements were identified that, given time and educational investment, could be implemented to further streamline this process and provide further cost savings. These included creation of a database for complete electronic data capture, elimination of paper records, elimination of in-house barcodes for reagents, and moving to regional reagent ordering to maximize discounts and minimize discards. In staff time alone this translates to an estimated minimum 26% further reduction in the average time for reagent entry or 4 cents per vial entered and $480 annually.

Conclusion
Streamlining of even the simplest processes can translate to sizeable cost savings and efficiencies when human and financial resources are significantly limited. However, staff need to be trained in the identification of waste and improving inefficiencies at the front line level.
Clinical, Poster Presentation

Roya Mojarab MLT, Mount Sinai Hospital
Nadine Shehata MD, Mount Sinai Hospital

The aim of study is to describe the utilization and effectiveness of platelet transfusion at a tertiary care center.

Methods
We reviewed all transfusion requisitions, which were received by Transfusion Medicine Service over period of six months. The transfusion requisitions were used to extract patient diagnosis and platelet transfusion data. Pre and post transfusion platelet counts were derived from the hospital’s Laboratory Information System. The numbers are the average of the result over one stay.

Results
Within the 6-month period 54 patients were transfused 316 units of platelets.

Platelet products were single donor, random donor, or HLA matched platelets. Among the 54 patients, 53% were male and 47% were female. The median age was 46 years. The number of units transfused ranged from 1-51 units.

The median hospital duration of 55 days, the numbers of transfused units to one patient were: minimum of one and the highest of 51 units for 1 patient.

The pre and post transfusion platelet counts are described in the table below.

<table>
<thead>
<tr>
<th>Clinical condition (Adult)</th>
<th>Mean Pre-transfusion count</th>
<th>Mean Post-transfusion count</th>
<th>Number of Patients</th>
<th>Number of units transfused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia / Lymphoma</td>
<td>$8.5 \times 10^9$/L</td>
<td>$27 \times 10^9$/L</td>
<td>14</td>
<td>111</td>
</tr>
<tr>
<td>Bone Marrow Transplant</td>
<td>$12 \times 10^9$/L</td>
<td>$26.4 \times 10^9$/L</td>
<td>7</td>
<td>81</td>
</tr>
<tr>
<td>Hematology /oncology</td>
<td>$20 \times 10^9$/L</td>
<td>$48 \times 10^9$/L</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Surgery /bleeding/ Massive transfusion</td>
<td>$39 \times 10^9$/L</td>
<td>$98 \times 10^9$/L</td>
<td>12</td>
<td>55</td>
</tr>
<tr>
<td>Other: Critically Ill</td>
<td>$29 \times 10^9$/L</td>
<td>$74 \times 10^9$/L</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td>Immune-mediated</td>
<td>$13 \times 10^9$/L</td>
<td>$43 \times 10^9$/L</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neonatal transfusion</th>
<th>Pre-transfusion count</th>
<th>Post-transfusion count</th>
<th>Patient Numbers</th>
<th>Transfused platelet time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected sepsis / Live birth</td>
<td>$14 \times 10^9$/L</td>
<td>$59 \times 10^9$/L</td>
<td>7</td>
<td>20</td>
</tr>
</tbody>
</table>
Transfusion Protocol to Support Massively Bleeding Patients: Descriptive outcomes based on blood components transfused

Clinical, Poster Presentation

Doris Neurath ART, MBA, EORLA, The Ottawa Hospital
Ayman Kafal PhD, CSL Behring Canada
Melanie Tokessy MLT, EORLA, The Ottawa Hospital
Nancy Cober ART, EORLA, The Ottawa Hospital
Shauna Love MLT, EORLA, The Ottawa Hospital
Antonio Giulivi MD, The Ottawa Hospital

Background
Massive bleeding requiring multiple blood product transfusions during medical interventions is associated with high mortality rates. Our purpose was to evaluate mortality and blood product utilization following implementation of the massive transfusion protocol.

Methods
Transfusion data was extracted from the information system at a Canadian tertiary care facility. Retrospective data was collected over 5 months on patients who had experienced massive bleeds during medical interventions and had received over 5 units of red blood cells (RBC) in combination with other blood components according to the hospital massive transfusion protocol.

Results
113 patients were selected in our study. 92 patients (83%) were undergoing surgical procedures, mainly cardiac surgery (46%). Half of our sample (54%) needed more than 8 RBC units. 21 patients (20%) were transfused with only one additional blood component (either frozen plasma (FP) or platelets) as well as RBC. 24 patients (21%) received both frozen plasma and platelets in addition to RBC. 48 patients (42%) received a combination of frozen plasma, platelets and cryoprecipitate and 18 patients (16%) required additional products such as recombinant factor VIIa (rFVIIa). Ratio of FP to RBC used was 1:1.5 and an average of 20 units of cryoprecipitate was required. Overall mortality was 18.5%.

Summary / Conclusions
With increased awareness of blood transfusion indications and the appropriate combination of blood components transfused we have seen improvement in blood utilization. Our data confer an advantage to transfusing a combination of blood components according to a systematic well defined protocol. Further research is needed to explore the influence of the sequence of blood components transfused on clinical outcomes of massively bleeding patients.
30. Transfusion-Related Acute Lung Injury Following Intravenous Immune Globulin Transfusion: A Case Report

Clinical, Oral Presentation

Graeme Quest MD, MSc, Alberta Health Services and University of Alberta
Hilda Gaal MLT, Alberta Health Services
Gwen Clarke MD, FRCP, University of Alberta and Alberta Health Services
Susan Nahirniak MD, FRCP, University of Alberta and Alberta Health Services

Background
Transfusion Related Acute Lung Injury (TRALI) is a severe adverse transfusion related event that typically occurs within 6 hours of the infusion of plasma containing products. Intravenous immune globulin (IVIG) is prepared from large pools of human plasma and therefore may also cause TRALI, though reports of this are scarce.

Case Report
A 77 year old female with common variable immune deficiency (CVID) has been receiving IVIG since 1996. The patient had previously experienced transfusion reactions to two IVIG products, Gamimune and Iveegam, and is known to have anti-IgA antibodies. Her current IVIG therapy for CVID, since September 2011, includes transfusion of 40g Gammagard S/D monthly, with acetaminophen and benedryl premedication. This therapy had been without incident until November of 2011. The infusion was started at 0840h and completed at 1605h. The patient experienced nausea with vomiting at 1430h, followed by dyspnea with decreasing oxygen saturations at 1605h. The patient’s blood pressure increased as oxygen saturations continued to fall by 1635h. The patient was given 8 liters oxygen, but continued to deteriorate requiring intubation and admission to the intensive care unit (ICU). Post-reaction chest x-rays demonstrated the new appearance of bilateral hazy groundglass opacifications with previously documented changes including and related to bronchiectasis, with the resolution of the opacities within 48 hours. Measurement of BNP following the reaction was 72 pg/mL (less than 100 pg/mL interpreted as heart failure unlikely). Trans-thoracic echocardiography demonstrated a Left Ventricular Ejection Fraction of 45-50%. Serological investigations on the day of the reaction showed her to be group B Rh positive with a negative antibody screen and a negative DAT. The patient was extubated within 24 hours, transferred from the ICU within five days, and discharged from hospital within eight days of the reaction. The reaction was classified as a TRALI and reported to Health Canada. The patient had not received this particular lot number of product before, and has since received 5 infusions of Gammagard S/D with different lot numbers without reaction.

Conclusion
This case report documents a case of TRALI following Gammagard S/D transfusion, confirming that TRALI can occur following IVIG infusion.
The Prophylactic Use of C1 Esterase Inhibitor (Berinert®) in HAE Patients Undergoing Invasive Procedures

Clinical, Poster Presentation

Stephanie Santucci RN, Yang Medicine Professional Corp
Genevieve Gavigan MASc, MD, University of Ottawa
Rachel Harrison BAScH, Yang Medicine
William H. Yang MD, FRCPC, FAAAAI, University of Ottawa and Yang Medicine

Rationale
For a patient with Hereditary Angioedema (HAE), physiological and/or psychological stress can cause insufficient control of local inflammatory pathways. This leads to complement and contact system activation and excess bradykinin resulting in angioedema. Therefore, an invasive procedure or surgery can trigger an HAE attack; this in turn can cause further medical complications and pose an added danger to the post-procedure patient. C1 inhibitor, Berinert®, was approved in the US and Canada in 2009 and 2010, respectively, for the treatment of acute attack. However, there is no approved indication for its prophylactic use for HAE attacks. We aim to demonstrate the effectiveness of C1 esterase inhibitor, Berinert®, as a prophylactic treatment for HAE patients undergoing invasive procedures.

Method
We performed a retrospective chart review from our Canadian Tertiary Care Allergy and Asthma Clinic of our entire database of HAE patients.

Results
Between 1997 and 2012, we administered C1 esterase inhibitor (Berinert®) for prophylactic use prior to invasive procedures. There were a total of 22 procedures, performed on 11 patients.

The 22 procedures breakdown as follows:
• 7 dental surgeries
• 4 open heart surgeries (3 coronary aneurysms, 1 heart valve replacement)
• 5 other surgical procedures (1 Melanoma in situ, 1 liver biopsy, 1 bronchial cyst, 1 hemia, 1 hysterectomy)
• 1 child birth
• 5 invasive procedures (2 Port-a-cath placements, 2 angiograms, 1 Hickman placement)

At the time of their respective procedures, 10 of these patients were adults, and one was a teenager.

In all 22 procedures, there was no incidence of post-procedure HAE attacks after prophylactic administration of C1 esterase inhibitor (Berinert®).

Conclusions
We found that C1 esterase inhibitor (Berinert®) decreased the incidence of post-procedure HAE attacks and was an effective prophylactic treatment.
Clinical, Poster Presentation

Lawrence Sham MLT, Vancouver General Hospital
David Pi MD, FRCPC, Vancouver Coastal Health
Brian Berry MD, FRCPC, Vancouver Island Health Authority
Jason Pal, Vancouver General Hospital
Kate Chipperfield MD, FRCPC, Vancouver Coastal Health
Teresa Sharp MLT, Richmond Hospital

Background
Traditionally, Crossmatch/Transfusion Ratio (C/T Ratio) is considered a useful index to assess the appropriateness of blood reserve in inventory for patients with serological crossmatch or patients with anticipatory transfusion needs in surgical or other clinical specialties. A major benefit of electronic crossmatching (EXM) is the expediency in blood order assignment, resulting in reduced and more flexible allocation of blood units to patients. In EXM, the computer generates a global (G)-C/T Ratio, based on the standardized process requiring a common entry to assigned inventory for all blood units (Crossmatch) prior to the final issue for transfusions (T). The objectives of this study are to explore the relationship of G-C/T Ratio and specialty-specific (SS)-C/T Ratio, and to evaluate the prevalent SS-C/T Ratios in hospitals using EXM in Greater Vancouver and Vancouver Island, British Columbia.

Method
The reasons for crossmatches were categorized into serological crossmatch, and specific types of surgical procedure, clinical diagnosis and patient location. We use a manual and/or a mathematically derived method (based on lead time analysis) to determine the blood units in the assigned inventory in the study period.

Results
Preliminary data from 2/7 hospitals analyzed to-date are presented in the abstract - Hospital A and B: The volume breakdown (%) and SS-C/T ratio respectively – Hospital A: Open heart surgery (7%, 1.98), other surgeries (21%, 2.47), gastrointestinal (GI) bleeds (2%, 1.14), medical inpatients (56%, 1.36), and outpatients (14%, 1.26). Hospital B: Obstetrics (6%, 6.00), other surgeries (7%, 3.75), GI bleeds (13%, 1.08), medical inpatients (56%, 1.36), and outpatients (16%, 1.18). Total SS-C/T Ratio and G-C/T Ratio: Hospital A: 2.62 and 1.51, Hospital B: 2.42 and 1.43. A linear correlation exists between SS-C/T Ratio and G-C/T Ratio; the slope of this equation is determined by the blood return rate and the proportion of transfusion in the assigned inventory (T1) over T.

Conclusion
In hospitals with electronic crossmatch, the significance of G-C/T ratio, conveniently generated by blood bank computers, need to be understood. G-C/T Ratio can be used as a good screening method to study the appropriateness of blood order assignment practices.
Clinical, Poster Presentation

Maryse St-Louis PhD, Héma-Québec
Josée Perreault PhD, Héma-Québec
Carole Éthier MT, Héma-Québec
Jessica Constanzo-Yanez BSc, Héma-Québec
Marie-Josée Paré MT, Héma-Québec
Brigitte St-Onge MT, Héma-Québec
Nicole Cormier MT, Héma-Québec
Élaine Deschênes MSc, Héma-Québec

Case
A 28-year old group B Black woman was referred to our Immunohematology Laboratory (IRL) in October 2012 for red blood cell antibody identification (G6P2A3). Her serum reacted strongly on every panel cell tested. This patient was first seen in September 2001 to confirm her D antigen status. The DNA-based genotype assay done at Héma-Québec at the time concluded for a DVI type 1 variant. No alloantibodies were detected at the time.

Serology results
The 2012 sample was looked at closely the IRL. Since the patient received no transfusion three months prior, her phenotype was done by approved serology techniques: C-c+E-e+, K-, M-N+S-s+, Fy(a-b-), Jk(a+b-). Again her serum reacted strongly on all panel cells (3+). Autocontrol and DAT were negative. Allodabsptions demonstrated the presence of anti-e and anti-f. Rare red blood cells were tested. Few negative reactions were obtained on on some Black origin cells. More Black rare cells were targeted for screening. Three hrB+w/- cells gave weak reactions in gel-LISS, while three others gave 11/2-2+ strength. Two hrS- cells were negative in gel-LISS and gel-papain, and two hrB-hrS+ cells were negative in gel-LISS and positive in gel-papain. Molecular biology results: RH messenger RNA were amplified and sequenced. The previous DNA-based assay predicted a DVI type 1 variant. However, the RHD sequencing results indicated a RHD*DAR variant (polymorphisms in exons 4, 5 and 7). The RHCE results gave a homozygous RHCE*ceAR variant often linked to RHD*DAR variant. This RHCE variant presents a variation in the following antigen expression: C-, partial c, E-, partial e, VS-V+ and hrs-.

Conclusion
This patient was notified that she has rare blood and was advised to give autologous donations. No compatible blood was found yet, despite the thorough screening of our self-identified Black donors.
Case
An 80-year old man with a myeloproliferative syndrome was first referred to our Immunohematology Laboratory in 2006. At the time, his blood group was phenotyped several times D+ and genotyped C+E- c+ e+. An anti-Jka was identified in his serum. In 2012, more samples from the same patient were sent, because a discrepancy was observed at the hospital. The patient was phenotyped D- C- E- c+ e+ on a pretransfusion sample. These new samples were examined more closely by the Research and Development group. Methods: To avoid samples mixed-up and mislabeling, three samples collected at different times were received from the hospital. RHD and RHCE mRNA were amplified. Resulting cDNA was sequenced and compared to a normal sequence. RHD and RHCE DNA genotype were also performed to confirm the previous 2006 results.

Results
No sequence was possible for the RHD mRNA/cDNA. No amplification was observed with two RNA prepared from two different samples. However, the RHCE mRNA/cDNA sequence gave a RHCE*ce/RHCE*ce result. As for the DNA based assay, results indicated a very weak amplification for the RHD, much weaker than the one observed with control DNA. The RHCE assay predicted a C+c+ phenotype.

Discussion
According to the DNA results, this patient should be D+C+c+, even if the RHD amplification was very weak. On the other hand, mRNA/cDNA sequencing results indicated a D-C-c+ result. Myelodysplastic syndrome was previously responsible for an ABO discrepancy. No reports could be found involving for RH antigens as shown here. We believe that methylation could be the reason why some mRNA are not expressed during the onset of the disease. Why the RHCE*C allele is silenced and not the RHCE*c? It could be due to the evolution of the RH genes, the RHCE*ce being older and less sensitive to modification. More work is needed to elucidate this interesting case.
Major Red Blood Cell Antigens in Self-Identified Black Blood Donors in Quebec

Clinical, Poster Presentation

Maryse St-Louis PhD, Héma-Québec
Jessica Constanzo-Yanez BSC, Héma-Québec
Carole Éthier MT, Héma-Québec
Josée Lavoie MSc, Héma-Québec
Élaine Deschênes MSc, Héma-Québec
Josée Perreault PhD, Héma-Québec

Background
The demand for extensively phenotyped red blood cells is on the rise, especially for sickle cell patients. To better answer their needs, Héma-Québec put forward great efforts to increase the recruitment of blood donors among the diverse cultural communities. Blood drives were organized in these communities, along with other activities to aware them of the importance of blood donations.

Study Design and Methods
A section was added on the record of donation filled out by the donors to indicate their ethnic background. Self-identified Black donors were extensively phenotyped by the Immunohematology Laboratory. The most interesting ones were referred to the Research and Development to complete the genotype/phenotype picture to identify rare donors.

Results
After four years, close to 1500 self-identified Black donors have donated blood at least once. Among the 1058 Black donors tested in R&D, 15 S-s-, 3 U-, 12 U+var, 10 Js(a+b-), 2 Hy- and 3 Jo(a)- were found. Variants in the RHCE were also identified: 30 hrB+w/- and 8 hrB-. Conclusion: These donors are precious to the patient cohort depending on blood transfusions and to our organisation as blood provider for the whole province of Quebec.
The Kidd blood group includes the antithetical Jka and Jkb antigens to which alloantibodies are made in ~1/3rd of severe delayed hemolytic transfusion reactions (DHTRs). The high incidence of anti-Kidd antibody-related DHTRs is due in part to their high immunogenicity, difficulty in detection (low titres, dosage effects), rapid evanescence and strong anamnestic responses. Given their potent hemolytic potential, identifying anti-Jk antibodies is of clinical importance. Here we describe a delayed serologic transfusion reaction (DSTR) to Jka in a 34 year old man with no transfusion history until receipt of 4 units of Jka+ RBCs amid extracorporeal membrane oxygenation (ECMO) for RSV-associated respiratory failure. For logistical reasons, his first three screening samples were analyzed by Ortho Diagnostics agglutination-based gel columns, and the fourth by solid-phase Capture® technology on the Immucor NEO. Although the 1st three screens were negative, anti-Jka was detected and confirmed on the fourth (2-3+), while remaining undetectable by gel columns even after enzyme treatment of double-dose Jka screening cells. Two weeks later, his anti-Jka response had increased to 3-4+ by Capture® but still remained negative by gel. To determine if anti-Jka could have been identified earlier, we re-analyzed his first 3 screens using solid-phase technology. While no anti-Jka was found by Capture® prior to receiving blood products, anti-Jka IgG became detectable just 3 days after presumed first exposure to Jka+ RBCs, in keeping more with memory response than primary alloimmunization. This discordance between the Jka antibody kinetics and the transfusion history may be explained by maternal-fetal exposure to Jka or a naturally-occurring anti-Jka, neither of which could be definitively ruled out. The septic recipient context around transfusion may have also contributed. Fortunately, there were no clinical sequelae and his investigation for hemolysis was negative by the time of DSTR recognition. He recovered from his pneumonia and was made aware of his anti-Jka serology through a multi-lingual “antibody” card detailing his alloantibody status and future blood product requirements. Although serious clinical consequences were avoided, this case highlights difficulties encountered with the Kidd blood group and technical properties of anti-Jka antibodies that can make their identification method dependent.
Hemoglobin Concentrations in Local Patients with Acute GI Bleeding before and After Massive Hemorrhage Protocol

Clinical, Poster Presentation

Elona Turley MD, University of Alberta
Tanya McKelvey MLT, Alberta Health Services
Heather Blain MLT, Alberta Health Services
Susan Nahiriak MD, FRCPC, University of Alberta and Alberta Health Services
Gwen Clarke MD, FRCPC, University of Alberta and Alberta Health Services

Background
Hemoglobin (Hb) triggers for transfusion in patients with acute gastrointestinal (GI) bleeds have been a contentious issue. The authors of a recently published randomized control trial found that patients with acute upper GI bleeding assigned to a restrictive transfusion strategy (transfusion trigger Hb 70g/L; post-transfusion Hb target 70-90g/L) experienced significantly improved outcomes when compared to patients assigned to a liberal strategy (trigger Hb 90g/L; post-transfusion Hb target 90-110g/L). We sought to retrospectively evaluate local transfusion practices in GI bleed patients with massive hemorrhage protocol (MHP) activations as part of a quality assurance project.

Methods
Local patients with MHP activations for GI bleeds from January 2011 to June 2012 were identified. Following the above study, the pre-MHP and post-MHP Hb concentrations were obtained, and the patients grouped according to pre-MHP Hb concentration.

Results
Of the 78 patients with MHP activations for GI bleeding in the study period, 51 had pre-MHP and post-MHP Hb measured. Pre-MHP Hb was ≤70g/L in 25 patients (49%), >70g/L but <90g/L in 16 patients (31%), and ≥90g/L in 11 patients (22%). The average pre- and post-MHP Hb in the ≤70g/L group was 50.2g/L (s=12.2g/L), and 91.2g/L (s=19.1g/L), respectively. The average pre-MHP Hb in the ≥90g/L group was 105.0g/L (s=9.5g/L) with an average post-MHP Hb of 98.5g/L (s=22.7g/L). The >70g/L but <90g/L group had an average pre-MHP Hb of 79.9g/L (s=7.8g/L) and average post-MHP Hb of 82.6g/L (s=26.4g/L).

Conclusion
Our review suggests that there is no currently defined local strategy with respect to Hb triggers and transfusion targets in patients with MHP activations for GI bleeding. Future educational initiatives may assist with practice changes to a more restrictive strategy given recent literature indicating the benefit of such an approach in these patients.
Validation of 5 day post thaw Octaplasma®

Clinical, Poster Presentation

Carolyn Villeneuve, University of Alberta
Hilda Gaal hilda.gaal@albertahealthservices.ca, University of Alberta
Linda Stang, University of Alberta
Gwen Clarke MD, FRCPC, University of Alberta
Susan Nahiriak MD, FRCPC, University of Alberta

Background
Octaplasma®, a solvent/detergent plasma product has been approved for use in Canada. The manufacturer states an expiry of 8 hours (h) post thaw; stored at 4°C. As Octaplasma® is a pooled plasma product, and fresh frozen plasma has been validated for use up to 5 days post thaw, we hypothesized that Octaplasma® could also be used up to 5 days post thaw when stored at 4 -6°C. The purpose of this study was to determine the integrity of Octaplasma® 5 days post-thaw. If clinically significant changes to clotting factor levels were not seen, extension of the expiration from 8h to 120h could be considered.

Methods
Thirty eight (38) units of European source Octaplasma® were thawed at 37°C then kept at 4-6°C for 5 days. Aliquots for testing were made at time 0, 24, 48, and 120h and frozen at -70°C. Levels of coagulation Factors V, VII, and VIII were performed on the STA-R Evolution analyzer. Testing was initially performed on 0h and 120h aliquots with follow-up at 48 and 24h if 120h aliquots showed levels < 0.50 IU/mL.

Results
Although Factors VII and V decreased on average by 11.9 % from baseline during the 120h storage, all aliquots had levels above 0.50 IU/mL. Factor VIII levels were less than 0.50 IU/mL (averaging 0.41 IU/mL) at time 0 and were reduced further at 120h averaging 16.5 % decrease from baseline.

Conclusion
The reduction of factors V and VII levels were not detrimental to the integrity of the product and are sufficient for transfusion. Factor VIII levels were severely reduced; but this is not due to degradation during storage as minimal levels were present at the time of thawing. Reduced Factor VIII levels are not a concern since usually the levels of Factor VII are sufficient to initiate the clotting cascade through the extrinsic pathway and Factor VIII typically increases in vivo as an acute phase reactant. The diminished VIII levels may not represent a clinically significant problem. Octaplasma® should be effective for treatments outlined by Canadian Blood Services for up to 5 days post thaw if stored at 4-6°C.
40. **Auto-anti Rh(D) in a patient with severe sepsis**

Clinical, Poster Presentation

Di Cindy Wu BSc, MLT, Surrey Memorial Hospital
Lina Kitani BSc, MLT, Surrey Memorial Hospital
Chui Shan Khan BSc, MLT, Surrey Memorial Hospital
Taralynn Le Clair MLT, Surrey Memorial Hospital
Lucia Wojtyniak MLT, Surrey Memorial Hospital
Samuel Krikler MBChB, FRCPC, Surrey Memorial Hospital and University of British Columbia

We describe a 39 year old man with "spontaneous" bacterial peritonitis who developed a strongly positive DAT (IgG 3+, C3d 1+) concomitant with his sepsis. The patient has a background history of cerebral palsy with multiple medical problems. He had been transfused previously and an allo-anti E was present in his plasma. His most probable Rh genotype is CDe/cde. An eluate prepared from the patient's washed red cells showed antibodies with a pattern consistent with anti-D and anti-E. Similar antibodies were identified in the plasma. The last wash recovered from the eluate also demonstrated anti-E, which appears to be an example of the Matuhashi-Ogata phenomenon since his red cells lack the E antigen. Our serologic findings were confirmed by CBS (Vancouver) and a sample has been referred to the National Reference Laboratory for Rh(D) genotyping by molecular (DNA-based) techniques. Auto-antibodies with anti-D specificity are rarely described and must be distinguished from allo-antibodies to epitopes within the D antigen. This patient showed no demonstrable reduction in red cell survival and there was no clinical or laboratory evidence of a generalized auto-immune disorder such as SLE. The positive DAT preceded anti-microbial therapy and does not appear to be drug-related. Rh(D) negative units were selected for transfusion.
A High-throughput One-step Method for the Screening of IgA Deficiency in Blood Donors

Scientific, Poster Presentation

Renée Bazin PhD, Héma-Québec
Tony Tremblay, Héma-Québec
Julie Coutemanche, Héma-Québec
Isabelle Paré, Héma-Québec
Pascal Roleau, Héma-Québec
Hélène Émond, Héma-Québec
Mathieu Drouin, Héma-Québec

At Héma-Québec, we maintain a registry of IgA-deficient donors to provide compatible blood components to IgA deficient patients, at the hospital’s request. We recently reinitiated screening of blood donors for IgA deficiency to compensate for the loss of active donors in our registry. Several screening methods mostly based on ELISA exist but, while being very reliable, they are also time-consuming. Typically, sample preparation and loading in microplates take about 2 and 7 hours for 1000 samples respectively, for one technician. The ELISA technique itself requires about 4 hours to be completed. To improve the efficiency of screening, we set up a fluorescent-based competition assay requiring only one incubation step. The workload for sample distribution in microplates has been considerably reduced by using the Hamilton Microlab AT Plus 2 automated dispenser (located at our regulatory testing lab in Montreal) to collect plasma samples (50 ul) from the left-over tubes used for ABO/Rh testing, in IgA-coated 96-well microplates. The microplates are then frozen to prevent spillage and shipped to the R&D department in Quebec City. For testing, the microplates are first thawed and anti-human IgA-FITC conjugate is added directly to each well. The plates are incubated for one hour at 37oC, washed, emptied and read on a fluorometer. The anti-human IgA-FITC conjugate is neutralized by the IgA contained in normal plasma, resulting in background fluorescence. In contrast, IgA-deficient plasma does not interfere with the binding of anti-human IgA FITC conjugate to IgA-coated wells, yielding high fluorescence and allowing the easy identification of IgA-deficient plasma. The overall procedure is completed within 2 hours, compared to more than 12 hours with our previous screening method, for the same number of samples. Using this new method, we tested 10 763 samples from January 16 to January 31 2013 and identified 23 IgA-deficient donors (frequency of 1/468). These samples were retested using our in-house cytometry-based assay for preliminary confirmation of the IgA deficiency status. All samples were IgA-negative, showing the specificity of our screening method. In conclusion, we have developed a cost-effective and efficient method for the screening of thousands of blood donors for IgA deficiency.
Intravenous immunoglobulin (IVIg) is a therapeutic preparation of human IgG isolated from thousands of plasma donations and has been proposed as a therapy to improve the rate of graft survival in patients with a high risk for antibody-mediated rejection. The rapid effect of IVIg in these patients was proposed to be due to blocking of anti-HLA antibodies by anti-idiotypic IgG present in IVIg. However, peripheral tolerance beyond the half-life of IVIg is observed in IVIg-treated patients, suggesting that IVIg modulates the functions of immune cells in addition to neutralizing anti-HLA antibodies. In the present study we show, using the allogeneic mixed lymphocyte reaction (MLR) as an in vitro model of allograft rejection and GvHD, that IVIg strongly inhibits T cell activation, as determined by IL-2 secretion, and modulates the level of other pro- and anti-inflammatory cytokine secretion (IL-6, INF-γ, IL-1RA). To further define the mechanisms underlying the IVIg-mediated inhibition of T cell activation in MLR, we studied the effect of IVIg on the phenotype of the cells involved in MLR (mainly T cells and monocytes). Our results reveal that MLR inhibition by IVIg correlates with the induction of an anti-inflammatory monocyte population (CD14+, HLA-DR+++) with a low expression of CD80 (a co-stimulatory receptor) and high expression of PDL1 (an inhibitory receptor). To evaluate the importance of PDL1 on the IVIg-mediated MLR inhibition, anti-PDL1 was added together with IVIg during the MLR. Blocking of PDL1 restored the MLR, as evaluated by IL-2 secretion by activated T cells. We thus propose that IVIg-mediated induction of anti-inflammatory monocytes expressing low levels of CD80 and high levels of PDL1 plays a central role in the inhibition of MLR.
A New Casing Design for the Storage of Frozen Blood Products to Reduce the Breakage

Scientific, Poster Presentation

Claudia Bédard MSc, Héma-Québec, R&D
Annie Jacques MSc, Héma-Québec, R&D
Éric Ducas MSc, Héma-Québec, R&D
Nathalie Dussault MSc, Héma-Québec, R&D
Pierre Lalonde, Héma-Québec, Finance and Administration
Sylvie Thibault, Héma-Québec, Exploitation
Michel Beaudin Ing, Héma-Québec, Project Direction
Louis Thibault PhD, Héma-Québec, R&D

Purpose
Héma-Québec delivers about 90,000 frozen blood products to hospitals yearly. Frozen products are stored at ≤-18°C and distributed to hospitals on dry ice (-78°C). The transport and handling of these products at this low temperature increase the risk of breakage and cracking of plastic bags. Last year, nearly 2,000 frozen products were lost. The aim of this project was to develop a “green” packaging that would reduce the incidence of breakage by immobilizing the frozen product in its casing.

Methods
The new casing design must meet specific criteria: adaptable to all types of storage bags of frozen products, economical, reusable, recyclable and have the least possible impact on our operations and those of hospitals. Drop tests from a height of 90 cm, a worst case scenario that simulates a fall from a work station, were conducted to evaluate prototype performance. The results were compared with our current casing.

Results
The new design has been easily adapted to plasmapheresis units as well as whole blood derived frozen products. The casing, made of corrugated cardboard, includes an insert which firmly holds the bag in place to reduce its mobility. The lid has a large window to give access to the product label and is firmly held in place with Velcro type closures. During drop tests, the new design reduced the breakage rate from about 78% to 16% when compared to our actual packaging. For 250-mL Frozen Plasma units and 500-mL Fresh Frozen Plasma units, the new design has reduced breakages by 60% and 70%, respectively.

Conclusions
The new casing design should reduce the rate of breakage and the loss of frozen blood products. The new casings will be put into operations shortly. However, it will take several months to assess their effectiveness in reducing the loss of frozen products.
Anti-D therapy has been highly successful in preventing hemolytic disease of the fetus and newborn, but the suppressive mechanism involved (referred as antibody-mediated immune suppression; AMIS) remains poorly understood. A major limitation to study the mechanisms implicated in AMIS to Rh antigens has been the lack of an animal model of D protein immunization. Normal mice do not mount an immune response to RhD. However, it has been suggested that the human HLA class II allele DRB1*1501 could be a major restricting element for human T-helper cells specific for RhD protein and DRB1*1501 positive mice responded to immunization with purified soluble RhD protein.

As an approach to develop a mouse model of RhD immunization, both human red blood cells (RBC) expressing the D antigen and Rh synthetic peptides corresponding to extracellular and putative immunogenic regions of human Rh proteins were separately used to immunize mice where mouse MHC class II was replaced with HLA class II DRB1*1501. Red blood cells were injected alone or mixed with CPG ODN adjuvant and administered in one and two doses. The three RhD peptides synthesized (Peptide 1 aa 34-46; Peptide 2 aa 228-238; peptide 3 aa 350-358) were linked to a carrier protein (KLH) through a cysteine added at the C-terminal region of each peptide and emulsified in Freund’s adjuvant (complete for the first and incomplete for the second and third dose).

DRB1*1501 transgenic mice challenged with RhD positive RBC developed antibodies that recognized human RBC by flow cytometry but no antibodies specific to the RhD protein were detected. For the peptide study, immunization of mice with the three RhD peptides induced a response specific for the immunizing peptides as detected by ELISA. Two of these sera also reacted with human RBC when tested by flow cytometry. Interestingly, RhD negative cells (i.e., Rhce+) were better recognized by the polyclonal antibodies than RhD positive cells. These results are consistent with inducing an antibody response in DRB1*1501 mice against antigenic regions of human Rh but not with specificity for the RhD antigen itself.
Integrating Blood Donor West Nile Virus Screening and Public Health Surveillance in British Columbia

Scientific, Poster Presentation

Mark Bigham MD, Canadian Blood Services
Alice Cheung, Canadian Blood Services
Patrick Loftus MN, Canadian Blood Services
Judy Hrytzak MLT, Canadian Blood Services
Sima Ashrafinia, Canadian Blood Services
Gershon Growe MD, Canadian Blood Services
Marsha Taylor MSc, BC Centre for Disease Control
Bonnie Henry MD, BC Centre for Disease Control

Purpose
Canadian Blood Services (CBS) performs year-round West Nile Virus (WNV) blood donor screening. In British Columbia (BC), seasonal WNV surveillance is conducted by public health, coordinated by the BC Centre for Disease Control (BCCDC). Here, we outline results of a partnership between CBS and BCCDC for WNV testing and surveillance that enhances both blood safety and the sensitivity of public health WNV surveillance.

Methods
A review and compilation of WNV surveillance processes and outcomes was undertaken, using unpublished WNV blood donor screening and program data collected annually by CBS BC and Yukon (BCY) Centre, and published provincial WNV surveillance program results.

Results
CBS began universal WNV screening by Nucleic Acid Testing, in July 2003, leaving a low residual risk of “window period” transfusion transmitted WNV. Suspected transfusion transmissible infections are reportable to Public Health in BC. In 2004, CBS BCY Centre and BCCDC developed processes for BCCDC to report clinically suspect cases of WNV (i.e. on receipt of a laboratory specimen for WNV testing) to CBS to further reduce the risk. If a registered blood donor with a recent donation is identified, CBS takes immediate precautionary action to quarantine or recall the unit. An anonymized data linkage was validated to maximize protection of privacy. Between 2004-2012, a mean 662 (range 427-1017) cases tested for WNV were reported annually by BCCDC to CBS; a mean 41(6.2%) were blood donors; and a mean 1.4 (range 0-4) product recalls per year were done.
Conversely, since 2008, CBS BCY Centre has provided BCCDC with biweekly or weekly, aggregate, non-nominal donor WNV test data, geographically stratified by health service delivery area of donor clinics. In 2012, weekly data on a cumulative 44,000 donor WNV screening test results between 30 May-2 Oct were provided to BCCDC. Blood donor WNV screening approximates population-based sampling, and provides close-to-real time human data that strengthen the sensitivity of the public health WNV surveillance system.

Conclusion
CBS and public health WNV-related activities are highly complementary and strengthen transfusion safety and public health WNV surveillance in BC.
Panreactive Antibodies and Autoantibodies in Prenatal Serological Testing

Scientific, Poster Presentation

Judith Hannon MD, Canadian Blood Services
Gwen Clarke MD, Canadian Blood Services and Alberta Health Services
Jean Ashdown BSc, MLT, Canadian Blood Services
Gerri Barr MLT, Canadian Blood Services

Background
Serological testing of blood group and antibody screening are a cornerstone of routine prenatal care in Canada. In addition to allowing for assessment of need for Rh immune globulin prophylaxis, antibody screening and identification allows for appropriate prenatal follow up and management of hemolytic disease of the fetus/newborn (HDFN). Our province wide perinatal testing program tests 75,000 maternal blood samples annually. Between January 1, 2010 and December 31, 2013, 1116 clinically significant antibodies were identified. In addition, a number of panreactive antibodies and autoantibodies were detected.

Results
A total of 231 autoantibodies or panreactive antibodies were detected. 8 cases showed a distinct antibody specificity (2 anti-D, 1 anti-C, 2 anti-e and 3 anti-Jka) but were positive for the corresponding antigen so were termed autoantibodies. 86 cases were panreactive with a negative DAT. All but one were reactive by solid phase method only. 137 cases were panreactive antibodies with a positive DAT (47 reactive with anti-IgG, 38 with anti-C3, 52 with both). 82 of these panreactive autoantibodies were reacting by solid phase method only. 55 were reactive by both solid phase and PEG IAT method. Autoabsorptions were performed on those reactive by PEG IAT to exclude or identify underlying allo-antibodies. 9% of patients with a panreactive antibody or autoantibody also had an allo-antibody (1 anti-D, 6 anti-E, 7 anti-S, 2 anti-Jka, 2 anti-Lea, 1 anti-M, 1 anti-N). Titres were not performed on the panreactive or autoantibodies. Repeat samples were requested monthly for autoantibodies with specificity and at 26 weeks gestation for all panreactive antibodies.

Outcomes
While individual outcomes were not assessed, the overall clinical impression is that autoantibodies in pregnancy are NOT usually implicated in HDFN. Laboratory protocols in the setting of autoantibodies must be optimized to ensure that clinically significant alloantibodies have been excluded. Follow up testing of patients for persistence of autoantibody, reassessment for possible alloantibody development and recommendations around assessment for hemolysis in the mother and in the newborn are not well established and should be considered. Future studies will attempt to document the clinical outcomes of neonates born to mothers with a red cell autoantibody.
Cord Testing on the Provue™

Scientific, Poster Presentation

Laura Harrison ART, Trillium Health Partners, Credit Valley Hospital
Janice Zeng MD, FRCP, Trillium Health Partners, Credit Valley Hospital
Shauna Dowsley ART, Trillium Health Partners, Credit Valley Hospital

Purpose
Cord testing was being performed manually, with ABO/Rh testing done by tube and Direct Antiglobulin Testing (DAT) performed using ID-MTS™ Anti-IgG gel card. Considerable technologist time was required and testing was limited to daily batch testing unless a STAT request was received.

Method
A total of 81 cord sample were tested manually and then repeated on the Provue™. Reproducibility and carryover testing was performed and samples up to 7 days old were tested. All samples were cleared of dots using applicator sticks prior to centrifugation and testing on the Provue™. Extra reagents needed on the Provue included ID-MTS™ Anti-IgG gel cards loaded into the room temperature incubator and MTS™ Diluent 2.

Results
There were no errors in blood group interpretation by the Provue™. Four of five samples (4/5) that showed "No Results Determined" (NRD) ABO/Rh interpretations due to questionable or mixed field results were resolved by tube testing. One cord sample appeared to have possible contamination with maternal blood. No follow up sample was provided. DAT comparison between manual gel testing and Provue™ testing showed 93.8% overall agreement. Consistent with the original validation between tube and gel testing, samples with very weak positive DAT due to ABO incompatibilities or presence of maternal Passive Anti-D, were difficult to reproduce. No issue with carryover was seen and 7 day old samples gave acceptable results. Within the samples tested, no clots were detected resulting in a failure to run.

Conclusion
Cord testing can be performed on the Provue™, resulting in improved turn-around-time (TAT) and decreased technologist resources. Questionable or NRD results will be repeated manually.
Transfusions of ABO incompatible platelets have been associated with clinically significant and severe hemolysis. ABO and Rh identical or plasma compatible platelets should be used when available. Often in urgent settings or where platelet inventories are limited, ABO plasma incompatible platelets may be required. In Dec 2010 the Blood Transfusion Laboratory (BTL) at London Health Sciences Centre began evaluating the titres of Anti-A and Anti-B in plasma of all group A, B and O platelet units received from the Canadian Blood Services (CBS). The aim of this one year retrospective review was to evaluate the impact of potential hemolysis using a set titration level of ABO isohemaglutins in O platelets donor units transfused to non-O recipients in Hematology/Oncology patients. A dilution of 1:150 was tested against A1, B and pooled A1 and B cells using Ortho Clinical Diagnostics MTS Buffered Gel Cards with no incubation and a 10 minute centrifugation. Those units identified with titres greater than 150 were only transfused to ABO identical recipients. Overall, our method of ABO isohemaglutinin titration appears to be safe in this population. Both study, 129, and control, 287, groups had a similar rate of mild and severe post transfusion hemoglobin drops. The post transfusion drops in hemoglobin likely are not due to hemolysis in this high risk population as anemia due to exposure to chemotherapy, radiation, infections and bleeding are very common in these individuals. However, similar rates of drops in hemoglobin between the study and the control groups seen in our study are reassuring. With the available evidence thus far, we believe that ABO plasma incompatible platelets with a titre less than 150 can be safely transfused to patients if necessary and that this method can be routinely performed in blood transfusion laboratories.
Reducing the IgA Content of Red Blood Cell Units Using the Haemonetics ACP 215 Automated System

Scientific, Poster Presentation

Annie Jacques MSc, Héma-Québec, R&D
Hana Daoud MSc, Héma-Québec, R&D
Claudia Bédard BSc, Héma-Québec, R&D
Marie-Claire Chevrier MSc, Héma-Québec, Reference and Stem Cell Laboratory
Louis Thibault PhD, Héma-Québec, R&D

Purpose
In Canada, it is generally agreed that IgA-deficient patients should be transfused with washed red blood cells (RBCs) when blood products made from IgA-deficient blood donors are not available. According to current guidelines, RBC units should be washed with a minimum volume of 2 L of saline. The closed-system ACP 215 (Haemonetics) enables automated washing and 14 days of post-wash storage. In this work, the ability of the ACP 215 system for washing SAGM and AS-3 RBC units to comply with the CSA recommendation of less than 0.5 µg of IgA per mL was investigated.

Methods
For this work, the ACP 215 washer was programmed using a standard hematocrit of 60%. Only leukoreduced AS-3 and SAGM RBC units weighting ≥250 g were used. After washing, RBCs were stored in AS-3 at 4°C. Three washing protocols were evaluated: 1.84 L (control), 3.0 L and 2 consecutive procedures of 1.84 L (n=6 per arm). Wash efficiency, assessed by measuring the volume of residual plasma and IgA concentration, and in vitro quality parameters of RBCs, were analyzed.

Results
The IgA content of washed RBC units using 1.84 L and 3.0 L of saline remained above the acceptable limit of 0.5 µg of IgA per mL, at 1.4 ± 0.7 µg/mL and 1.3 ± 0.9 µg/mL, respectively. By performing two washes, the IgA content of RBC units was reduced from 202 ± 129 µg/mL to 0.007 ± 0.003 µg/mL. The RBC recovery was 90% for AS-3 units and 89% for SAGM units. After 7 days of post-wash storage, the level of hemolysis of double-washed units was 0.4 ± 0.1%, and the residual volume of plasma was ≤ 2 µL.

Conclusions
This study shows that two sequential washes can reduce the IgA concentration of RBC units below the limit of 0.5 µg/mL, thereby complying with Canadian regulatory guidelines. Double-washed RBC units can be stored for up to 7 day post-wash when suspended in AS-3. A validation study is underway to confirm the routine use of the ACP 215 system to reduce the IgA content of RBC units.
Scientific, Poster Presentation

Annie Jacques MSc, Héma-Québec, R&D
Hana Daoud MSc, Héma-Québec, R&D
Claudia Bédard BSc, Héma-Québec, R&D
Audrey Laforce-Lavoie MSc, Héma-Québec, R&D
Marie-Claire Chevrier MSc, Héma-Québec, Reference and Stem Cell Laboratory
Louis Thibault PhD, Héma-Québec, R&D

Purpose
Currently, deglycerolized rare red blood cell (RBC) units are processed using COBE 2991 cell processors. Thawed RBC units that are suspended without nutrient solution have a limited shelf life of 24 hours at 1-6°C. The ACP 215 automated closed system from Haemonetics allows for prolonged postglycerolization storage. In this study, the post-thaw quality of SAGM and AS-3 RBC units previously frozen on day 14 was investigated on day 7 following deglycerolization and suspension in AS-3 solution.

Methods
Five leukoreduced AS-3 and 5 leukoreduced SAGM RBCs were frozen with 40% glycerol on day 14 using the automated ACP 215. After at least 48 hours of storage at -80°C, frozen RBC units were deglycerolized with the ACP 215 and suspended in about 100 mL of AS-3 additive solution. The instrument was set with a standard hematocrit of 0.60 L/L. Thawed RBC units were stored at 1 to 6°C. Samples were collected on day 7 to study the effects of deglycerolization on the quality of RBCs (ATP, 2,3-DPG, free hemoglobin, lactate and potassium).

Results
After thawing, the percentage of recovery was comparable for both types of RBCs regardless of the nature of the nutrient solution used before freezing (85% vs. 88% for AS-3 and SAGM RBC units, respectively). The hemoglobin content was 46 ± 2 g vs. 39 ± 7 g for AS-3 and SAGM RBC units. After 7 days of storage, the mean level of hemolysis and ATP were also comparable for both types of RBCs (0.4 ± 0.1% vs. 0.5 ± 0.1% and 2.1 ± 0.5 µmol/g Hb vs. 1.9 ± 0.5 µmol/g Hb for AS-3 and SAG-M RBC units, respectively). No bacterial contamination was detected in these products.

Conclusions
These results show that AS-3 and SAGM RBCs can be stored for up to 14 days before glycerolization and deglycerolization with the ACP 215 system. Once suspended in AS-3, packed red blood cells can be stored for up to 7 days to 1 to 6°C. A validation study has been undertaken to confirm these results.
Quality of Red Blood Cell Units Washed with the Haemonetics ACP 215 Automated System and Stored in AS-3 Additive Solution

Scientific, Poster Presentation

Annie Jacques MSc, Héma-Québec, R&D
Hana Daoud MSc, Héma-Québec, R&D
Claudia Bédard BSc, Héma-Québec, R&D
Marie-Claire Chevrier MSc, Héma-Québec, Reference and Stem Cell Laboratory
Louis Thibault PhD, Héma-Québec, R&D

Purpose
Currently, washed red blood cells (RBCs) are washed with COBE 2991 and stored in saline solution for up to 24 hours to reduce the risk of bacterial contamination. The closed-system ACP 215 (Haemonetics) enables automated washing and 14-day post-wash storage in SAGM solution. We studied the quality parameters of SAGM and AS-3 RBC units washed at various storage times and stored suspended in AS-3 solution.

Methods
Leukoreduced AS-3 and SAGM RBC units (450 mL) were washed using the ACP 215 system after 7, 14, 21 or 28 days of storage and stored post-wash for up to 14 days (n=5 per arm). After washing, RBCs were stored in 80 mL of AS-3 solution. Product quality (volume, hematocrit, and hemolysis) and RBC metabolism (ATP, pH, lactate, potassium, and sodium) were analyzed before and after post-wash storage.

Results
Recovery was 96% for AS-3 RBCs and 90% for SAGM RBCs. Hemoglobin content in washed AS-3 RBC units was 55 ± 4 g/unit, and 44 ± 3 g/unit in SAGM RBC units. The hematocrit of AS-3 and SAGM RBC units was 0.54 ± 0.01 L/L and 0.49 ± 0.03 L/L, respectively. The percentage of hemolysis in RBC units washed after up to 14 days of storage and stored 7 days post-wash was approximately 0.5% for both SAGM and AS-3 RBC units. Immediately after washing, the average potassium level was 1.0 ± 0.4 mmol/L, and increased to 10.4 ± 1.3 mmol/L after 7 days of post-wash storage. The residual volume of plasma was 0.40 ± 0.14 mL in AS-3 RBC units and 0.13 ± 0.02 mL in SAGM RBC units.

Conclusions
Our data show that RBC units stored for up to 14 days before washing can efficiently be washed with the ACP 215 system and stored for up to 7 days post-wash when suspended in AS-3. The nutrient solution in which RBCs are stored before washing has little impact on their in vitro parameters. A validation study is underway to confirm the routine use of the ACP 215 system to wash blood for transfusion.
55. **Method of predicting cryopreserved RBC quality while preserving units for transfusion**

Scientific, Poster Presentation

Jayme Kurach MSc, Canadian Blood Services
Tracey Turner MLT, Canadian Blood Services
Adele Hansen BSc, Canadian Blood Services
Jason Acker PhD, MBA, Research and Development, Canadian Blood Services and Department of Laboratory Medicine and Pathology, University of Alberta

**Background**
Cryopreservation of RBCs is useful in maintaining an inventory of rare blood types. CBS uses the COBE 2991 to deglycerolize frozen RBCs in preparation for transfusion; which has been validated on normal RBC units to avoid unnecessary discard of rare blood units. However, these validation conditions do not consider that some rare blood types may be more susceptible to injury caused by cryopreservation.

**Objective**
To develop a method to predict the quality of rare blood types post cryopreservation while still allowing the RBC unit to be utilized for transfusion.

**Method**
Leukoreduced CPD-SAGM RBCs produced using the whole blood method were collected (n=6), ABO-matched and divided into two groups. Each group (n=3) was pooled, split and glycerolized according to standard CBS procedures. Before segments were made 6 Fenwal sample pouches were individually sterile docked, filled with 4 mL of glycerolized RBCs, and detached. The RBC units and sample pouches were frozen at -80 °C for ≥ 24 h. Each unit and its corresponding pouches were thawed and deglycerolized together. Units were deglycerolized using standard CBS procedures. Pouches were deglycerolized using a simulated COBE process in tubes. To simulate the COBE process in tubes each wash step was replicated using the same saline solutions (12 % NaCl, 1.6 % NaCl, and 0.9 % NaCl/0.2 % dextrose) to achieve similar glycerol concentration after each step. Both the units and pouch test samples were tested 24 h post-deglycerolization for hemolysis, extracellular potassium, and recovery.

**Results**
There was no statistical differences (p>0.05) between the units and corresponding pouches at 24 h post-deglycerolization for hemolysis (1.41 ± 0.19 %, 1.29 ± 0.22 % respectively) or recovery (77 ± 4 %, 76 ± 5 % respectively). Deglycerolized units (13 ± 2 mmol/L) had significantly higher (p<0.01) extracellular potassium than corresponding pouches (9 ± 1 mmol/L).

**Conclusion**
This method was able to obtain samples from glycerolized units without compromising unit integrity. The simulated method resulted in samples that were of comparable quality to their corresponding units. With further development this process could be used to predict the quality of frozen RBC units.
Red Cell Transport in Ambient Temperatures of 0°C and Greater

Scientific, Poster Presentation

Jennifer LeFrense BSc, MLT, Nova Scotia Provincial Blood Coordinating Program
Craig Jenkins BSc, MLT, Canadian Blood Services

Purpose
Nova Scotia redistributes blood components between hospitals as a means of continuously improving efficiency. Hospitals use the Canadian Blood Services (CBS) shipping containers which have been validated for the transport of red blood cells in ambient temperatures of 0°C or greater. J82 shipping containers are used with ice packs conditioned at temperatures between -8°C and -14°C. These conditioning temperatures posed some difficulty for several hospitals due to lack of freezers available in this temperature range.

Various attempts to validate a packing scheme using ice packs stored at -30°C were performed without success. The Nova Scotia Provincial Blood Coordinating Program (NSPBCP) liaised with CBS to seek a solution to obtain a validated RBC transport system which maintains red cells at a temperature between 1°C and 10°C for a maximum transit time of 24 hours as required by CSA standards.

Method
CBS’ Quality Monitoring Program provided information on the conditioning and behavior of the ice packs when subjected to different temperatures. Data suggested that removing the ice packs from current storage conditions until they reached the required conditioning temperature may help meet the necessary conditioning requirements. CBS QMP provided NSPBCP with temperature charts showing this ‘warming’ could be achieved in approximately 30 minutes.

The ice packs were removed from freezers and allowed to warm while resting at room temperature. An infrared thermometer was used to confirm the desired temperature was reached and packing continued according to CBS’ packing scheme. Using CBS temperature monitoring devices (TMDs), outdated red blood cells were shipped between facilities to validate that this method would sustain product temperatures between 1°C and 10°C.

Results
Trial shipments were successful with all shipping containers maintaining an internal temperature between 1°C and 10°C.

Conclusion
Allowing ice packs to warm followed by packing according to CBS’ validated packing scheme maintains red cell unit temperatures during shipping as required by CSA and CSTM standards. This approach supported one packing scheme that simplified verification at time of receipt as well as saving significant dollars as the need to purchase additional freezers was eliminated.
Purpose
To validate the use of the MTS Gel IgG card technology (MTS Gel) for testing of eluates prepared by the Immucor ELU KIT Plus.

Method
Eluates were prepared and tested following the manufacturer's recommended Modified Antiglobulin Test (MAT). The same eluate was then tested in parallel by MTS Gel. A total of twenty eluates were tested, eighteen patient samples and two control samples. The first control was a known anti-Fya sample which was adsorbed onto known Fy(a+) cells and eluted. The second control was a known anti-D sample which was adsorbed onto D+ cells and eluted. Reactions were graded 0 to 4+.

Observation
In all twenty cases, the eluates reacted significantly more strongly in the MTS Gel assay than in the MAT. In seven cases, the eluate reacted 4+ by MTS Gel and 3+ by the MAT; in ten cases, the eluate reacted 3+ to 4+ in MTS Gel and w+ to 1+ by MAT. In one case, the eluate reacted 2+ in MTS Gel and was non-reactive in MAT. The two eluates from the anti-Fya and anti-D control samples reacted 2+ in MTS Gel and weakly by MAT.

Discussion
The advantages of the MTS Gel IgG card technology are that it is easy to use, yields easy to read results, uses less sample, is more sensitive than the tube method and the cards can be stored for later review. The ELU Kit Plus does not recommend testing the eluate by MTS Gel, however in our lab we have demonstrated that the MTS Gel is a more sensitive test than the MAT for testing eluates. We have adopted the MTS Gel IgG card technology for testing eluates whenever we are presented with a limited volume of patient sample.
Alloimmunization Rates in Chronic Transfusion Patients in the Edmonton Zone

Scientific, Poster Presentation

Davita Ou, University of Alberta
Kristi Lew MLT, MSc, University of Alberta
Susan Nahirmiak MD, FRCPC, Alberta Health Services
Gwen Clarke MD, FRCPC, Alberta Health Services

Purpose
Alloimmunization among patients receiving chronic transfusion therapy is a rising concern as these patients are in constant need of antigen matched donor units. Chronic transfusion patients are defined in our zone as those receiving a transfusion of red cells at least once every two months for a minimum of six months. The specificity of these antibodies and the time of identification (before or after starting chronic transfusions) are of particular concern as well as the frequency of antibody groups and whether antibody development can be related to any specific diagnosis.

Methods
A retrospective study was performed using data collected from the Laboratory Information System (LIS) to identify patients chronically transfused from January to December 2012. Additionally, the number and type of antibody each patient formed, date of antibody identification, the primary diagnosis, and demographic information such as age and gender were determined.

Results
A total of 254 chronic transfusion patients were identified, with 62 (24.4%) of these patients being alloimmunized. The most commonly occurring antibodies were the Rh, Kell, and Kidd blood group antibodies, which were found in 28, 18, and 14 patients respectively. Of these alloimmunized patients, 35 (56.5%) formed at least one antibody following the onset of their chronic transfusion therapy. The 254 patients received a total of 7269 red cell units with an average of 29 units each.

Conclusion
Despite efforts to supply chronic transfusion patients with Rh and Kell matched units, anti-E and anti-K are still the most commonly identified antibodies in this patient population. Antibodies first identified during chronic transfusion therapy belonged most commonly to the Rh, Kell, and Kidd blood group systems. The data suggests that further improvements can be made in providing these patients with antigen matched units in order to prevent the development of alloantibodies.
Platelets Directly Bind Dengue Virus and Replicate its Positive-strand RNA Genome

Ayo Y. Simon DVM, PhD, Canadian Blood Services and Centre for Blood Research, University of British Columbia
Michael R. Sutherland PhD, Canadian Blood Services, and Centre for Blood Research, University of British Columbia
Edward L.G. Pryzdial PhD, Canadian Blood Services, and Centre for Blood Research, University of British Columbia

Dengue virus (DENV) is the most prevalent arboviral infection on the globe, annually infecting ~50 million people. Although DENV predominates in tropical regions, due to vacation travel, recent outbreaks in the southern United States and documented transfusion transmission Canadian Blood Services has listed it as an emerging pathogen. Four serotypes (DENV1-4) exist, manifesting from benign subclinical flu-like symptoms to severe life-threatening hemorrhagic fever and shock syndrome resulting in 500,000 hospitalizations per year and requiring extensive platelet and plasma replacement. Escalation to severe pathology likely occurs after infection by a second serotype introduces non-neutralizing cross-reactive antibodies that mediate infection of Fc-receptor bearing cells. To account for the thrombocytopenia typical of severe disease, earlier studies (Anderson et al, 1995) using washed platelets and semi-purified virus concluded that the dengue virus-platelet interaction requires this Fc-dependent mechanism. Since 10^5-10^6 DENV particles/ml of blood may exist in an asymptomatic blood donor without cross-reactive antibodies, we investigated the Fc-independent binding of purified DENV to purified platelets using sensitive qRT-PCR. Here, we present the first evidence that DENV1 and 2 bind directly to platelets independent of virus-specific antibody, further explaining the basis of platelet dysfunction during infection, but also implying that the virus may partition with platelets during blood cell fractionation. Binding at 37°C was saturable over the concentration range used for DENV1 and 2 at 4 and 3 viruses/platelet, respectively, which was confirmed antigenically. Fc had no effect on binding, which excludes a role for platelet Fc-receptors. After treatment of platelets with thrombin, enhanced binding indicated stimulus-induced expression of receptors, which may occur during pathology. Furthermore, pretreatment of platelets with purified DENV1 or 2 increased detection of P-selectin expression as measured by flow cytometry, suggesting that the virus can trigger platelet activation. Since the DENV genome consists of positive strand-RNA, replication by platelets was investigated. At 25°C, the temperature platelets are stored in the blood bank, the genome of DENV1-4 was replicated 5- to 7-fold after 7 days. At 37°C, only DENV2 and 3 genomes were replicated. These data suggest that platelets obtained from asymptomatic donors may harbor and enhance dengue virus during storage.
**Antibacterial Activity of Cord Blood**

Scientific, Poster Presentation

Louis Thibault PhD, Héma-Québec, R&D
Marie-Pierre Cayer MSc, Héma-Québec, R&D
Joanie Pichette, Héma-Québec, R&D
Diane Fournier, Héma-Québec, Public Cord Blood Bank
Gilles Delage MD, Héma-Québec, Medical Affairs
Marie-Claire Chevrier MSc, Héma-Québec, Public Cord Blood Bank

**Purpose**
Currently, no standards are available for microbial contamination testing in cord blood banks (CBBs). For instance, some organizations use cord blood plasma to inoculate culture bottles; others recover the leftover cell concentrate remaining in the bag or collect a small aliquot from the final unit before freezing. It is known that plasma might have an antimicrobial activity on some bacteria. In this work, we studied the antimicrobial activity of plasma extracted from cord blood units and adult whole blood.

**Methods**
The antimicrobial activity of plasma extracted from 60 cord blood units and 20 adult whole blood samples (controls) was analyzed using an inhibition test similar to that of an antibiogram. Blood agar dishes were first inoculated with Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Micrococcus luteus, Propionibacterium acnes, Bacteroides fragilis, Bacteroides uniformis, Klebsiella pneumoniae, Streptococcus agalactiae, or Enterococcus sp. Thirty µL of plasma or PBS (control) were next placed on 13 mm antibiotic assay disks deposited on the agar surface. Zones of inhibition were measured after an overnight incubation at 37°C. Aliquots of plasma showing an antibacterial activity were heated to 56°C for 30 minutes to inhibit complement proteins or treated with a β-lactamase enzyme to abolish penicillin activity.

**Results**
Significant antimicrobial activity was observed in 29% of cord blood samples, whereas none of the adult plasma samples inhibited the growth of tested bacteria. Only Gram-positive bacteria were growth-inhibited by cord blood plasma samples. The inhibitory activity was not destroyed by heating at 56°C, indicating that complement proteins are not involved. However, treatment of plasma samples with a β-lactamase enzyme abolished the antibacterial activity, confirming that antibiotics are present in these samples.

**Conclusions**
This study shows that antibiotics are often found in cord blood units. Consistent with this observation, antibiotic prophylaxis is frequently used to prevent infections in obstetrical procedures. Additional work will be required to better understand the impact of antibiotics on the efficacy of the sterility test done on each cord blood unit.
Purpose
Microbial contamination of umbilical cord blood (UCB) products represents a substantial risk of complications after transplantation to immunodeficient patients. Nowadays, sterility testing uses standard microbiological culture-based methods which are time-consuming and require high inoculum volumes. Recently, Patel and coworkers (Transfusion, 2012) have published on a new molecular strategy to detect microbial contaminations in platelet concentrates. Here, we have applied this qPCR assay to detect the presence of bacterial contaminants in UCB samples.

Methods
Cord blood samples were inoculated using four common UCB contaminants (E. coli, K. pneumonia, S. aureus and Enterococcus sp.) at 10, 100 and 500 colony-forming units (CFUs). DNA extractions were optimized using a commercial kit designed for isolation of bacterial DNA (Molzym). The qPCR assay was based on the amplification of a universal DNA sequence from the bacterial 16S ribosomal DNA. Mitochondrial DNA was co-amplified as an internal control to confirm the reliability of DNA extraction and amplification. We also compared the qPCR detection limits with our current bacterial contamination culture method (BacT/ALERT system).

Results
After optimizing the DNA extraction protocol and determining cut-off values for the qPCR assay, the average detection limit was about 300 CFUs per mL. No differences were observed between the detection of Gram-negative and Gram-positive bacteria. Despite efforts to optimize DNA extraction, the sensitivity and reproducibility of the qPCR assay seems to be affected by the presence of human DNA and inhibitors present in the UCB matrix. The total time for both DNA extraction and amplification was less than four hours, much shorter than the BacT/ALERT system, which may take several days to detect slow-growing microorganisms.

Conclusions
The qPCR method does not have the required sensitivity and efficiency to detect microbial contaminants in UCB samples. The BacT/ALERT system remains a more sensitive assay to detect bacterial contaminations in these samples. Further work is required to improve the sensitivity of the qPCR method.
66. Optimization of Whole Blood Centrifugation Process to Improve Plasma Recovery

Scientific, Poster Presentation

Louis Thibault PhD, Héma-Québec, R&D
Étienne Fisette MBA, Héma-Québec, Exploitation
Annie Jacques MSc, Héma-Québec, R&D
Jocelyne Dion, Héma-Québec, Quality Assurance
Bernard Renaud, Héma-Québec, Exploitation
Martine Richard PhD, Héma-Québec, R&D
Marie Joëlle de Grandmont MSc, Héma-Québec, R&D
Louis-Philippe Gagné BSc, Héma-Québec, Exploitation

Purpose
In recent years, Héma-Québec has introduced several changes in its blood processing operations. Automated extractors (MacoPharma) have now replaced manual extractors, thus improving the consistency and quality of blood products. The efficacy of these extractors is influenced by the settings applied during whole blood (WB) centrifugation prior to plasma extraction. Interestingly, our centrifugation settings have not been revised over the last 15 years. In this work, we have revised and validated a new centrifugation time to improve plasma recovery.

Methods
For centrifugation settings verification, WB (450 mL) was collected in CP2D using the Pall Medical Leukotrap WB collection set. Blood from 3 ABO-compatible donors was pooled and split to obtain 3 identical WB units. After filtration, WB was centrifuged for 5, 10 or 15 min. at 5 147 × g (4°C). Quality parameters of blood products were measured (volume, yield and hemolysis) to determine the optimal centrifugation time. Next, 63 WB units were centrifuged for 10 min. at 5 147 × g. Plasma expression and AS-3 addition to red blood cell (RBC) units were achieved using automated extractors. The amount of residual plasma in RBC units was determined, based on the ratio of IgG in RBC supernatants and their respective plasma. Results were compared to blood products (n=60) processed using our current centrifugation parameters (5 147 × g, 5 min.).

Results
By increasing centrifugation time from 5 to 10 minutes, the volume of plasma units increased from 263 ± 23 mL to 283 ± 16 mL, for an improved yield of 20 mL per bag (p < 0.05). Conversely, increasing the centrifugation time has reduced the residual volume of plasma in RBCs from 39 ± 12 mL to 20 ± 5 mL. The percentage of hemolysis in RBCs was comparable in both centrifugation conditions.

Conclusions
This work shows the importance of regularly reviewing the parameter settings applied to blood processing. An increase of only 5 minutes in the WB centrifugation time has little impact on operations, but significantly reduces the volume of residual plasma in RBCs. The average recovery of 20 mL of plasma per donation represents a potential gain of 4 000 liters of plasma annually.
**Standardization of Hematocrit in COBE 2991 Deglycerolized SAGM RBCs**

**Tracey R. Turner MLT, Research and Development, Canadian Blood Services**

**Adele Hansen BSc, Research and Development, Canadian Blood Services**

**Jayme D.R. Kurach MSc, Research and Development, Canadian Blood Services**

**Jason P. Acker PhD, Research and Development, Canadian Blood Services and Department of Laboratory Medicine and Pathology, University of Alberta**

**Background**

The COBE 2991 cell processor can be used to produce cryopreserved, deglycerolized and washed RBCs. Canadian standards require that the hematocrit (hct) in at least 90% of deglycerolized RBCs be ≤ 0.80 L/L. Historically, deglycerolized RBCs had an average hct of 0.80 ± 0.04 L/L, and a range of 0.71 L/L to 0.90 L/L, resulting in only 52% meeting the standard. The purpose of this study was to determine the optimal amount of saline/dextrose to suspend deglycerolized RBCs in order to reduce hcts.

**Methods**

Post-processing addition of saline/dextrose was used to reduce deglycerolized RBCs to the target hct range of 0.50 L/L to 0.70 L/L. Using historic quality control data for hct and volume of COBE 2991 deglycerolized RBCs, it was estimated that addition of 65 to 90 g (median 75 g) of saline/dextrose would result in a targeted mean hct of 0.60 L/L. A pool and split study was completed and 12 groups of 4 deglycerolized RBCs were used to examine four experimental conditions; suspension in 0 g (control), 65 g, 75 g, or 90 g of saline/dextrose. All RBCs were tested for weight, volume, hct, hemoglobin content, recovery, supernatant K+, hemolysis, and ATP concentration. Results: All resuspended RBCs were able to meet the hct standard of ≤ 0.80 L/L in at least 90% of units tested with a mean hematocrit of 0.66 ± 0.07 L/L. The addition of the median 75 g of saline/dextrose resulted in RBCs with mean hematocrit 0.62 ± 0.02 L/L, unit volume 286 ± 22 mL, hemoglobin content 51 ± 4 g/unit, recovery 92 ± 4 %, hemolysis 0.48 ± 0.06 % and a ATP concentration of 3.88 ± 0.50 μmol/g Hgb. The ATP concentrations for all resuspended RBCs were statistically higher than control RBCs (p<0.001).

**Conclusion**

The increase in ATP is likely due to the suspension solution containing dextrose that is known to preserve and stimulate ATP production. Deglycerolized RBCs suspended in a fixed mass of saline/dextrose had a more standardized hematocrit and were able to meet all standards with equivalent or improved in vitro quality.
1. **Moving Sri Lanka Towards a 100% Volunteer Blood Donor Collection System: Lessons learned from Canada**

Administrative, Poster Presentation

Pavithra Aarewatte MD, Canadian Blood Services
Mark Bigham MD, Canadian Blood Services
Gershon Growe MD, Canadian Blood Services

The lead author is a consultant transfusion physician from Sri Lanka presently undertaking a one year international professional education experience through Canadian Blood Services in Vancouver.

Sri Lanka has a nationally coordinated transfusion service which oversees 84 hospital based blood banks and is the sole agency that manages the vein to vein transfusion process in Sri Lanka. Sri Lanka collects 14 units per 1000 population, where 79% of donors are voluntary and non-remunerated. By contrast, in Canada, with 100% voluntary donation, the rate of donation is double that of Sri Lanka. The average donation rate in the countries with 100% voluntary blood donation is 31 per 1000 population, suggesting that Sri Lanka might achieve a higher national blood donation rate by moving towards a 100% voluntary blood donor system.

This report summarizes the author’s evaluation of donor recruitment and other practices at Canadian Blood Services that could be utilized by the Sri Lankan transfusion service, with the aim of increasing the proportion of volunteer donors to 100%. Key recommendations include:

1. Improving knowledge of donor counseling officers and introducing consistent, evidence-based criteria for selection and deferral of blood donors.
2. Strengthening procedures for maintaining confidentiality during donor counseling at mobile blood collection sites.
3. Establishing a computerized donor database for better analysis and information retrieval.
4. Engaging communities to increase awareness and sustain support for voluntary blood donation.
5. Continuous upgrading of blood collection skills.
6. Ensuring appropriate equipment at blood collection units.
7. Improving Quality Management, through audits and external assessment schemes.
8. Introducing Standard Operating Procedures for donor adverse reaction management.
9. Establishing a system of blood donor records and:
   10. Improving management and recall of deferred and lapsed donors.

According to WHO reports blood collection system in Sri Lanka maintains satisfactory standards compared to other South Asian countries. But further improvements in its quality are required with regard to blood safety and Canadian strategies in this respect are very useful.
2. **Plasma Products Redistribution Program Pilot**

Administrative, Oral Presentation

Tracy Cameron MLT, Ontario Regional Blood Coordinating Network - Northern and Eastern Ontario Region
Wendy Owens MLT, Ontario Regional Blood Coordinating Network - Northern and Eastern Ontario Region
Sarah Crymble BA, St. Michael's Hospital

**Background**
An effective cost-saving system for redistribution of factor concentrate products from Ontario hospital sites to Hemophilia clinics has been in place since 2007. However, this program does not support redistribution of other plasma protein products (PPP) such as Intravenous Immune Globulin (IVIG). A request was made by the Ministry of Health-Long Term Care (MOHLTC) Blood Programs Coordinating Office (BPCO), to evaluate the impact of expanding the current redistribution program to include all plasma protein products throughout Ontario, including any financial gains or losses. This project had two BPCO funded blood programs, the Factor Concentrate Redistribution Program and the Ontario Regional Blood Coordinating Network (ORBCoN) working collaboratively to develop and implement redistribution of all PPP. The Eastern Ontario Regional Lab Association (EORLA), consisting of 18 hospitals, agreed to pilot this project for 6 months.

**Method**
Based on the Canadian Blood Services (CBS) expiry report received every month by ORBCoN, product information was transferred onto a notification form and sent out to all pilot sites to complete and return the form. ORBCoN reviewed the completed forms and entered the information into a data sheet for tracking purposes. ORBCoN facilitated the transfer of the products to either another hospital in the EORLA region, a hospital in a neighbouring Local Health Integrated Network (LHIN) or with the Factor Concentrate Redistribution Program located at St. Michael’s Hospital in Toronto. Any costs for shipping and loss of the near to expired products was tracked as well as cost of product that was not wasted as a result of redistribution.

**Results**
A total of 14 transfers of 7 different products took place between sites during the pilot. Two specialty products (VZIg and IMIg) could not be redistributed as no receiving site could be identified. The total net costs savings of product redistributed was $384,380. Of this, $187,986 worth of IVIG was redistributed.

**Conclusion**
The pilot has shown that there is a cost savings benefit by redistributing all plasma products throughout Ontario. The program will be rolled out across the province in 2013.
3. **Reinventing the Inventory Management Paradigm: Creation of a novel informatics-driven high-resolution red cell inventory map**

Administrative, Oral Presentation

Calvino Cheng MD, FRCPC, Capital District Health Authority, Dalhousie University
Stephanie Watson MLT, Capital District Health Authority
Joan Macleod MLT, Capital District Health Authority
Andrew Kumar-Misir BSc, Capital District Health Authority
Irene Sadek MD, FRCPC, Capital District Health Authority, Dalhousie University

**Background**
Current global inventory management paradigms are based on mathematical models and practices developed in the 1970s and 80s, and aggregated key performance/quality indicators, such as discard/expiry rates. Practically, these methodologies are approximations and proxies for understanding the internal working of the inventory ‘black box’, which the literature still has not fully characterized since the 1970s. We present a novel data-driven high-resolution inventory mapping technique which directly visualizes the red cell unit (RBC) flow inside the ‘black box’. This method can characterise inventory flow volume and performance, confirm policy adherence, and discover policy deviations at multiple abstraction levels. The technique can be scaled and applied to any information system-enabled transfusion service.

**Materials/Methods**
RBC data during May 1, 2009-January 31, 2013 was queried from the laboratory information system (Cerner Millennium) for all sites at Capital District Health Authority, Halifax, Nova Scotia. This data contained processing, handling, unit, and location attributes. Data validation, cleaning, and analysis was performed using commonly available database, spreadsheet and data mining software. Ethics approval was not required by institutional policies.

**Results**
There were 574,245 transactional states with 745 unique location and transfusion state specific activities involving 59,367 red cell units. There were 15994 transactional variants, with the most common variant involving 2811 units (4.74%) having 9 consistent temporally connected states (receivedHI>unconfirmedHI>confirmedHI>availableHI>transferredVG>crossmatchedVG>issuedMDU>transfused MDU), corresponding to a RBC commonly transfused at an outpatient oncology ward. RBC transition times through inventory obeyed a Pareto distribution, with 75% of cases completed before 9 days and 2 hours. High resolution inventory maps performance and volume data were also generated for all transfusion sites.

**Conclusion**
Using a revolutionary and novel high resolution process discovery technique, the flow of RBCs through our inventory was characterized. These maps directly reflected how inventory reacted to institutional transfusion practice, and allowed us to examine business practices more closely than via conventional aggregated key performance metrics data. This will allow for evaluation of policy changes, allow reverse-engineering of policies, and enable true lab-to-lab benchmarking, ultimately enabling cost savings and improved patient care in transfusion.
4. Transfusion Bits and Bytes: Transfusion Medicine Laboratory Technologist education videos

Administrative, Poster Presentation

Susanna Darnel ART, BC Provincial Blood Coordinating Office (BCPBCO)
Aimee Beauchamp, BC Provincial Blood Coordinating Office (BCPBCO)
Anne Lucas RT, BC Provincial Blood Coordinating Office (BCPBCO)
Sheazin Premji MBA, BC Provincial Blood Coordinating Office (BCPBCO)
Sonia Chau, BC Provincial Blood Coordinating Office (BCPBCO)

Background
A common thread running through the 2010 Canadian MLT’s Learning Network Survey Report, administered by the Canadian Blood Services (CBS), was the inconvenience of continuing education. Respondents indicated that busy work and family schedules and high costs prohibited them from accessing educational tools that are currently offered. As a result of this study, CBS initiated the BloodTechnNet Learning Competition, designed to find innovative learning projects for Medical Laboratory Technologists in those parts of Canada served by CBS.

Proposal
The BC Provincial Blood Coordinating Office (PBCO), in collaboration with the BC Technical Resource Group, submitted a proposal named "Transfusion Bits and Bytes". The goal of Transfusion Bits and Bytes is to assist Canadian Medical Technologists in keeping up to date with current knowledge and changing practice specific to the field of transfusion medicine. The key points of the proposal included: web-based education that could be accessible free of charge from any location with internet access (whether at work, at home or elsewhere), and educational units that would be kept short so they could easily fit into busy schedules.

Results
The proposal was awarded a $25 000 prize by a judging panel. The monetary award was used to consult with an education coordinator; to develop a script; to evaluate electronic delivery platforms and to record, format, and upload lectures. The funding was also used to consult with a computer software engineer to develop applications for smartphones.

The first presentation topics chosen were relevant to new and rotating core technologists as well as those working in Transfusion Medicine in large centres. The idea was to begin with basic Transfusion Science presentations and work up to more complex or difficult topics, and also to include presentations related to management experience.

Conclusion
A series of six educational videos, ranging from 13-26 minutes on Transfusion Science related topics, were recorded and can be accessed through the internet on the BC Provincial Blood Coordinating Office website www.pbco.ca. The presentations are also accessible free of charge as downloadable apps on the iPhone smartphone and iPad tablet.
5. **ORBCoN’s Transfusion Committee Forum: 5 years of successful collaboration**

Administrative, Poster Presentation

Denise Evanovitch MLT, Dipl. Adult Ed., ORBCoN, McMaster University
Kate Gagliardi MLT, ART, BA, ORBCoN
Deborah Lauzon MLT, ART, BHA, ORBCoN
Wendy Owens MLT, ART, BComm, ORBCoN
Stephanie Cope MLA, ORBCoN
Troy Thompson MLT, ASQ-CQA, ORBCoN
Tracy Cameron MLT, ORBCoN
Heather Nesrallan BA, ORBCoN
Laurie Young MLT, ORBCoN

**Background**
One mandate of the Ontario Regional Blood Coordinating Network (ORBCoN) is to provide hospital transfusion services with best practice tools that support transfusion service delivery. Standards and requirements mandate the establishment of a transfusion committee (TC) and their responsibilities at transfusion service organizations. In 2006, only 57% of the responding hospitals in Ontario (response rate of 80%) had a TC in place; 7% had no TC activities at all. The Ontario Blood Programs Coordinating Office (BPCO) recognized this deficiency and recommended in December 2007 that ORBCoN develop a Transfusion Committee Toolkit and host an accompanying forum, which has continued as a popular event for the past 5 years. It is funded by the BPCO of the Ministry of Health and Long-Term Care (MOHLTC) and is accredited by the RCPSC.

**Methods**
A multidisciplinary, province-wide committee plans each forum. Toronto is the host city based on audience feedback from all Ontario regions, accessibility to the rest of the province and its attractions. Funding is provided for transportation and accommodation costs for all TC chairs, or their alternates if they cannot attend, to encourage their participation in this event. This is crucial for attendees from more remote areas of the province. The first forum was held in March 2008 and Transfusion Committees and the toolkit were selected as the theme. Subsequent themes were selected based on forum feedback and environmental scanning.

**Results**
Participant satisfaction ratings for the past 5 years have averaged 4.2-4.6/5 in each rating category, where a ‘1’ rating indicates poor and a ‘5’ indicates excellent. An overwhelming majority of the participants indicated that they learned something that will influence their practice and that they plan on attending future ORBCoN events. Despite competition from many other transfusion-related educational events, the number of attendees remains consistently between 135 – 150 each year. In 2012, the launch of a new TC handbook occurred along with videos of veteran TC members that are posted to www.transfusionontario.org.

**Conclusion**
The Transfusion Committee forum is a successful and popular event as evidenced by the consistent attendance and high feedback ratings by the participants.
6. **Competency Assessment: Do we ask the right questions?**

   Administrative, Poster Presentation

   Shelley Feenstra RN, Vancouver Coastal Health
   Caitlyn Skeith BSc (C), University of Waterloo

   The therapeutic intervention of a transfusion involves several critical steps. It is essential that those participating in the transfusion process are fluent in the related policies and competent in their skills and practice. Many key elements should be visualized when developing and/or maintaining an “on-going training process” established for the interdisciplinary team members who practice transfusion medicine. In addition to determining the timeframe, level of content and the media modality that best facilitates the learning experience, the trainer must also consider the key challenges of measuring competency in the theory or the practical skill.

   Competency of a skill can be measured through return demonstration of a task i.e. the technical skill of preparing filtered red cells in a syringe for neonatal transfusion or a change in spiking technique related to a new product container. However, in addition there are key principles and fundamental theory that support best practices. Historically, the application of a pre and / or post-test has been utilized to measure knowledge. To evaluate the level of knowledge and assure safe clinical and laboratory practice, a realistic expectation of the learner is to achieve a pre-determined test score i.e. 100%. Test questions should assess practical knowledge. Failure or multiple repeat test attempts can indicate questions that focus heavier on reading comprehension. This can lead to mounting frustration and non-compliance. At Vancouver Coastal Health, anecdotal evidence of non-compliance and negative feedback initiated a review and rewrite of transfusion competency test questions. Although the existing program was efficient and effective when developed, it no longer satisfied the needs of the user and a new battery of questions was required.

   Our objective is to evaluate the level of knowledge with the community of transfusion medicine and evaluate whether structural change in the series of questions leads to improved test scores, decreased frustration for the user and improved compliance with the on-going training process. Health care providers will trial the individually randomized online cache of new questions and we will collate the responses. A statistical comparison of the two batteries of questions will be completed and evaluated.
A massive hemorrhage protocol (MHP) has been available from our transfusion service since 2008. Although the protocol involves more than just product support, an integral part of the MHP is the blood cooler of 6 units of packed red blood cells, 6 units of plasma, and one pool of platelets transportable with the patients. Given the recent literature regarding transfusion practices in patients with upper gastrointestinal (GI) bleeding, and lack of evidence for 1:1:1 transfusion, we sought to evaluate massive hemorrhage protocol (MHP) activations in GI bleeding patients in our region from January 2011 to June 2012.

Over that period, 339 MHP activations occurred in the region with 78 (23%) of these resulting from GI bleeds. Thirty-nine MHP packs were issued for GI bleeds at the University of Alberta Hospital (UAH - the local major academic hospital) and the remaining 39 among the community-based hospitals in Edmonton Zone. Increased hemoglobin level post-MHP was noted in 35 (44.9%) patients. Of those, 91.4% had hemoglobin level pre-MHP <90 g/L. Sixteen (20.5%) showed decreased levels post-MHP; of which 50% had hemoglobin level <90 g/L. Utilization of issued pack components at the community hospital sites was 34.2%, 66.4%, and 70.4% compared to 48.5%, 56.2%, and 60.3% at UAH for PRBCs, plasma, and platelets, respectively. For every pack issued, the community hospitals issued an average 3.92 units RBCs, 0.90 units plasma, and 0.05 pools of platelets prior to the initiation of the MHP. UAH issued an average of 3.07 units RBCs, 0.67 units plasma, and 0.10 pools of platelets pre-MHP per pack issued. After the MHP, on average, an additional 2.38 units of RBCs, 0.79 units of plasma, and 0.33 pools of platelets were issued for every MHP pack by the community hospitals. An additional 1.92 units RBCs, 0.897 units plasma, and 0.25 platelet pools were issued per MHP pack, on average, by UAH.

Conclusion: MHP activations for GI bleeds constitute a significant number of our total MHP use. The hemoglobin level was more likely to increase if pre-MHP level was <90 g/L. The appropriateness of this utilization and approach is currently under review.
8. **Competency Tools for Health Professionals Involved in Transfusion Activities**

Administrative, Poster Presentation

Deborah Lauzon MLT, ART, BHA, The Ontario Regional Blood Coordinating Network (ORBCoN)
Wendy Owens MLT, ART, BComm, The Ontario Regional Blood Coordinating Network (ORBCoN)
Kate Gagliardi MLT, ART, BA, The Ontario Regional Blood Coordinating Network (ORBCoN)
Troy Thompson MLT, The Ontario Regional Blood Coordinating Network (ORBCoN)
Denise Evanovitch MLT, The Ontario Regional Blood Coordinating Network (ORBCoN)
Laurie Young MLT, The Ontario Regional Blood Coordinating Network (ORBCoN)
Tracy Cameron MLT, The Ontario Regional Blood Coordinating Network (ORBCoN)
Yulia Lin MD, FRCPC, CTBS, Sunnybrook Health Sciences Centre
Ana Lima RN, HP(ASCP), Sunnybrook Health Sciences Centre

The Ontario Regional Blood Coordinating Network (ORBCoN) was established in 2006 by the Ontario Ministry of Health and Long-Term Care to facilitate implementation of a provincial strategy for blood utilization. Inherent in this strategy is improving patient safety related to blood transfusion. The Canadian Standards Association (CSA) and the Canadian Society for Transfusion Medicine (CSTM) publish national standards for Transfusion Medicine. In Ontario, the Ontario Laboratory Accreditation division (OLA) of the Quality Management Program for Laboratory Services (QMPLS) is the primary agent that assesses health care facilities’ compliance with these standards through participation in a mandatory accreditation process. Both national standards require ongoing education and competency assessment for all health professionals involved in transfusion activities including: technologists, nurses, nurse practitioners, physicians and perfusionists. In Ontario, meeting these competency requirements continues to be one of the top 10 reported non-conformances.

In collaboration with other health professionals, ORBCoN developed three online educational programs to assist health care facilities in meeting the competency requirements: Bloody Easy Tech Assess, primary target audience- technologists, launched in 2008; Bloody Easy for Nurses, primary target audience- nurses, and also applicable for perfusion and anesthesia, launched in 2009; Bloody Easy Lite, primary audience-prescribing physicians, launched in 2012. Each of these programs offers a registration and tracking system for health care facilities in Ontario, enabling them to track participation and performance and provide evidence of ongoing competency assessment for accreditation purposes. Registration for the Tech Assess program is performed by a site administrator assigned by each health care facility.

Assessments and learning resources to augment the tech assess program are updated annually. Participants in the other two programs self register and are able to affiliate themselves with one or more facilities. Site administrators at the affiliate facility are able to monitor and track performance electronically. Reports of participation can be generated on demand by site administrators. Current registration numbers for Ontario include: 2,000 MLT’s, 7550 RN’s and 275 MD’s. These programs provide Ontario health care facilities with a confidential, standardized, no cost solution to meeting the accreditation requirements for ongoing education and competency assessment of health professionals.
Development and Implementation of a Procedure for RBC Syringe Aliquot Preparation for Neonatal Resuscitation

Administrative, Poster Presentation

Darlene Mueller MA, ART, Fraser Health Authority
Diana Kobes RT, Fraser Health Authority
Doug Morrison FRCP, MD, Fraser Health Authority

In response to patient safety quality reviews of neonatal resuscitation events, we recognized the need for a health authority wide approach to provision of red blood cells in these critical clinical situations. As a result, the Fraser Health Transfusion Medicine Laboratory (TML), with input from the maternal and neonatal program, developed a procedure to provide pre-filtered syringes of unmatched O Negative red blood cells for immediate transfusions.

This procedure was implemented at eleven acute care facilities in Fraser Health where newborns are delivered. Implementation included the use of a training and competency assessment document and direct observation of over 200 technologists that rotate through the transfusion medicine laboratory.

One year after implementation, a TML process audit was initiated in response another patient safety review. Internal audit results revealed the need for technologists to perform this procedure on a regular basis with a focus on the physical preparation, computer steps, and communication with the clinical unit. On the clinical side, the need to notify the TML, as early in the event as possible, using consistent, clear, and predetermined language was identified as an area in need of improvement.

A revised procedure was developed, validated, and implemented with direct observation. This included a laminated, large font job aide for posting at the bench. Pre-packaged, standardized supply kits were developed to ensure that technologists could provide the syringe within 10 minutes of receiving the request. Informal feedback from technologists during implementation of these revisions indicated that the ease in preparation of this component had been improved.

Throughout this quality improvement cycle, engagement of the laboratory leadership team and the maternal/neonatal program has resulted in increased support for this critical procedure. The laboratory leadership team has endorsed implementation of an enhanced yearly competence assessment program for this and other selected low frequency, high acuity procedures. The maternal/neonatal program has committed to include information on the availability and ordering of this component as part of their health authority wide bi-annual neonatal resuscitation certification program.
10. Implementation of Two Anti-D Reagents in a Hospital-based Transfusion Service: Detection of weakened RhD serological expression

Administrative, Poster Presentation

Chantale Pambrun MD, FRCPC, IWK Health Centre
Katherine Gough MLTII, IWK Health Centre
Catherine McAuley MLTII, IWK Health Centre
Patti Burrell MLT, BHSC, DHSA, IWK Health Centre

Approximately 0.2-1% of Caucasian individuals will react weakly with anti-D reagents. (1) The detection of a weakened serological expression, as well as a discrepant result between reagents, highlight a difference in the RhD antigen, weak D or partial D genotype. It is important to detect the serological discrepancy in order to determine the underlying genetics and the risk of alloimmunization to RhD. The risk of alloimmunization to the D antigen has implications in hemolytic disease of the fetus and newborn, as well as the decision to administer Rh immunoglobulin (RhIg). The IWK Health Centre is the prenatal reference laboratory in Nova Scotia, and has recently implemented the use of two D antisera for all blood bank samples. Seventy-five percent of the samples received are from females, which is the group of interest for detection of weak D and partial D genotype. The implementation of the new policy included technical staff education, clinical input and coordination with the National Immunohematology Reference Laboratory. The most difficult element to implement was the workflow to accommodate the automated gel methodology and the manual tube methodology. The use of standard operative procedures, flow charts, and interpretative tables were an asset. Daily checks by senior technologists, were vital to pick up process errors during early implementation. To date, the published incidence of weak D and partial D is reflected in our population. Moving forward, women with a weak D genotype will not receive RhIg unnecessarily and those with a partial D genotype will be treated as Rh negative to avoid D alloimmunization and hemolytic disease of the fetus and newborn. Could Canadian hospital-based transfusion services be doing more to avoid blood product exposure (RhIg) and alloimmunization of females with child-bearing potential?

(1) Transfusion 2005;45:1547–51.
11. Transfusion Education for BC Pathologists

Administrative, Poster Presentation

Sheazin Premji MBA, BC Provincial Blood Coordinating Office
Kate Chipperfield MD, FRCPC, Vancouver Coastal Health
Jason Doyle MD, FRCPC, Interior Health
Doug Morrison MD, FRCPC, Fraser Health
Louis Wadsworth MD, FRCPC, University of British Columbia
Shelley Feenstra RN, Vancouver Coastal Health
Donna Miller RN, Vancouver Island Health Authority
Maureen Wyatt ART, Interior Health

Background
In order to identify and respond consistently to transfusion reactions in BC, new provincial minimum standards of practice have been endorsed for transfusion reaction reporting. New standardized forms, process and procedure documents for laboratory investigation, and training materials were developed, and to complement these materials, a six module online web-based education program entitled Transfusion Reaction Education for BC Pathologists was developed and launched in October 2011.

Proposal
Three cycles of the education program were offered between October 2011 and September 2012. The course was initially targeted at BC pathologists but was subsequently opened to other clinicians in BC and other provinces, and other stakeholders including doctors, nurses and technologists. The education program was accredited and provided Section 1 Credit as defined by the Maintenance of Certification program of the Royal College of Physicians and Surgeons of Canada.

The objectives for the education program included:
- Enhancing knowledge and understanding of transfusion surveillance activities in BC.
- Presenting the new provincial minimum standards of practice for transfusion reactions and supporting process and procedure documentation.
- Reviewing types, signs and symptoms of transfusion reactions.
- Introducing standardized guidelines for pathologist conclusions for transfusion reaction reporting.
- Using case studies to enhance knowledge uptake.

Results
A total of 936 completions were recorded for all three cycles (one completion is equivalent to one module completed with quiz passed) – 199 in cycle 1, 180 in cycle 2 and 557 in cycle 3. Interest in the online course was widespread, with people participating from BC, Alberta, Ontario, Saskatchewan, Nova Scotia, Manitoba, Yukon and even outside of Canada. Participant types included Doctors, Technologists, Nurses, Clinicians, Transfusion Safety Officers, and Medical Services Coordinators.

Conclusion
Participants were asked to complete an optional online evaluation at the end of each module. Out of 936 completions, 608 (65%) participant evaluations were received. The majority of the respondents ranked the overall quality of presentations as excellent (43%) or good (49%).

Outcomes and results from before and after the education initiative will be measured and reviewed to assess the effectiveness of this initiative and to help identify further education opportunities.
Clinical Abstracts

12. A Closer Look at the Emergency Department STAT Type & Screens: When is it really an emergency?

Clinical, Poster Presentation

Salah Aboughouche Grad Student (pre-residency), University of Saskatchewan
Karen L. Dallas, Saskatoon Health Region
Linda Friesen MLT, Saskatoon Health Region

Introduction
Saskatoon’s biggest Emergency Department (ED) routinely sends STAT type and screen test to the Transfusion Medicine Laboratory (TML). Some of these test are clearly emergencies and sometimes blood must be issued out prior to the completion of this pre-transfusion testing. However, at other times, testing is sent without apparent rationale. With increasing constraints on our budget and workforce in TML and with an increasing focus on patient-first care, we felt it prudent to look into possible unnecessary testing.

Objective
Objective of this study was to assess the significance and clinical utility of STAT type and screen testing sent from the ED to TML.

Method
All of the patients who had STAT type and screens sent from the ED during the months of April and May 2012 were studied. Data was manually collected on a spreadsheet and included Diagnosis, Hgb level, whether or not blood was requested, whether the patient got admitted and whether the patient had a positive antibody screen. Data was then entered into an Excel format for analysis.

Results
624 patient had data collected. Of these patients, 514 (82.4%) had Hgb levels of >100 g/dL. Additionally, 555 patients (88.9%) did not have a requisition for blood. 269 (43.1%) patients went on to be admitted to hospital while the rest were discharged. Also important for the lab, nearly 5% of patients had positive antibody screens which required a full work-up.

Conclusions
In conclusion, we have uncovered a large source of wastage when it comes to the utilization of pre-transfusion testing. Not only does this waste time and resources for the lab, it affects patient care – both for those patients we are needlessly drawing blood on and potentially for other patients who are not getting as timely testing in TML because of these unnecessary STAT specimens. Clearly there is opportunity here for improvement in our local practice and we are currently in active discussion with our clinical colleagues about this matter.
Background
Transfusion-related acute lung injury (TRALI) is a devastating transfusion-associated adverse event reported after transfusion. There is a paucity of data on the incidence and characteristics of TRALI cases that occur preoperatively. We classified suspected perioperative TRALI cases reported to Canadian Blood Services, between 2001 and 2012, and compared them to non-perioperative cases, to elucidate specific surgical factors that make them particularly at risk for developing TRALI.

Study Design and Methods
Using Canadian Consensus Conference definitions, all suspected TRALI cases reported to Canadian Blood Services (CBS) since 2001 were reviewed by two experts or, from 2006 to 2012, an expert TRALI Medical Review Group (TMRG) were classified and detailed in a database. Two additional reviewers further categorized them as occurring within 72 hours from the onset of surgery (perioperative) or not in that period (non-perioperative). Various demographic and characteristic variables of each case were collected and compared between the groups.

Results
In Canada, between 2001-2012, a total of 303 suspected TRALI cases were reported to Canadian Blood Services. Of those, 38% (112) were identified as occurring during the perioperative period. Patients who underwent cardiac surgery requiring cardiopulmonary bypass (25.0%), general surgery (18.0%) and orthopedics patients (12.5%) represented the three largest surgical groups. Perioperative TRALI cases comprised of more men (53.6 vs. 41.4, p=0.0395) than non-perioperative patients. More perioperative TRALI patients required supplemental O2 (14.3% vs. 3.1%, p=0.0003), required mechanical ventilation (18.8% vs. 3.1%), or were from the ICU (14.3% vs. 3.7%, p=0.0043), than non-perioperative TRALI patients before their operations. The surgical patients were transfused approximately 673 products and on average were transfused more products than non-perioperative patients (6.0[SD=8.3] vs. 3.6[5.2], p=0.0002). Perioperative TRALI patients were transfused more frozen and fresh frozen plasma and cryoprecipitate than non-TRALI patients. Non-perioperative patients utilized more cryosupernatant plasma. There was no difference between donor antibody test results between the groups.

Conclusion
CBS data has given more insight into the nature of TRALI cases that occur perioperatively; this group represents a large proportion of TRALI cases. Perioperative transfusion that occurs within this specific population should be carefully considered and monitored, especially in patients with at-risk characteristics. Further research will be required to delineate the exact mechanisms behind perioperative TRALI.
Intravenous immune globulin (IVIg) use for neurological conditions accounted for 36% of total IVIg use in BC at a cost of $11.9 million in 2011/12. Neurology is the second fastest-growing specialty user of IVIg; up 5.6% last fiscal year and up 6.2% in the last three fiscal years (2009/10 through 2011/12), compared to an overall provincial IVIg increase of 5.3% (1 year) and 11.7% (3 years). Neurology is also the highest dose specialty, with a mean use of 449 grams/patient in 2011/12 compared to a mean use of 293 grams/patient for all specialties.

The IVIg Neurology project entailed working with a task force of provincial Neuromuscular (NM) Neurology representatives to define the approaches and develop the systems to effectively manage patients who require IVIg for neuromuscular conditions. The project involved finalizing a provincial approach for IVIg use for approved clinical conditions including treatment, patient follow-up and reassessment for IVIg efficacy and dose reduction. The use of the IVIg dose calculator which was brought into effect in BC last year as a part of the IVIg directives was incorporated into guiding NM prescribing practices. The IVIg NM task force put together a physician toolkit for physicians across BC to use to support standardization of clinical approaches. This will help improve patient care and product utilization across the province. The IVIg NM task force also established a provincial Neurology IVIg screening panel to assess the appropriateness of using IVIg to treat rare and complicated cases through the evaluation of treatment outcomes.
15. Loss of RhD Expression Coinciding with Relapsed Acute Myelogenous Leukemia

Clinical, Oral Presentation

Signy Chow MD, University of Toronto
Vikas Gupta MD, FRCPC, FRCPATH, Princess Margaret Hospital, Toronto
Jacob Pendergrast MD, FRCPC, University Health Network, Toronto
Chistine Cserti-Gazdewich MD, FRCPC, FASCP, University Health Network, Toronto

Background
RHD expression is expected to be stable outside of hematopoietic stem cell transplantation (HSCT), although spontaneous loss due to chimerism, mosaicism or somatic mutation has been described, with such events predominantly associated with malignancy.

Case
We describe a case of a 50 year old acute myelogenous leukemia (AML) patient whose loss of RHD expression coincided with relapsed malignancy. The original diagnostic bone marrow suggested preceding chronic myelomonocytic leukemia (CMML) in the company of splenomegaly, while JAK2 and BCR-ABL studies were negative. Standard induction chemotherapy was followed by bone marrow evidence of morphologic remission two months later, and no cytogenetic abnormalities were noted at diagnosis or post-induction.

His presenting transfusion laboratory sample typed as O, RHD-positive with a negative red cell antibody screen, and stable repeat grouping for two months. Platelet transfusion refractoriness developed (PRA 99%), and he qualified for procurement of HLA-matched platelets. The AML relapsed, and he was then found to have mixed field reactions on RHD grouping (despite no transfusions with O- RBC or HSCT), with two distinct populations of O+ and O- cells. Cytogenetic re-analysis of marrow did not demonstrate any abnormalities by G-banding.

Analysis of a peripheral blood sample demonstrated the RH genotype ccEe with D (R2r), although the ratio of e/E was out of the usual range for heterozygosity, with e present more abundantly than E. Repeat testing with alternative sequencing primers ruled out unequal amplification of allele-specific polymorphisms at primer sites, while the absence of other out-of-range data for other antigens excluded chimerism. With the mixed field reactions indicating loss of the RHD, this suggested a specific deletion of an entire DcE (R2) allele at a clonal pre-erythroid level.

Discussion
Loss of heterozygosity (LOH) on chromosome 1 has been shown to be an important mechanism of RHD loss. While such loss may be benign, the role of LOH in leukemogenesis is also well known, with cases of the loss and resurgence of RHD expression paralleling the development and remission of malignancy. Alteration in RH expression may be either a surrogate for relapsed malignancy or the effect of an ongoing clonal evolutionary process.
Adult Thalassemia Patients Are Not Disadvantaged By Matching Less Rigorously Than The Sickle Cell Standard

Clinical, Poster Presentation

Christine Cserti-Gazdewich MD, FRCPC, University Health Network and University of Toronto
Michael Angers MLT, University Health Network
Anumithraa Niranjan BHSc(Cand), University of Ontario Institute of Technology
Gisele Thorpe MLT, University Health Network
Jacquie Beal MLT, University Health Network
Sally Balmer ART, University Health Network
Janice Hawes BA, MLT, University Health Network
Elana Tsiokos MLT, University Health Network
Jacob Pendergrast MD, FRCPC, University Health Network and University of Toronto
Marciano Reis MD, FRCPC, Sunnybrook Health Sciences Centre & University Health Network, University of Toronto

Background
Transfused patients are at risk of alloimmunization according to their “immune responder” status and the extent of their exposures to erythrocytes (RBCs) bearing unfamiliar antigen types. Sickle cell disease patients (SCD) are known to be strong responders, with transfusions occurring intermittently amid inflammatory/ischemic-hemolytic crises, while facing ancestrally more divergent (predominantly non-African) donor antigen profiles. Conversely, chronically transfused thalassemia major (TM) patients are exposed in a more tolerizing schedule since their infancy, with the quality of blood having greater incidental phenotypic similarity but the quantity of donor exposures unsurpassed. In SCD, guidelines promote prophylactic antigen matching (PAM) towards RHCE and KELL, with extensive antigen matching (EAM) towards Duffy, Kidd, and Ss after sensitization towards any significant minor red cell antigen, therein avoiding further seroconversions and sequelae thereof.

Problem
At our 1000-bed acute care teaching hospital, policies since 1991 advise PAM/EAM to SCD patients by blood group profile and serostatus. TM patients are not phenotyped, but are empirically given units tagged as Kell-negative, with antigen-negative blood only as needed. Whether TM warrants the SCD standard of matching is unsettled.

Study Design and Methods
On-site summary alloimmunization and transfusion prevalences for SCD and thalassemia patients were compared to determine if the latter were disadvantaged by our policy. Patients registered within the Laboratory Information System (HCLL 4.6.0.2, Mediware Info Sys Inc, Oakbrook IL) were filtered by diagnosis-related instruction and dates of first-to-last transfusions within a 19y post-policy period (11/1993–11/2012).

Results
Twice as many SCD (538) as thalassemia (257) were registered, although only 38% (208) of the former were transfused compared with 66% (169) of the latter. RBC sensitization occurred in 83 SCD patients (40% of the transfused [95% CI: 33–47%], or 15% overall), and in 75 thalassemia patients (44% of the transfused [95% CI: 37–52%], or 29% overall); transfusees were thus not statistically different (2-tailed Z-score 0.87). Despite policy, 74 SCD RHCE/KELL antibodies (46%) and 20 thalassemia KELL antibodies (13%) occurred/pre-existed, while insignificant antibodies dominated in thalassemia (81 or 52% vs 45 or 28% in SCD).

Conclusion
Resource-intensive matching, although of limited efficacy, is best reserved for SCD.
Hospital-based Transfusion Error Tracking from 2005 to 2010: Identifying the key errors threatening patient transfusion safety

Clinical, Poster Presentation

Helen Downie MLT, Sunnybrook Health Sciences Centre
Carolyn Maskens BSc, University of British Columbia
Alison Wendt MLT, Sunnybrook Health Sciences Centre
Lisa Merkley MLT, Sunnybrook Health Sciences Centre
Ana Lima RN, Sunnybrook Health Sciences Centre
Yulia Lin MD, Sunnybrook Health Sciences Centre
Jeannie Callum MD, Sunnybrook Health Sciences Centre

Background
This report provides a comprehensive analysis of transfusion errors occurring at a large teaching hospital and aims to determine key errors that are threatening transfusion safety, despite implementation of safety measures.

Study Design and Methods
Errors were prospectively identified from 2005-2010. Error data was coded on a secure online database called the Transfusion Error Surveillance System (TESS). Errors were defined as any deviation from established standard operating procedures. Errors were identified by clinical and laboratory staff. Denominator data for volume of activity were used to calculate rates.

Results
A total of 15,134 errors were reported with a median number of 215 errors per month (range 85-334). Overall, 9083 (60%) errors occurred on the transfusion service and 6051 (40%) on the clinical services. In total, 23 errors resulted in patient harm: 21 of these errors occurred on the clinical services and 2 in the transfusion service. Of the 23 harm events, 21 involved inappropriate use of blood. Errors with no harm were 657 times more common than events that caused harm. The most common high severity clinical errors were sample labeling (37.5%) and inappropriate ordering of blood (28.8%). The most common high severity error in the transfusion service was sample accepted despite not meeting acceptance criteria (18.3%). The cost of product loss due to errors was $593,337.

Conclusion
Errors occurred at every point in the transfusion process, with the greatest potential risk of patient harm resulting from inappropriate ordering of blood products and errors in sample labeling.
Background
Chagas Disease is caused by a protozoan parasite, Trypanosoma cruzi, transmitted by triatomine bugs in Mexico, Central and South America. Increasing immigration to North America from these countries has prompted the screening of blood donors for Chagas Disease. In May 2010, Canadian Blood Services (CBS) began testing ‘at risk’ donors for Chagas antibody. A seroprevalence study was set up in September 2010 to test a subset of ‘no risk’ donors. This was done primarily to assess the efficacy of the risk questions in identifying donors for Chagas testing.

Method
A target of at least 60,000 donors from clinics in areas reflecting immigration from endemic countries was selected for Phase I and included donors from southern Ontario, Manitoba, Alberta and British Columbia. Phase II was carried out in Manitoba only. Donors who answered no to all three Chagas risk questions were tested for Chagas antibody using the Abbott PRISM Chagas assay. All repeat reactives were sent to the National Reference Centre for Parasitology (NRCP) at McGill University, Montreal, for testing by ELISA, immunoblot and Polymerase Chain Reaction (PCR). Samples were sent to Quest Laboratories (U.S.) for the Radioimmunoprecipitation assay (RIPA) and to Blood Systems Inc. laboratories for the Ortho Chagas assay used for donor screening in the U.S.

Results
From September 2010 to October 2012, 84,078 donors were tested, with 62,117 tested in Phase I and 21,961 in Phase II. Only one confirmed positive donor was identified in Phase I. Further investigation revealed that the donor was infected via vertical transmission from her mother who had been transfused, around the time of the donor’s birth. This donor would not have been detected by any risk questions. The recipient of the only product transfused from this donor, tested negative for Chagas Disease. Phase II found no additional confirmed positive donors.

Conclusions
Based on the finding of only one confirmed positive donor of more than 80,000 ‘no risk’ donors tested, CBS has maintained the strategy of testing only at risk donors, identified by questioning. This strategy represents a new paradigm in infectious disease testing at CBS.
19. Investigation of a Possible Case of WNV Transfusion Transmission: Summer 2012 season

Clinical, Poster Presentation

Elaine Fournier RN, Canadian Blood Services
Peter Lesley MD, Canadian Blood Services
Melanie Tokessy MLT, The Ottawa Hospital
Barbara Hannach MD, Canadian Blood Services
Kai Makowski MLT, Public Health Agency of Canada
Vito Scalia MSc, Canadian Blood Services
Margaret Fearon MD, Canadian Blood Services

Background
On August 24, 2012, a sixty five year old man presented to the Ottawa Hospital Emergency Room with pancytopenia and progressive fatigue. Five units of red cells and one unit of pooled platelets were transfused over the course of a week. A diagnosis of myelodysplastic syndrome was made and the patient was discharged home on August 30. On September 1, after being home for one full day, he returned to the Emergency Room with fever, sore throat, nausea and neurological symptoms. The patient was transfused with more platelets and red cells. On September 12, the Ottawa Hospital reported a possible transfusion transmitted West Nile Virus infection. Canadian Blood Services was advised the recipient had tested positive for WNV IgM on September 7 and was confirmed to have WNV encephalitis.

Method
As the patient was at home for less than forty eight hours and did not report any mosquito bites during that time, the source of the West Nile Virus infection was suspected to have been from a transfusion. Five units of red cells and two units of pooled platelets (four donors each) were possibly associated with the West Nile Virus infection. All thirteen associated donors were contacted by Canadian Blood Services and requested to return for follow up testing. All of the donors complied with the request. Testing was performed at Canadian Blood Services for WNV NAT and serology (IgM and IgG).

Results
All donors tested negative for WNV NAT. Serological testing for WNV IgM and IgG identified one donor positive for IgG and two donors as IgG equivocal. All donors were IgM negative. The manufacturer of the antibody test kits subsequently recalled the IgG kits being used by Canadian Blood Services due to inaccurately high readings. Consequently, Canadian Blood Services retested the three donor samples (which had tested IgG positive or IgG equivocal) using antibody test kits with new lot numbers, supplied by the manufacturer. The three initially IgG reactive donors were found to be negative on retest. The three donor samples were also tested by the PHAC, National Microbiology Lab in Winnipeg and all IgG results were confirmed negative. One donor tested WNV IgM equivocal in the Winnipeg lab but was NAT and IgG negative. The donor who initially tested IgG positive and the one who tested IgG indeterminate (on the recalled test kits) were antibody negative on follow up samples three weeks after the initial test. The third donor was unavailable to provide a second sample for follow up antibody testing.

Conclusion
Although the source of WNV infection could not be identified, it is likely that the infection occurred as a result of mosquito borne transmission, despite the narrow window of opportunity. There is no laboratory evidence to support that this was a transfusion transmitted West Nile Virus infection.
20. Use of Intravenous immunoglobulin in Neurological Conditions

Clinical, Poster Presentation

Angela Genge MD, FRCP(C), Montreal Neurological Hospital

Introduction
This abstract reviews the uses of immunoglobulin across the spectrum neurological diseases. In addition it will include the range of doses and the schedules in use as well as the practical approach to determining the ideal dose frequency. Finally the outcome measure used to determine eventual long-term scheduling and discontinuation will be discussed.

Methods/Design
This is review of all patients who have received intravenous immunoglobulin for neurological conditions at the Montreal Neurological Institute, both as inpatients and outpatients for the last 12 months ending March 15, 2013. Currently over 50 percent of the intravenous immunoglobulin used at the McGill University Health Center is prescribed for neurological indications. The doses used are significantly higher than used in immunodeficiency indications. The longest period of time that any current patient has been receiving intravenous immunoglobulin at our institution is 23 years.

Results
There are 208 outpatients and 20 inpatients who have received intravenous immunoglobulin over the last 12 months in our institution. The diagnoses include NMDA encephalitis, rasmussen’s encephalitis, guillian barre syndrome, chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy, neuromyelitis optica, myasthenia, gravis, dermatomyositis, polymyositis, inclusion body myositis, polyarteritis nodosa, diabetic neuropathy/diabetic amyotrophy and necrotizing myositis secondary to statin use.

Modes of therapy presented in this review are exclusively intravenous. Doses range from 0.4 mg per kg per day to 1 gm per kg per day. Frequency of infusions range from 3-5 consecutive days to weekly to every 6 weeks.

The initiation of IVIG for all conditions in which it is used is based on the evidence in each condition that it is autoimmune in etiology. Further evidence supporting the use of IVIG is in the form of published case series essentially over the past 25 years. Initial dosing was determined arbitrarily to be 0.4 mg/kg per day in a study in Guillian Barre Syndrome. A subsequent series of studies in multifocal motor neuropathy have led to using an induction dose of 1g/kg/day for 3 days. In the neuromuscular condition strength assessment and functional deficits are used to guide frequency. In patients who are quadriplegic dosing starts at weekly up to 1g/kg/day. As specific goals are achieved in ambulation the frequency is reduced. IVlg alone does not induce remission in autoimmune neurological disease. However it returns patients to a normal level of functioning and maintenance therapy enables return to work.

The only neurological disease for which intravenous immunoglobulin is approved is chronic inflammatory demyelinating polyneuropathy.

Conclusions
IVlg is an extremely effective and important treatment for otherwise untreatable or poorly treated neurological conditions. Using various doses and frequencies IVlg is an important therapy in neurological diseases.
Pre-Transfusion Testing: Are we wasting our time?

Clinical, Poster Presentation

Chaturika Herath, Saskatoon Health Region
Karen L. Dallas, Saskatoon Health Region
Linda Frieson MLT, Saskatoon Health Region

Introduction
Pre-transfusion testing ensures ABO compatibility between donor and recipient and detects most clinically significant red cell alloantibodies that could react with donor red cells and cause a hemolytic transfusion reaction. According to current Saskatoon Health Region Guidelines, this testing must be performed ≤ 96 hours prior to the administration of red cells to determine ABO and Rh compatibility and, more importantly, whether new alloantibodies have developed. Over time, some clinical areas have adopted a practice of repeating testing every 96 hours regardless of whether or not a patient is being transfused, thus being “ready” just in case the patient does need a transfusion.

Objective
The objective of this study was to assess the significance and clinical utility of this every-96 hour group and screen practice on the Hematology-Oncology ward.

Method
All of the patients who had grouping and screening performed during the month of April 2012 were retrieved from the computer data base. Data on those who had red cell transfusions during this period was also retrieved.

Patients who had grouping and screening every 96 hours (regardless of plans to transfuse) and the others (i.e. patients who had pre-transfusion testing done less frequently) were categorized into Groups A and B, respectively. Transfusion trends among Groups A and B were assessed.

Results
Total of 51 patients had grouping and screening during the month of April. Three were excluded as they were admitted towards the last day of the month. Total of 47 patients were included in the study. Among 47 patients, 28 (59.6%) had every 96 hour grouping and screening (Group A). The rest (19/47, 40.4%) had infrequent grouping and screening (Group B) 11 out of 28 (39.2%) Group A patients had no transfusions. 12 out of 19 (63.1%) Group B patients had no transfusions. 42.9% of Group A patients had just one transfusion. Only 17.9% of Group A patients had more than one transfusion.

Conclusion
The every-96 hour group and screen practice – in anticipation of possible transfusion – does not appear to hold any value. It is likely increasing patient discomfort, leading to iatrogenic anaemia, and increasing the work load of nursing and laboratory staff. Certainly, it leads to significant resource wastage within our health region. Discussions are currently underway between the laboratory and the clinical team to change this practice.
An In-house Method to Assess T Activation in Paediatric Patients with Pneumococcal Hemolytic Uremic Syndrome

Clinical, Poster Presentation

Wendy Lau MD, The Hospital for Sick Children
Archana Bhatt MLT, The Hospital for Sick Children

Background
Hemolytic uremic syndrome (HUS) is often associated with Escherichia coli or Shigella infection, with a small proportion caused by Streptococcus pneumoniae. Neuraminidase released by Strep pneumoniae can lead to de-sialylation of red cell membrane glycoproteins exposing the cryptic Thomson-Friedenreich antigen (T-antigen), resulting in hemolytic anemia. The lectins for T activation testing are not routinely available in most transfusion laboratories, so we devised an in-house method to test for T activation when results are needed urgently.

Case Report
A four year old girl was admitted with Pneumococcal HUS. Her admission Hb was 86g/L, which dropped to 40g/L a few hours later. She was transfused with washed red cells. The Transfusion Service was asked to look for T-activation as her Partial Thromboplastin Time (PTT) was elevated and the clinician was contemplating plasma transfusion prior to line insertion for dialysis. Our transfusion service does not have the lectins to test for T activation, and this being Friday afternoon, the patient’s sample would not arrive at the reference laboratory until the following Monday.

Methods
Monoclonal antisera routinely used for ABO typing do not contain anti-T, so the patient’s red cells were tested against plasma samples from patients over 10 years of age, as well as against plasma samples from neonates.

Results
Patient is B positive, antibody screen negative, DAT negative.

<table>
<thead>
<tr>
<th>Patient cells</th>
<th>Monoclonal anti-A</th>
<th>Monoclonal anti-B</th>
<th>Plasma (Gp A)</th>
<th>Plasma (Gp B)</th>
<th>Plasma (Gp AB)</th>
<th>Plasma (Gp O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Prewarmed SIAT</td>
<td></td>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baby plasma (Gp A)</td>
<td></td>
<td>Baby plasma (Gp B)</td>
<td>Baby plasma (Gp AB)</td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td></td>
<td>0 to wk</td>
<td></td>
<td>wk</td>
<td>0 to wk</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions
These results suggest the presence of an IgM antibody, present in plasma of all ABO groups, reacting with patient’s red cells. Given the clinical history, this antibody is most likely anti-T, indicating the patient’s cells are T activated. It was recommended to the clinician that plasma transfusion be avoided if possible. Thus, on a Friday afternoon, this in-house test allowed us to make informed decisions about transfusion before T activation can be confirmed by a reference laboratory.
A Case of Drug-induced Hemolytic Anemia Due to Ceftriaxone in a Paediatric Patient

Clinical, Poster Presentation

Wendy Lau MD, The Hospital for Sick Children
Archana Bhatt MLT, The Hospital for Sick Children

Case Report
A six year boy with HbSC disease was transferred from another hospital with fever and upper respiratory tract infection. He was being treated with ceftriaxone. On admission, his Hb was baseline (123g/L), showing a slow decline over a few days with evidence of intravascular hemolysis. On the fourth day, a precipitous drop to 13g/L occurred, with loss of consciousness and grand mal seizures. He was transfused urgently but Ceftriaxone was continued for 2 more days before it was stopped. A few days later, the Blood Transfusion Laboratory was asked to look for evidence of ceftriaxone antibody.

Serological Testing
Patient is O positive with no history of clinically significant antibody. On admission, his antibody screen was negative, DAT positive with complement. The initial diagnosis was paroxysmal cold hemoglobulinuria, but the Donath-Landsteiner test was negative. When ceftriaxone-induced hemolytic anemia was suspected, we tested the eluate and the patient’s plasma by adding ceftriaxone in a 1:1 ratio.

Results

<table>
<thead>
<tr>
<th></th>
<th>12/25</th>
<th>12/30</th>
<th>01/01</th>
<th>01/02</th>
<th>01/04</th>
<th>01/05</th>
<th>01/09</th>
<th>01/12</th>
<th>01/15</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DAT (C3)</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>wk</td>
<td></td>
</tr>
<tr>
<td>DAT (IgG)</td>
<td>0</td>
<td>wk</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eluate</td>
<td>wk</td>
<td>wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eluate + Ceftriaxone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma + Ceftriaxone</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The test for ceftriaxone antibody was finally negative a month after admission. During this time, the patient received a total of 20 units of red cells, and underwent plasma exchange multiple times, using 132 plasma units in total.

Conclusions
On reviewing the patient’s history, it was discovered that he had been briefly DAT positive with anti-complement a year ago, when he was treated with ceftriaxone. This second exposure to ceftriaxone resulted in much more severe hemolysis. The hypoxemia has resulted in ischemic lesions and gangrene of his fingers and toes. Ceftriaxone is a commonly prescribed antibiotic for children with sickle cell disease. This case illustrates the severity of ceftriaxone-induced hemolytic anemia and the importance of early recognition and diagnosis.
24. **Two Episodes of Anaphylactoid/Anaphylactic Reactions in One Paediatric Patient Related to Food Allergies**

Clinical, Poster Presentation

Wendy Lau MD, The Hospital for Sick Children
Kathleen McShane MLT, The Hospital for Sick Children
Mary Huang RN, Canadian Blood Services

**Case Report**

A nine year boy was diagnosed with medulloblastoma in March, and was treated with chemotherapy and tandem autologous stem cell transplants in Aug, Sept and Oct. On Nov 12, he ate salmon at home and 5 minutes later started coughing and complaining of foreign body in throat and swollen lip. On arrival in Emergency, he was hypotensive and tachycardic, but was conscious and complained of a bone stuck in his throat. He responded to treatment with benadryl. Immunology recommended radioallergosorbent test (RAST), avoid salmon and vertebrate fish, and carry an EpiPen. On Nov 16, while at home, he ate one Reese peanut butter cup and within 10 minutes had vomiting and swelling of his top lip. He did not have an EpiPen yet, so he went to Emergency and was treated with benadryl. On Nov 18, he was admitted for his fourth transplant. Skin tests showed strong reactions to mixed fish, salmon, peanut and tree nut mix. The patient had no known allergies to fish or nuts, and had been eating these foods without incident until his last transplant. As it is highly unusual to develop new allergies at this age, we reported the reactions to the blood centre to investigate the blood donors involved. Since Nov 1, he had received one red cell unit, 2 platelet pools and two apheresis platelet units. The red cell unit, which contained little plasma, was not a concern, so we started with the apheresis donors, who denied any allergies. Then the male donors who provided the plasma for the platelet pools were interviewed; one donor had no allergies, the other reported allergies to shellfish and fish and all nuts including peanuts. This platelet pool was transfused on Nov 3. The patient’s RAST subsequently came back positive for both salmon and peanut.

**Conclusion**

Two episodes of anaphylactoid/anaphylactic reactions, after eating different foods, occurred 9 and 13 days after one transfusion from a donor with allergies to the same foods. The most likely cause is passive transfer of IgE antibodies from donor to patient. This donor has been deferred from future donations.
25. **Red Cell Recycling: An Update**

Clinical, Poster Presentation

Tanya McKeIvey MLT, BSc (MLS), Alberta Health Services
Gwen Clarke MD, FRCPC, University of Alberta and Alberta Health Services
Susan Nahiriak MD, FRCPC, University of Alberta and Alberta Health Services

**Background**
In April 2003, a red blood cell redistribution system was implemented that allowed recycling of near outdate red cell units to the University of Alberta Hospital (UAH) Transfusion Medicine laboratory. In the first year of the program, 4 rural sites from Northern Alberta recycled a total of 138 units. These four sites had success rates (defined as a unit being acceptable for inventory entry at the UAH) ranging from 59%-78%, with an overall success rate of 69%. Of the units accepted into inventory, 89% were group O. The majority of the failures were temperature failures (49%), tamper evident seal missing/broken (28%), and other (23%). This prompted re-education of participating sites with re-evaluation of packing configurations and standardization of requirements allowing an additional 2 sites to come on board in each of the third and fourth year of the program.

**Results**
By the end of 2012, a total of 9 sites participated in the program. In 2012, a total of 950 units were returned. The success rate for these sites ranged from 69%-100% with an average rate of 92%. The average age of the units on receipt was 29 days. Of the units considered acceptable for inventory 67% were group O, 98% were utilized and only 0.2% were outdated (≤ 1 day from outdate upon receipt). The majority of failures were temperature failures (28%), data logger failures (23%), transport time > 24 hours (16%), and other miscellaneous problems (35.1%).

**Conclusion**
Although we have increased the number of units recycled by 688% and more than doubled the number of participating sites, up to 8% of recycled units are still being discarded because they are not acceptable for inventory. Root cause analysis and attention must be given as to how we can improve this process further so we can maximize the utility of these resources, especially as we have recently expanded the program to accept redistribution of plasma protein products and have been approached to expand to additional sites.
Troubleshooting the Automated Blood Bank Analyzer

Clinical, Poster Presentation

Tanya McKelvey MLT, BSc (MLS), Alberta Health Services
Gwen Clarke MD, FRCPC, University of Alberta and Alberta Health Services
Susan Nahiriak MD, FRCPC, University of Alberta and Alberta Health Services

Background
In June 2009, automation was introduced in Edmonton Transfusion Medicine laboratories. Effective troubleshooting of automation breakdowns is critical to manage loss of functionality and tech time during downtime. Automated Blood Bank analyzers contain numerous interconnected hardware modules that must be running at peak performance to achieve accurate test results. Identification of components with recurring breakdowns and exploration of the root cause can highlight areas where preventative maintenance not addressed by the manufacturer would be of benefit.

Results/Case Study
Over the past 4 years, the fluidics module has been identified by “primary operators” (technologists with advanced training) as a recurring source of breakdowns. The most common breakdowns are 1) blocked probe rinse station, 2) blocked wash station, 3) sensor degradation in the saline and waste containers, and 4) corrosion of fluidics hardware including the peristaltic pump. Extensive troubleshooting of these issues has provided experience based training towards efficient troubleshooting. For example, leakage from the probe rinse station is often due to overflow from a blocked filter. Increased equivocal results can be due to saline crystallization in the wash station. Quick identification of such blockages translates to downtime resolution in minutes versus > 24 hours for a service call and in the latter case, immediate result quality improvement. Efficient identification of breakdowns that require a service call, like degraded sensors or a corroded peristaltic pump, can further save valuable tech time otherwise spent on excessive and unnecessary troubleshooting. All these experiences have highlighted easy to perform preventative maintenance tasks not otherwise recommended or performed by the manufacturer which include 1) internal cleaning of the probe rinse station semi-annually, 2) checking and, if necessary, replacing the filter in the probe rinse station quarterly, and 3) in depth cleaning and dusting of the instrument monthly.

Conclusions
Identification and efficient troubleshooting of problematic hardware components, and developing preventative maintenance plans that exceed the manufacturer recommendations can reduce loss of functionality to downtime, save tech time and improve result quality. Advanced training is currently limited to “primary operators” but should be expanded to include all frontline staff to maximize troubleshooting potential in the lab.


27. **Streamlining and Process Improvement in the Transfusion Medicine Laboratory**

Clinical, Poster Presentation

Tanya McKelvey MLT, BSc (MLS), Alberta Health Services
Gwen Clarke MD, FRCPC, University of Alberta and Alberta Health Services
Hilda Gaal, Alberta Health Services
Angela Goulard, Alberta Health Services
Susan Nahiriak MD, FRCPC, University of Alberta and Alberta Health Services
Amanada Oleksy, Alberta Health Services

**Background**
With current fiscal constraints in healthcare, the need for efficiency and streamlining of processes is critical to manage operational costs. To promote staff engagement in streamlining of processes, select frontline staff were offered the opportunity to participate in workshops and training modules focused on identifying waste and streamlining processes in the workplace.

**Process**
A Six Sigma Yellow Belt project to reduce inefficiencies and duplication of work within the reagent entry process was performed between November 2011 and February 2012. By defining opportunities and applying the basic streamlining principles of eight wastes identification (with emphasis on duplication of work), value stream maps, and change management to the reagent entry process three “quick wins” were identified. The quick wins were: 1) better standardize the procedure for reagent entry; 2) eliminate the need for manual documentation in a circular binder; and 3) create a database to track reagent discards. These small but effective changes to the process decreased the average time for reagent entry by 19.3% within just 2 months. In tech time savings alone this translates to 7 seconds and 8 cents per vial entered or an estimated 15 hours and $600 annually. In addition, numerous other process improvements were identified that, given time and educational investment, could be implemented to further streamline this process and provide further cost savings. These included creation of a database for complete electronic data capture, elimination of paper records, elimination of in-house barcodes for reagents, and moving to regional reagent ordering to maximize discounts and minimize discards. In staff time alone this translates to an estimated minimum 26% further reduction in the average time for reagent entry or 4 cents per vial entered and $480 annually.

**Conclusion**
Streamlining of even the simplest processes can translate to sizeable cost savings and efficiencies when human and financial resources are significantly limited. However, staff need to be trained in the identification of waste and improving inefficiencies at the front line level.
Clinical, Poster Presentation

Roya Mojarab MLT, Mount Sinai Hospital
Nadine Shehata MD, Mount Sinai Hospital

The aim of study is to describe the utilization and effectiveness of platelet transfusion at a tertiary care center.

Methods
We reviewed all transfusion requisitions, which were received by Transfusion Medicine Service over period of six months. The transfusion requisitions were used to extract patient diagnosis and platelet transfusion data. Pre and post transfusion platelet counts were derived from the hospital’s Laboratory Information System. The numbers are the average of the result over one stay.

Results
Within the 6-month period 54 patients were transfused 316 units of platelets.

Platelet products were single donor, random donor, or HLA matched platelets. Among the 54 patients, 53% were male and 47% were female. The median age was 46 years. The number of units transfused ranged from 1-51 units.

The median hospital duration of 55 days, the numbers of transfused units to one patient were: minimum of one and the highest of 51 units for 1 patient.

The pre and post transfusion platelet counts are described in the table below.

<table>
<thead>
<tr>
<th>Clinical condition (Adult)</th>
<th>Mean Pre-transfusion count</th>
<th>Mean Post-transfusion count</th>
<th>Number of Patients</th>
<th>Number of units transfused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia / Lymphoma</td>
<td>8.5 x10^9/L</td>
<td>27 x10^9/L</td>
<td>14</td>
<td>111</td>
</tr>
<tr>
<td>Bone Marrow Transplant</td>
<td>12 x10^9/L</td>
<td>26.4 x10^9/L</td>
<td>7</td>
<td>81</td>
</tr>
<tr>
<td>Hematology / oncology</td>
<td>20 x10^9/L</td>
<td>48 x10^9/L</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Surgery / bleeding / Massive transfusion</td>
<td>39 x10^9/L</td>
<td>98 x10^9/L</td>
<td>12</td>
<td>55</td>
</tr>
<tr>
<td>Other: Critically Ill</td>
<td>29 x10^9/L</td>
<td>74 x10^9/L</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td>Immune-mediated</td>
<td>13 x10^9/L</td>
<td>43 x10^9/L</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neonatal transfusion</th>
<th>Pre-transfusion count</th>
<th>Post-transfusion count</th>
<th>Patient Numbers</th>
<th>Transfused platelet time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected sepsis / Live birth</td>
<td>14 x10^9/L</td>
<td>59 x10^9/L</td>
<td>7</td>
<td>20</td>
</tr>
</tbody>
</table>
Transfusion Protocol to Support Massively Bleeding Patients: Descriptive outcomes based on blood components transfused

Clinical, Poster Presentation

Doris Neurath ART, MBA, EORLA, The Ottawa Hospital
Ayman Kafal PhD, CSL Behring Canada
Melanie Tokessy MLT, EORLA, The Ottawa Hospital
Nancy Cober ART, EORLA, The Ottawa Hospital
Shauna Love MLT, EORLA, The Ottawa Hospital
Antonio Giulivi MD, The Ottawa Hospital

Background
Massive bleeding requiring multiple blood product transfusions during medical interventions is associated with high mortality rates. Our purpose was to evaluate mortality and blood product utilization following implementation of the massive transfusion protocol.

Methods
Transfusion data was extracted from the information system at a Canadian tertiary care facility. Retrospective data was collected over 5 months on patients who had experienced massive bleeds during medical interventions and had received over 5 units of red blood cells (RBC) in combination with other blood components according to the hospital massive transfusion protocol.

Results
113 patients were selected in our study. 92 patients (83%) were undergoing surgical procedures, mainly cardiac surgery (46%). Half of our sample (54%) needed more than 8 RBC units. 21 patients (20%) were transfused with only one additional blood component (either frozen plasma (FP) or platelets) as well as RBC. 24 patients (21%) received both frozen plasma and platelets in addition to RBC. 48 patients (42%) received a combination of frozen plasma, platelets and cryoprecipitate and 18 patients (16%) required additional products such as recombinant factor VIIa (rFVIIa). Ratio of FP to RBC used was 1:1.5 and an average of 20 units of cryoprecipitate was required. Overall mortality was 18.5%.

Summary / Conclusions
With increased awareness of blood transfusion indications and the appropriate combination of blood components transfused we have seen improvement in blood utilization. Our data confer an advantage to transfusing a combination of blood components according to a systematic well defined protocol. Further research is needed to explore the influence of the sequence of blood components transfused on clinical outcomes of massively bleeding patients.
Transfusion-Related Acute Lung Injury Following Intravenous Immune Globulin Transfusion: A Case Report

Clinical, Oral Presentation

Graeme Quest MD, MSc, Alberta Health Services and University of Alberta
Hilda Gaal MLT, Alberta Health Services
Gwen Clarke MD, FRCP, University of Alberta and Alberta Health Services
Susan Nahiriak MD, FRCP, University of Alberta and Alberta Health Services

Background
Transfusion Related Acute Lung Injury (TRALI) is a severe adverse transfusion related event that typically occurs within 6 hours of the infusion of plasma containing products. Intravenous immune globulin (IVIG) is prepared from large pools of human plasma and therefore may also cause TRALI, though reports of this are scarce.

Case Report
A 77 year old female with common variable immune deficiency (CVID) has been receiving IVIG since 1996. The patient had previously experienced transfusion reactions to two IVIG products, Gamimune and Ivecagam, and is known to have anti-IgA antibodies. Her current IVIG therapy for CVID, since September 2011, includes transfusion of 40g Gammagard S/D monthly, with acetaminophen and benedryl premedication. This therapy had been without incident until November of 2011. The infusion was started at 0840h and completed at 1605h. The patient experienced nausea with vomiting at 1430h, followed by dyspnea with decreasing oxygen saturations at 1605h. The patient's blood pressure increased as oxygen saturations continued to fall by 1635h. The patient was given 8 liters oxygen, but continued to deteriorate requiring intubation and admission to the intensive care unit (ICU). Post-reaction chest x-rays demonstrated the new appearance of bilateral hazy groundglass opacifications with previously documented changes including and related to bronchiectasis, with the resolution of the opacities within 48 hours. Measurement of BNP following the reaction was 72 pg/mL (less than 100 pg/mL interpreted as heart failure unlikely). Trans-thoracic echocardiography demonstrated a Left Ventricular Ejection Fraction of 45-50%. Serological investigations on the day of the reaction showed her to be group B Rh positive with a negative antibody screen and a negative DAT. The patient was extubated within 24 hours, transferred from the ICU within five days, and discharged from hospital within eight days of the reaction. The reaction was classified as a TRALI and reported to Health Canada. The patient had not received this particular lot number of product before, and has since received 5 infusions of Gammagard S/D with different lot numbers without reaction.

Conclusion
This case report documents a case of TRALI following Gammagard S/D transfusion, confirming that TRALI can occur following IVIG infusion.
The Prophylactic Use of C1 Esterase Inhibitor (Berinert®) in HAE Patients Undergoing Invasive Procedures

Clinical, Poster Presentation

Stephanie Santucci RN, Yang Medicine Professional Corp
Genevieve Gavigan MASc, MD, University of Ottawa
Rachel Harrison BAScH, Yang Medicine
William H. Yang MD, FRCPC, FAAAAI, University of Ottawa and Yang Medicine

Rationale
For a patient with Hereditary Angioedema (HAE), physiological and/or psychological stress can cause insufficient control of local inflammatory pathways. This leads to complement and contact system activation and excess bradykinin resulting in angioedema. Therefore, an invasive procedure or surgery can trigger an HAE attack; this in turn can cause further medical complications and pose an added danger to the post-procedure patient. C1 inhibitor, Berinert®, was approved in the US and Canada in 2009 and 2010, respectively, for the treatment of acute attack. However, there is no approved indication for its prophylactic use for HAE attacks. We aim to demonstrate the effectiveness of C1 esterase inhibitor, Berinert®, as a prophylactic treatment for HAE patients undergoing invasive procedures.

Method
We performed a retrospective chart review from our Canadian Tertiary Care Allergy and Asthma Clinic of our entire database of HAE patients.

Results
Between 1997 and 2012, we administered C1 esterase inhibitor (Berinert®) for prophylactic use prior to invasive procedures. There were a total of 22 procedures, performed on 11 patients.

The 22 procedures breakdown as follows:
- 7 dental surgeries
- 4 open heart surgeries (3 coronary aneurysms, 1 heart valve replacement)
- 5 other surgical procedures (1 Melanoma in situ, 1 liver biopsy, 1 bronchial cyst, 1 hemia, 1 hysterectomy)
- 1 child birth
- 5 invasive procedures (2 Port-a-cath placements, 2 angiograms, 1 Hickman placement)

At the time of their respective procedures, 10 of these patients were adults, and one was a teenager.

In all 22 procedures, there was no incidence of post-procedure HAE attacks after prophylactic administration of C1 esterase inhibitor (Berinert®).

Conclusions
We found that C1 esterase inhibitor (Berinert®) decreased the incidence of post-procedure HAE attacks and was an effective prophylactic treatment.
The Interpretation of Crossmatch-to-Transfusion Ratio in Hospitals with Electronic Crossmatch

Clinical, Poster Presentation

Lawrence Sham MLT, Vancouver General Hospital
David Pi MD, FRCPC, Vancouver Coastal Health
Brian Berry MD, FRCPC, Vancouver Island Health Authority
Jason Pal, Vancouver General Hospital
Kate Chipperfield MD, FRCPC, Vancouver Coastal Health
Teresa Sharp MLT, Richmond Hospital

Background
Traditionally, Crossmatch/Transfusion Ratio (C/T Ratio) is considered a useful index to assess the appropriateness of blood reserve in inventory for patients with serological crossmatch or patients with anticipatory transfusion needs in surgical or other clinical specialties. A major benefit of electronic crossmatching (EXM) is the expediency in blood order assignment, resulting in reduced and more flexible allocation of blood units to patients. In EXM, the computer generates a global (G)-C/T Ratio, based on the standardized process requiring a common entry to assigned inventory for all blood units (Crossmatch) prior to the final issue for transfusions (T). The objectives of this study are to explore the relationship of G-C/T Ratio and specialty-specific (SS)-C/T Ratio, and to evaluate the prevalent SS-C/T Ratios in hospitals using EXM in Greater Vancouver and Vancouver Island, British Columbia.

Method
The reasons for crossmatches were categorized into serological crossmatch, and specific types of surgical procedure, clinical diagnosis and patient location. We use a manual and/or a mathematically derived method (based on lead time analysis) to determine the blood units in the assigned inventory in the study period.

Results
Preliminary data from 2/7 hospitals analyzed to-date are presented in the abstract - Hospital A and B: The volume breakdown (%) and SS-C/T ratio respectively – Hospital A: Open heart surgery (7%, 1.98), other surgeries (21%, 2.47), gastrointestinal (GI) bleeds (2%, 1.14), medical inpatients (56%, 1.36), and outpatients (14%, 1.26). Hospital B: Obstetrics (6%, 6.00), other surgeries (7%, 3.75), GI bleeds (13%, 1.08), medical inpatients (56%, 1.36), and outpatients (16%, 1.18). Total SS-C/T Ratio and G-C/T Ratio: Hospital A: 2.62 and 1.51, Hospital B: 2.42 and 1.43. A linear correlation exists between SS-C/T Ratio and G-C/T Ratio; the slope of this equation is determined by the blood return rate and the proportion of transfusion in the assigned inventory (T1) over T.

Conclusion
In hospitals with electronic crossmatch, the significance of G-C/T ratio, conveniently generated by blood bank computers, need to be understood. G-C/T Ratio can be used as a good screening method to study the appropriateness of blood order assignment practices.
Case
A 28-year old group B Black woman was referred to our Immunohematology Laboratory (IRL) in October 2012 for red blood cell antibody identification (G6P2A3). Her serum reacted strongly on every panel cell tested. This patient was first seen in September 2001 to confirm her D antigen status. The DNA-based genotype assay done at Héma-Québec at the time concluded for a DVI type 1 variant. No alloantibodies were detected at the time.

Serology results
The 2012 sample was looked at closely the IRL. Since the patient received no transfusion three months prior, her phenotype was done by approved serology techniques: C-c+E-e+, K-, M-N+S-s+, Fy(a-b-), Jk(a+b-). Again her serum reacted strongly on all panel cells (3+). Autocontrol and DAT were negative. Alloabsorptions demonstrated the presence of anti-e and anti-f. Rare red blood cells were targeted for screening. Three hrB+w/- cells gave weak reactions in gel-LISS, while three others gave 11/2-2+ strength. Two hrS- cells were negative in gel-LISS and gel-papain, and two hrB-hrS+ cells were negative in gel-LISS and positive in gel-papain. Molecular biology results: RH messenger RNA were amplified and sequenced. The previous DNA-based assay predicted a DVI type 1 variant. However, the RHD sequencing results indicated a RHD*DAR variant (polymorphisms in exons 4, 5 and 7). The RHCE results gave a homozygous RHCE*ceAR variant often linked to RHD*DAR variant. This RHCE variant presents a variation in the following antigen expression: C-, partial c, E-, partial e, VS-V+ and hrs-.

Conclusion
This patient was notified that she has rare blood and was advised to give autologous donations. No compatible blood was found yet, despite the thorough screening of our self-identified Black donors.
Clinical, Poster Presentation

Maryse St-Louis PhD, Héma-Québec
Élaine Deschênes MSc, Héma-Québec
Josée Perreault PhD, Héma-Québec
Carole Éthier MT, Héma-Québec
Julie Paquet MT, Héma-Québec
Vicky Beauchesne MT, Héma-Québec
Josée Perreault PhD, Héma-Québec
Carole Éthier MT, Héma-Québec
Vicky Beauchesne MT, Héma-Québec
Jessica Constanzo-Yanez BSc, Héma-Québec

Case
An 80-year old man with a myeloproliferative syndrome was first referred to our Immunohematology Laboratory in 2006. At the time, his blood group was phenotyped several times D+ and genotyped C+E-c+e+. An anti-Jka was identified in his serum. In 2012, more samples from the same patient were sent, because a discrepancy was observed at the hospital. The patient was phenotyped D-C-E-c+e+ on a pretransfusion sample. These new samples were examined more closely by the Research and Development group. Methods: To avoid samples mixed-up and mislabeling, three samples collected at different times were received from the hospital. RHD and RHCE mRNA were amplified. Resulting cDNA was sequenced and compared to a normal sequence. RHD and RHCE DNA genotype were also performed to confirm the previous 2006 results.

Results
No sequence was possible for the RHD mRNA/cDNA. No amplification was observed with two RNA prepared from two different samples. However, the RHCE mRNA/cDNA sequence gave a RHCE*ce/RHCE*ce result. As for the DNA based assay, results indicated a very weak amplification for the RHD, much weaker than the one observed with control DNA. The RHCE assay predicted a C+c+ phenotype.

Discussion
According to the DNA results, this patient should be D+C+c+, even if the RHD amplification was very weak. On the other hand, mRNA/cDNA sequencing results indicated a D-C+c+ result. Myelodysplastic syndrome was previously responsible for an ABO discrepancy. No reports could be found involving for RH antigens as shown here. We believe that methylation could be the reason why some mRNA are not expressed during the onset of the disease. Why the RHCE*C allele is silenced and not the RHCE*c? It could be due to the evolution of the RH genes, the RHCE*ce being older and less sensitive to modification. More work is needed to elucidate this interesting case.
35. **Major Red Blood Cell Antigens in Self-Identified Black Blood Donors in Quebec**

Clinical, Poster Presentation

Maryse St-Louis PhD, Héma-Québec  
Jessica Constanzo-Yanez BSC, Héma-Québec  
Carole Éthier MT, Héma-Québec  
Josée Lavoie MSc, Héma-Québec  
Élaine Deschênes MSc, Héma-Québec  
Josée Perreault PhD, Héma-Québec

**Background**
The demand for extensively phenotyped red blood cells is on the rise, especially for sickle cell patients. To better answer their needs, Héma-Québec put forward great efforts to increase the recruitment of blood donors among the diverse cultural communities. Blood drives were organized in these communities, along with other activities to aware them of the importance of blood donations.

**Study Design and Methods**
A section was added on the record of donation filled out by the donors to indicate their ethnic background. Self-identified Black donors were extensively phenotyped by the Immunohematology Laboratory. The most interesting ones were referred to the Research and Development to complete the genotype/phenotype picture to identify rare donors.

**Results**
After four years, close to 1500 self-identified Black donors have donated blood at least once. Among the 1058 Black donors tested in R&D, 15 S-s-, 3 U-, 12 U+var, 10 Js(a+b-), 2 Hy- and 3 Jo(a-) were found. Variants in the RHCE were also identified: 30 hrB+w/- and 8 hrB-. Conclusion: These donors are precious to the patient cohort depending on blood transfusions and to our organisation as blood provider for the whole province of Quebec.
The Kidd blood group includes the antithetical Jka and Jkb antigens to which alloantibodies are made in ~ 1/3rd of severe delayed hemolytic transfusion reactions (DHTRs). The high incidence of anti-Kidd antibody-related DHTRs is due in part to their high immunogenicity, difficulty in detection (low titres, dosage effects), rapid evanescence and strong anamnestic responses. Given their potent hemolytic potential, identifying anti-Jk antibodies is of clinical importance. Here we describe a delayed serologic transfusion reaction (DSTR) to Jka in a 34 year old man with no transfusion history until receipt of 4 units of Jka+ RBCs amid extracorporeal membrane oxygenation (ECMO) for RSV-associated respiratory failure. For logistical reasons, his first three screening samples were analyzed by Ortho Diagnostics agglutination-based gel columns, and the fourth by solid-phase Capture® technology on the Immucor NEO. Although the 1st three screens were negative, anti-Jka was detected and confirmed on the fourth (2-3+), while remaining undetectable by gel columns even after enzyme treatment of double-dose Jka screening cells. Two weeks later, his anti-Jka response had increased to 3-4+ by Capture® but still remained negative by gel. To determine if anti-Jka could have been identified earlier, we re-analyzed his first 3 screens using solid-phase technology. While no anti-Jka was found by Capture® prior to receiving blood products, anti-Jka IgG became detectable just 3 days after presumed first exposure to Jka+ RBCs, in keeping more with memory response than primary alloimmunization. This discordance between the Jka antibody kinetics and the transfusion history may be explained by maternal-fetal exposure to Jka or a naturally-occurring anti-Jka, neither of which could be definitively ruled out. The septic recipient context around transfusion may have also contributed. Fortunately, there were no clinical sequelae and his investigation for hemolysis was negative by the time of DSTR recognition. He recovered from his pneumonia and was made aware of his anti-Jka serology through a multi-lingual “antibody” card detailing his alloantibody status and future blood product requirements. Although serious clinical consequences were avoided, this case highlights difficulties encountered with the Kidd blood group and technical properties of anti-Jka antibodies that can make their identification method dependent.
37. **Loss of Serologically Detectable B Antigen in a Chronically Transfused Child with Thalassemia Major**

Clinical, Oral Presentation

Elona Turley MD, University of Alberta  
Hilda Gaal MLT, Alberta Health Services  
Suzanne Miller MLT, Alberta Health Services  
Gwen Clarke MD, FRCPC, University of Alberta and Alberta Health Services  
Susan Nahirniak MD, FRCPC, University of Alberta and Alberta Health Services

**Introduction**

Lack of expected reactivity on forward ABO typing can be associated with weak antigen subgroups, chimerism, non-group matched transfusion, and malignancy. In this case, a group AB Rh positive child with thalassemia major lost anti-B, but maintained anti-A forward reactivity. This patient’s chronic transfusion dependence complicated our investigations, and both clinical and laboratory information was important in the workup of his ABO discrepancy. This case is remarkable in that, despite our workup, the etiology of his apparent acquired loss of B antigen remains unclear. A literature review did not reveal an association between ABH antigen loss and disorders of hemoglobin or chronic transfusion.

**Case description**

A 6-year-old male with transfusion dependence due to thalassemia major, previously determined to be group AB+, was found to have lost his previous and expected anti-B reactivity on forward typing by solid phase and manual tube methods. Transfusion history revealed the patient received RBCs every three weeks with the RBC group depending on local inventory, and the last A+, B+, and O+ units transfused 21, 104, and 35 days previously, respectively. The absent anti-B despite a strong anti-A reactivity persisted even after transfusions were changed to group O RBCs only, and adequate time for clearance of transfused non-group O cells had passed. Absence of anti-B reactivity was confirmed on repeat testing with washed cells, and on all subsequent collections. There was no history of transplant or immunosuppressive medications, and no clinical or laboratory findings suspicious for hematologic malignancy, hence bone marrow investigation was not performed. Papain proteolytic enzyme treatment of patient cells collected 161 days after the last non-group O transfusion did not result in detectable agglutination with either of two different anti-B reagents (ImmuCor and BioRad). Patient genotype was confirmed to be AB.

**Discussion**

This is an unusual case of acquired loss of expected anti-B reactivity in a thalassemic child, and highlights the challenge of investigating transfusion-dependent patients as well as the importance of both clinical and laboratory information in the workup to ABO discrepancies.
Background
Hemoglobin (Hb) triggers for transfusion in patients with acute gastrointestinal (GI) bleeds have been a contentious issue. The authors of a recently published randomized control trial found that patients with acute upper GI bleeding assigned to a restrictive transfusion strategy (transfusion trigger Hb 70g/L; post-transfusion Hb target 70-90g/L) experienced significantly improved outcomes when compared to patients assigned to a liberal strategy (trigger Hb 90g/L; post-transfusion Hb target 90-110g/L). We sought to retrospectively evaluate local transfusion practices in GI bleed patients with massive hemorrhage protocol (MHP) activations as part of a quality assurance project.

Methods
Local patients with MHP activations for GI bleeds from January 2011 to June 2012 were identified. Following the above study, the pre-MHP and post-MHP Hb concentrations were obtained, and the patients grouped according to pre-MHP Hb concentration.

Results
Of the 78 patients with MHP activations for GI bleeding in the study period, 51 had pre-MHP and post-MHP Hb measured. Pre-MHP Hb was ≤70g/L in 25 patients (49%), >70g/L but <90g/L in 16 patients (31%), and ≥90g/L in 11 patients (22%). The average pre- and post-MHP Hb in the ≤70g/L group was 50.2g/L (s=12.2g/L), and 91.2g/L (s=19.1g/L), respectively. The average pre-MHP Hb in the ≥90g/L group was 105.0g/L (s=9.5g/L) with an average post-MHP Hb of 98.5g/L (s=22.7g/L). The >70g/L but <90g/L group had an average pre-MHP Hb of 79.9g/L (s=7.8g/L) and average post-MHP Hb of 82.6g/L (s=26.4g/L).

Conclusion
Our review suggests that there is no currently defined local strategy with respect to Hb triggers and transfusion targets in patients with MHP activations for GI bleeding. Future educational initiatives may assist with practice changes to a more restrictive strategy given recent literature indicating the benefit of such an approach in these patients.
39. Validation of 5 day post thaw Octaplasma®

Clinical, Poster Presentation

Carolyn Villeneuve, University of Alberta
Hilda Gaal hilda.gaal@albertahealthservices.ca, University of Alberta
Linda Stang, University of Alberta
Gwen Clarke MD, FRCPC, University of Alberta
Susan Nahiriak MD, FRCPC, University of Alberta

Background
Octaplasma®, a solvent/detergent plasma product has been approved for use in Canada. The manufacturer states an expiry of 8 hours (h) post thaw; stored at 4°C. As Octaplasma® is a pooled plasma product, and fresh frozen plasma has been validated for use up to 5 days post thaw, we hypothesized that Octaplasma® could also be used up to 5 days post thaw when stored at 4-6°C. The purpose of this study was to determine the integrity of Octaplasma® 5 days post-thaw. If clinically significant changes to clotting factor levels were not seen, extension of the expiration from 8h to 120h could be considered.

Methods
Thirty eight (38) units of European source Octaplasma® were thawed at 37°C then kept at 4-6°C for 5 days. Aliquots for testing were made at time 0, 24, 48, and 120h and frozen at -70°C. Levels of coagulation Factors V, VII, and VIII were performed on the STA-R Evolution analyzer. Testing was initially performed on 0h and 120h aliquots with follow-up at 48 and 24h if 120h aliquots showed levels < 0.50 IU/mL.

Results
Although Factors VII and V decreased on average by 11.9 % from baseline during the 120h storage, all aliquots had levels above 0.50 IU/mL. Factor VIII levels were less than 0.50 IU/mL (averaging 0.41 IU/mL) at time 0 and were reduced further at 120h averaging 16.5 % decrease from baseline.

Conclusion
The reduction of factors V and VII levels were not detrimental to the integrity of the product and are sufficient for transfusion. Factor VIII levels were severely reduced; but this is not due to degradation during storage as minimal levels were present at the time of thawing. Reduced Factor VIII levels are not a concern since usually the levels of Factor VII are sufficient to initiate the clotting cascade through the extrinsic pathway and Factor VIII typically increases in vivo as an acute phase reactant. The diminished VIII levels may not represent a clinically significant problem. Octaplasma® should be effective for treatments outlined by Canadian Blood Services for up to 5 days post thaw if stored at 4-6°C.
40. Auto-anti Rh(D) in a patient with severe sepsis

Clinical, Poster Presentation

Di Cindy Wu BSc, MLT, Surrey Memorial Hospital
Lina Kitani BSc, MLT, Surrey Memorial Hospital
Chui Shan Khan BSc, MLT, Surrey Memorial Hospital
Taralynn Le Clair MLT, Surrey Memorial Hospital
Lucia Wojtyniak MLT, Surrey Memorial Hospital
Samuel Krikler MBChB, FRCPC, Surrey Memorial Hospital and University of British Columbia

We describe a 39 year old man with "spontaneous" bacterial peritonitis who developed a strongly positive DAT (IgG 3+, C3d 1+) concomitant with his sepsis. The patient has a background history of cerebral palsy with multiple medical problems. He had been transfused previously and an allo-anti E was present in his plasma. His most probable Rh genotype is CDe/cde. An eluate prepared from the patient's washed red cells showed antibodies with a pattern consistent with anti-D and anti-E. Similar antibodies were identified in the plasma. The last wash recovered from the eluate also demonstrated anti-E, which appears to be an example of the Matuhasi-Ogata phenomenon since his red cells lack the E antigen. Our serologic findings were confirmed by CBS (Vancouver) and a sample has been referred to the National Reference Laboratory for Rh(D) genotyping by molecular (DNA-based) techniques. Auto-antibodies with anti-D specificity are rarely described and must be distinguished from allo-antibodies to epitopes within the D antigen. This patient showed no demonstrable reduction in red cell survival and there was no clinical or laboratory evidence of a generalized auto-immune disorder such as SLE. The positive DAT preceded anti-microbial therapy and does not appear to be drug-related. Rh(D) negative units were selected for transfusion.
41. Inhibition of Fc Receptor-mediated Phagocytosis by Anti-CD44 Antibodies: A possible mechanism for amelioration of ITP

Scientific, Oral Presentation

Alaa Amash PhD, Canadian Blood Services and the Li Ka Shing Knowledge Institute of St. Michael's Hospital
Alan Lazarus PhD, Canadian Blood Services and the Li Ka Shing Knowledge Institute of St. Michael's Hospital

Introduction
We have recently shown that anti-CD44 antibodies can mimic the effect of IVIg in ameliorating immune thrombocytopenia (ITP) in mice (Crow AR, Blood, 2011). However, the mechanism underlying this therapeutic effect is unclear. Here we directly evaluated the ability of anti-CD44 antibodies to inhibit Fc receptor mediated-phagocytosis in macrophages.

Objective
To examine the potential effect of anti-CD44 antibodies on Fc-receptor mediated-phagocytosis in macrophages.

Study Design
To first assess the capacity of the anti-CD44 antibody (KM114) to bind to different phagocytic cell types, leukocytes from peripheral blood, spleen and bone marrow, as well as red blood cells and platelets from C57BL/6 mice were isolated and stained with KM114 and analyzed by flow cytometry. To examine the potential effect of KM114 on phagocytosis, primary murine peritoneal macrophages (PerMac); pretreated with KM114 or an isotype control IgG, were used as phagocytic cells while sheep red blood cells (sRBCs) labeled with CM-Green™ and opsonized with rabbit anti-sRBCs IgG were used as targets. After different incubation times, unbound sRBCs were washed away and the PerMac cells were fixed and stained with AlexaFluor633-goat anti-rabbit F(ab’)2 to detect bound vs internalized sRBCs. Evaluation of phagocytosis was done by flow cytometry and by confocal microscopy.

Results and Discussion
CD44 antibody bound well to leukocytes from bone marrow, spleen and peripheral blood, but not to RBCs. Interestingly, the antibody bound at the highest levels to granulocytes and macrophages/monocytes from these tissues. These data suggest that granulocytes and macrophages/monocytes, which are phagocytic cells could potentially be direct targets of the CD44 antibody. Pre-treatment of PerMac cells with KM114 inhibited phagocytosis of opsonized-sRBCs by those cells, in a dose dependent manner, as demonstrated by flow cytometry. Evaluation of phagocytosis by confocal microscopy confirmed the inhibition of phagocytosis. These data suggest that the therapeuticeffects of the anti-CD44 antibody KM114 in ITP may be mediated by inhibition of Fc-receptor mediated-phagocytosis in macrophages.

Conclusion
Here we show that anti-CD44 antibodies can bind to macrophages and inhibit their Fc receptor-mediated phagocytic function directly suggesting a possible mechanism by which these antibodies can ameliorate ITP.
At Héma-Québec, we maintain a registry of IgA-deficient donors to provide compatible blood components to IgA deficient patients, at the hospital’s request. We recently reinitiated screening of blood donors for IgA deficiency to compensate for the loss of active donors in our registry. Several screening methods mostly based on ELISA exist but, while being very reliable, they are also time-consuming. Typically, sample preparation and loading in microplates take about 2 and 7 hours for 1000 samples respectively, for one technician. The ELISA technique itself requires about 4 hours to be completed. To improve the efficiency of screening, we set up a fluorescent-based competition assay requiring only one incubation step. The workload for sample distribution in microplates has been considerably reduced by using the Hamilton Microlab AT Plus 2 automated dispenser (located at our regulatory testing lab in Montreal) to collect plasma samples (50 ul) from the left-over tubes used for ABO/Rh testing, in IgA-coated 96-well microplates. The microplates are then frozen to prevent spillage and shipped to the R&D department in Quebec City. For testing, the microplates are first thawed and anti-human IgA-FITC conjugate is added directly to each well. The plates are incubated for one hour at 37°C, washed, emptied and read on a fluorometer. The anti-human IgA-FITC conjugate is neutralized by the IgA contained in normal plasma, resulting in background fluorescence. In contrast, IgA-deficient plasma does not interfere with the binding of anti-human IgA FITC conjugate to IgA-coated wells, yielding high fluorescence and allowing the easy identification of IgA-deficient plasma. The overall procedure is completed within 2 hours, compared to more than 12 hours with our previous screening method, for the same number of samples. Using this new method, we tested 10,763 samples from January 16 to January 31 2013 and identified 23 IgA-deficient donors (frequency of 1/468). These samples were retested using our in-house cytometry-based assay for preliminary confirmation of the IgA deficiency status. All samples were IgA-negative, showing the specificity of our screening method. In conclusion, we have developed a cost-effective and efficient method for the screening of thousands of blood donors for IgA deficiency.
IVIg Suppresses Allogeneic Mixed Lymphocyte Reactions by Induction of Anti-inflammatory Monocytes (CD14+, HLA-DR+++) with CD80+ and PDL1+++ Expression

Scientific, Poster Presentation

Renée Bazin PhD, Héma-Québec
Lauriane Padet, Héma-Québec

Intravenous immunoglobulin (IVIg) is a therapeutic preparation of human IgG isolated from thousands of plasma donations and has been proposed as a therapy to improve the rate of graft survival in patients with a high risk for antibody-mediated-rejection. The rapid effect of IVIg in these patients was proposed to be due to blocking of anti-HLA antibodies by anti-idiotypic IgG present in IVIg. However, peripheral tolerance beyond the half-life of IVIg is observed in IVIg-treated patients, suggesting that IVIg modulates the functions of immune cells in addition to neutralizing anti-HLA antibodies. In the present study we show, using the allogeneic mixed lymphocyte reaction (MLR) as an in vitro model of allograft rejection and GvHD, that IVIg strongly inhibits T cell activation, as determined by IL-2 secretion, and modulates the level of other pro- and anti-inflammatory cytokine secretion (IL-6, INF-γ, IL-1RA). To further define the mechanisms underlying the IVIg-mediated inhibition of T cell activation in MLR, we studied the effect of IVIg on the phenotype of the cells involved in MLR (mainly T cells and monocytes). Our results reveal that MLR inhibition by IVIg correlates with the induction of an anti-inflammatory monocyte population (CD14+, HLA-DR+++) with a low expression of CD80 (a co-stimulatory receptor) and high expression of PDL1 (an inhibitory receptor). To evaluate the importance of PDL1 on the IVIg-mediated MLR inhibition, anti-PDL1 was added together with IVIg during the MLR. Blocking of PDL1 restored the MLR, as evaluated by IL-2 secretion by activated T cells. We thus propose that IVIg-mediated induction of anti-inflammatory monocytes expressing low levels of CD80 and high levels of PDL1 plays a central role in the inhibition of MLR.
A New Casing Design for the Storage of Frozen Blood Products to Reduce the Breakage

Claudia Bédard MSc, Héma-Québec, R&D
Annie Jacques MSc, Héma-Québec, R&D
Éric Ducas MSc, Héma-Québec, R&D
Nathalie Dussault MSc, Héma-Québec, R&D
Pierre Lalone, Héma-Québec, Finance and Administration
Sylvie Thibault, Héma-Québec, Exploitation
Michel Beaudin Ing, Héma-Québec, Project Direction
Louis Thibault PhD, Héma-Québec, R&D

Purpose
Héma-Québec delivers about 90,000 frozen blood products to hospitals yearly. Frozen products are stored at <-18°C and distributed to hospitals on dry ice (-78°C). The transport and handling of these products at this low temperature increase the risk of breakage and cracking of plastic bags. Last year, nearly 2,000 frozen products were lost. The aim of this project was to develop a “green” packaging that would reduce the incidence of breakage by immobilizing the frozen product in its casing.

Methods
The new casing design must meet specific criteria: adaptable to all types of storage bags of frozen products, economical, reusable, recyclable and have the least possible impact on our operations and those of hospitals. Drop tests from a height of 90 cm, a worst case scenario that simulates a fall from a work station, were conducted to evaluate prototype performance. The results were compared with our current casing.

Results
The new design has been easily adapted to plasmapheresis units as well as whole blood derived frozen products. The casing, made of corrugated cardboard, includes an insert which firmly holds the bag in place to reduce its mobility. The lid has a large window to give access to the product label and is firmly held in place with Velcro type closures. During drop tests, the new design reduced the breakage rate from about 78% to 16% when compared to our actual packaging. For 250-mL Frozen Plasma units and 500-mL Fresh Frozen Plasma units, the new design has reduced breakages by 60% and 70%, respectively.

Conclusions
The new casing design should reduce the rate of breakage and the loss of frozen blood products. The new casings will be put into operations shortly. However, it will take several months to assess their effectiveness in reducing the loss of frozen products.
45. Activating and Inhibitory Fcγ Receptors are not Required for Successful Antibody-mediated Immune Suppression to Foreign Erythrocytes

Scientific, Plenary Presentation

Lidice Bernardo Reyes PhD, Li Ka Shing Knowledge Institute of St. Michael’s Hospital
Honghui Yu, Canadian Blood Services; LKSKI of St. Michael’s Hospital
Alan Lazarus, Canadian Blood Services; LKSKI of St. Michael’s Hospital; University of Toronto

Hemolytic disease of the fetus and newborn (HDFN) is characterized by the maternal IgG-mediated destruction of fetal erythrocytes. Anti-D prophylaxis has been used to prevent RhD-negative mothers from becoming immunized against RhD positive erythrocytes during and after pregnancy and this mechanism has been referred as “antibody-mediated immune suppression” (AMIS). Although anti-D preventative therapy has been highly successful, the immune inhibitory mechanism remains poorly understood. Two major theories behind this mechanism involve the binding of the IgG mediating the AMIS to activating or inhibitory Fcγ receptors (FcγR) which induce either erythrocyte clearance or immune inhibition, respectively. In the case of the inhibitory FcγRIIB, this receptor has been suggested to inhibit cellular activation as a result of the co-crosslinking of the inhibitory receptor with the B-cell antigen receptor. In this work, we explored the absolute role of activating and inhibitory Fcγ receptors in the AMIS mechanism using the HOD mouse model of red blood cell (RBC) immunization. HOD transgenic mice contain a RBC specific triple fusion recombinant protein comprised of the hen egg lysozyme (HEL) in tandem sequence with ovalbumin and the human transmembrane Duffy antigen. HOD RBCs thus express a unique erythrocyte antigen that can be used to stimulate an immune response to the red cells. These HOD RBCs were injected into naïve C57BL/6 mice alone or together with anti-HEL antibodies (i.e., AMIS). To assess the contribution of activating and inhibitory Fcγ receptors to the induction of an AMIS effect, normal mice vs Fcγ chain -/- mice or FcγRIIB -/- mice were used as recipients of HOD RBCs and the resulting immune response to the HEL antigen evaluated. We show that anti-HEL polyclonal antibodies induce the same degree of AMIS in mice lacking the IgG binding receptors (activating as well as inhibitory) as compared to wild-type mice. In agreement with this, F(ab’)2 fragments of the AMIS antibody which cannot interact with both activating and inhibitory FcγR reduced the antibody response nearly as efficiently as anti-HEL antibodies. In conclusion, inhibition of in vivo antibody responses to HEL protein on HOD RBCs by polyclonal IgG can occur via an FcγR-independent pathway. These results have implications for the understanding of RhD prophylaxis.
46. Human Rh Immunogenic Regions in HLA DRB1*1501 Transgenic Mice

Scientific, Poster Presentation

Lidioe Bernardo Reyes PhD, Li Ka Shing Knowledge Institute of St. Michael’s Hospital
Kunjlata Shah, Department of Transfusion Medicine, St. Michael’s Hospital
Gregory Denomme, Immunohaematology Reference Laboratory, Blood Centre of Wisconsin
Alan Lazarus, Canadian Blood Services, LKSKI of St. Michael’s Hospital and University of Toronto

Anti-D therapy has been highly successful in preventing hemolytic disease of the fetus and newborn, but the suppressive mechanism involved (referred as antibody-mediated immune suppression; AMIS) remains poorly understood. A major limitation to study the mechanisms implicated in AMIS to Rh antigens has been the lack of an animal model of D protein immunization. Normal mice do not mount an immune response to RhD. However, it has been suggested that the human HLA class II allele DRB1*1501 could be a major restricting element for human T-helper cells specific for RhD protein and DRB1*1501 positive mice responded to immunization with purified soluble RhD protein.

As an approach to develop a mouse model of RhD immunization, both human red blood cells (RBC) expressing the D antigen and Rh synthetic peptides corresponding to extracellular and putative immunogenic regions of human Rh proteins were separately used to immunize mice where mouse MHC class II was replaced with HLA class II DRB1*1501. Red blood cells were injected alone or mixed with CPG ODN adjuvant and administered in one and two doses. The three RhD peptides synthesized (Peptide 1 aa 34-46; Peptide 2 aa 228-238; peptide 3 aa 350-358) were linked to a carrier protein (KLH) through a cysteine added at the C-terminal region of each peptide and emulsified in Freund’s adjuvant (complete for the first and incomplete for the second and third dose).

DRB1*1501 transgenic mice challenged with RhD positive RBC developed antibodies that recognized human RBC by flow cytometry but no antibodies specific to the RhD protein were detected. For the peptide study, immunization of mice with the three RhD peptides induced a response specific for the immunizing peptides as detected by ELISA. Two of these sera also reacted with human RBC when tested by flow cytometry. Interestingly, RhD negative cells (i.e., Rhce+) were better recognized by the polyclonal antibodies than RhD positive cells. These results are consistent with inducing an antibody response in DRB1*1501 mice against antigenic regions of human Rh but not with specificity for the RhD antigen itself.
Integrating Blood Donor West Nile Virus Screening and Public Health Surveillance in British Columbia

Scientific, Poster Presentation

Mark Bigham MD, Canadian Blood Services
Alice Cheung, Canadian Blood Services
Patrick Loftus MN, Canadian Blood Services
Judy Hrytzak MLT, Canadian Blood Services
Sima Ashrafinia, Canadian Blood Services
Gershon Growe MD, Canadian Blood Services
Marsha Taylor MSc, BC Centre for Disease Control
Bonnie Henry MD, BC Centre for Disease Control

Purpose
Canadian Blood Services (CBS) performs year-round West Nile Virus (WNV) blood donor screening. In British Columbia (BC), seasonal WNV surveillance is conducted by public health, coordinated by the BC Centre for Disease Control (BCCDC). Here, we outline results of a partnership between CBS and BCCDC for WNV testing and surveillance that enhances both blood safety and the sensitivity of public health WNV surveillance.

Methods
A review and compilation of WNV surveillance processes and outcomes was undertaken, using unpublished WNV blood donor screening and program data collected annually by CBS BC and Yukon (BCY) Centre, and published provincial WNV surveillance program results.

Results
CBS began universal WNV screening by Nucleic Acid Testing, in July 2003, leaving a low residual risk of “window period” transfusion transmitted WNV. Suspected transfusion transmissible infections are reportable to Public Health in BC. In 2004, CBS BCY Centre and BCCDC developed processes for BCCDC to report clinically suspect cases of WNV (i.e. on receipt of a laboratory specimen for WNV testing) to CBS to further reduce the risk. If a registered blood donor with a recent donation is identified, CBS takes immediate precautionary action to quarantine or recall the unit. An anonymized data linkage was validated to maximize protection of privacy. Between 2004-2012, a mean 662 (range 427-1017) cases tested for WNV were reported annually by BCCDC to CBS; a mean 41(6.2%) were blood donors; and a mean 1.4 (range 0-4) product recalls per year were done. Conversely, since 2008, CBS BCY Centre has provided BCCDC with biweekly or weekly, aggregate, non-nominal donor WNV test data, geographically stratified by health service delivery area of donor clinics. In 2012, weekly data on a cumulative 44,000 donor WNV screening test results between 30 May-2 Oct were provided to BCCDC. Blood donor WNV screening approximates population-based sampling, and provides close-to-real time human data that strengthen the sensitivity of the public health WNV surveillance system.

Conclusion
CBS and public health WNV-related activities are highly complementary and strengthen transfusion safety and public health WNV surveillance in BC.
48. Membrane Changes in Liposome-treated Rat Red Blood Cells

Scientific, Oral Presentation

Luciana da Silveira Cavalcante MSc, University of Alberta and Canadian Blood Services
Qingping Feng MD, PhD, University of Western Ontario
Ian Chin-Yee MD, University of Western Ontario and Canadian Blood Services
Jason Acker PhD, University of Alberta and Canadian Blood Services
Jelena Holovati PhD, University of Alberta and Canadian Blood Services

Background
Liposomes, microscopic synthetic lipid vesicles, improve red blood cell (RBC) in vitro quality by reducing membrane damage occurring during 42 day storage. The use of animal models is an essential step in preclinical studies for evaluating transfusion efficacy with novel manipulations of blood transfusion products.

Objectives
The aim of this study was to evaluate the effect of four liposome formulations on rat RBC membrane-related quality parameters, to determine which formulation will be subsequently used in a rat model for transfusion efficacy.

Methods
Unilamellar liposomes were synthesized using an extrusion method to contain lipid bilayer of unsaturated (1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC):cholesterol, 7:3 mol%), saturated (1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC):cholesterol, 7:3 mol%), unsaturated charged (DPPC:cholesterol: phosphatidylserine (PS), 6:3:1 mol%) and unsaturated charged (DOPC:cholesterol:PS, 6:3:1 mol%) phospholipids. The leukoreduced, packed RBCs from Sprague-Dawley rats (n=3) were incubated for 1 h at 37 °C in a mixture of AS-3 solution with either HEPES-NaCl solution (control) or 2 mM lipid from four different liposome compositions. RBC membrane quality was assessed by % hemolysis (Drabkins), deformability (ektacytometry), hematological indices (Coulter counter), morphology (microscopy), cholesterol and phospholipid concentration (colorimetry).

Results
Similar to human RBCs, all four liposome treatments resulted in significant decrease in percent hemolysis, with the effect most prominent with DOPC-treated RBCs (1.6 ± 0.1 vs. 3.1 ± 0.2, p = 0.05). Liposome treatment does not alter rat RBC membrane deformability or morphology, as there were no statistically significant differences in Elmax, KEI or hematological indices in any of the preparations. While DOPC liposome treatment resulted in significant increase in rat RBC phospholipid concentration, DPPC liposome treatment seemed to induce loss of phospholipids from the rat RBC membranes (0.30 ± 0.03 mg vs 0.15 ± 0.01 mg, p = 0.05). The cholesterol:phospholipid ratio in DOPC-treated rat RBCs was comparable to control (0.49 ± 0.06 vs 0.51 ± 0.08, p = 0.80).

Conclusions
The beneficial effect of liposome treatment on rat RBC membrane is related to the saturation level of liposome phospholipids, rather than liposome charge. DOPC-liposomes will be used in the upcoming in vivo rat studies to further evaluate the clinical effect of transfusing liposome-treated RBCs.
Panreactive Antibodies and Autoantibodies in Prenatal Serological Testing

Scientific, Poster Presentation

Judith Hannon MD, Canadian Blood Services
Gwen Clarke MD, Canadian Blood Services and Alberta Health Services
Jean Ashdown BSc, MLT, Canadian Blood Services
Gerri Barr MLT, Canadian Blood Services

Background
Serological testing of blood group and antibody screening are a cornerstone of routine prenatal care in Canada. In addition to allowing for assessment of need for Rh immune globulin prophylaxis, antibody screening and identification allows for appropriate prenatal follow up and management of hemolytic disease of the fetus/newborn (HDFN). Our province wide perinatal testing program tests 75,000 maternal blood samples annually. Between January 1, 2010 and December 31, 2013, 1116 clinically significant antibodies were identified. In addition, a number of panreactive antibodies and autoantibodies were detected.

Results
A total of 231 autoantibodies or panreactive antibodies were detected. 8 cases showed a distinct antibody specificity (2 anti-D, 1 anti-C, 2 anti-e and 3 anti-Jka) but were positive for the corresponding antigen so were termed autoantibodies. 86 cases were panreactive with a negative DAT. All but one were reactive by solid phase method only. 137 cases were panreactive antibodies with a positive DAT (47 reactive with anti-IgG, 38 with anti-C3, 52 with both). 82 of these panreactive autoantibodies were reacting by solid phase method only. 55 were reactive by both solid phase and PEG IAT method. Autoabsorptions were performed on those reactive by PEG IAT to exclude or identify underlying allo-antibodies. 9% of patients with a panreactive antibody or autoantibody also had an allo-antibody (1 anti-D, 6 anti-E, 7 anti-S, 2 anti-Jka, 2 anti-Lea, 1 anti-M, 1 anti-N). Titres were not performed on the panreactive or autoantibodies. Repeat samples were requested monthly for autoantibodies with specificity and at 26 weeks gestation for all panreactive antibodies.

Outcomes
While individual outcomes were not assessed, the overall clinical impression is that autoantibodies in pregnancy are NOT usually implicated in HDFN. Laboratory protocols in the setting of autoantibodies must be optimized to ensure that clinically significant alloantibodies have been excluded. Follow up testing of patients for persistence of autoantibody, reassessment for possible alloantibody development and recommendations around assessment for hemolysis in the mother and in the newborn are not well established and should be considered. Future studies will attempt to document the clinical outcomes of neonates born to mothers with a red cell autoantibody.
Cord Testing on the Provue™

Scientific, Poster Presentation

Laura Harrison ART, Trillium Health Partners, Credit Valley Hospital
Janice Zeng MD, FRCP, Trillium Health Partners, Credit Valley Hospital
Shauna Dowsley ART, Trillium Health Partners, Credit Valley Hospital

Purpose
Cord testing was being performed manually, with ABO/Rh testing done by tube and Direct Antiglobulin Testing (DAT) performed using ID-MTS™ Anti-IgG gel card. Considerable technologist time was required and testing was limited to daily batch testing unless a STAT request was received.

Method
A total of 81 cord sample were tested manually and then repeated on the Provue™. Reproducibility and carryover testing was performed and samples up to 7 days old were tested. All samples were cleared of clots using applicator sticks prior to centrifugation and testing on the Provue™. Extra reagents needed on the Provue included ID-MTS™ Anti-IgG gel cards loaded into the room temperature incubator and MTS™ Diluent 2.

Results
There were no errors in blood group interpretation by the Provue™. Four of five samples (4/5) that showed "No Results Determined" (NRD) ABO/Rh interpretations due to questionable or mixed field results were resolved by tube testing. One cord sample appeared to have possible contamination with maternal blood. No follow up sample was provided. DAT comparison between manual gel testing and Provue™ testing showed 93.8% overall agreement. Consistent with the original validation between tube and gel testing, samples with very weak positive DAT due to ABO incompatibilities or presence of maternal Passive Anti-D, were difficult to reproduce. No issue with carryover was seen and 7 day old samples gave acceptable results. Within the samples tested, no clots were detected resulting in a failure to run.

Conclusion
Cord testing can be performed on the Provue™, resulting in improved turn-around-time (TAT) and decreased technologist resources. Questionable or NRD results will be repeated manually.
51. Role of ABO isohemaglutinin titration in selection of ABO incompatible platelets and impact on recipient hemoglobin: A Single Centre Retrospective review

Scientific, Poster Presentation

Karen Hodgins ART, London Health Science Centre
Cyrus Hsia MD, FRCPC, London Health Science Centre
Jeff Kinney ART, London Health Science Centre

Transfusions of ABO incompatible platelets have been associated with clinically significant and severe hemolysis. ABO and Rh identical or plasma compatible platelets should be used when available. Often in urgent settings or where platelet inventories are limited, ABO plasma incompatible platelets may be required. In Dec 2010 the Blood Transfusion Laboratory (BTL) at London Health Sciences Centre began evaluating the titres of Anti-A and Anti-B in plasma of all group A, B and O platelet units received from the Canadian Blood Services(CBS). The aim of this one year retrospective review was to evaluate the impact of potential hemolysis using a set titration level of ABO isohemaglutins in O platelets donor units transfused to non-O recipients in Hematology/Oncology patients. A dilution of 1:150 was tested against A1, B and pooled A1 and B cells using Ortho Clinical Diagnostics MTS Buffered Gel Cards with no incubation and a 10 minute centrifugation. Those units identified with titres greater than 150 were only transfused to ABO identical recipients. Overall, our method of ABO isohemaglutinin titration appears to be safe in this population. Both study, 129, and control, 287, groups had a similar rate of mild and severe post transfusion hemoglobin drops. The post transfusion drops in hemoglobin likely are not due to hemolysis in this high risk population as anemia due to exposure to chemotherapy, radiation, infections and bleeding are very common in these individuals. However, similar rates of drops in hemoglobin between the study and the control groups seen in our study are reassuring. With the available evidence thus far, we believe that ABO plasma incompatible platelets with a titre less than 150 can be safely transfused to patients if necessary and that this method can be routinely performed in blood transfusion laboratories.
Reducing the IgA Content of Red Blood Cell Units Using the Haemonetics ACP 215 Automated System

Scientific, Poster Presentation

Annie Jacques MSc, Héma-Québec, R&D
Hana Daoud MSc, Héma-Québec, R&D
Claudia Bédard BSc, Héma-Québec, R&D
Marie-Claire Chevrier MSc, Héma-Québec, Reference and Stem Cell Laboratory
Louis Thibault PhD, Héma-Québec, R&D

Purpose
In Canada, it is generally agreed that IgA-deficient patients should be transfused with washed red blood cells (RBCs) when blood products made from IgA-deficient blood donors are not available. According to current guidelines, RBC units should be washed with a minimum volume of 2 L of saline. The closed-system ACP 215 (Haemonetics) enables automated washing and 14 days of post-wash storage. In this work, the ability of the ACP 215 system for washing SAGM and AS-3 RBC units to comply with the CSA recommendation of less than 0.5 µg of IgA per mL was investigated.

Methods
For this work, the ACP 215 washer was programmed using a standard hematocrit of 60%. Only leukoreduced AS-3 and SAGM RBC units weighting ≥250 g were used. After washing, RBCs were stored in AS-3 at 4°C. Three washing protocols were evaluated: 1.84 L (control), 3.0 L and 2 consecutive procedures of 1.84 L (n=6 per arm). Wash efficiency, assessed by measuring the volume of residual plasma and IgA concentration, and in vitro quality parameters of RBCs, were analyzed.

Results
The IgA content of washed RBC units using 1.84 L and 3.0 L of saline remained above the acceptable limit of 0.5 µg of IgA per mL, at 1.4 ± 0.7 µg/mL and 1.3 ± 0.9 µg/mL, respectively. By performing two washes, the IgA content of RBC units was reduced from 202 ± 129 µg/mL to 0.007 ± 0.003 µg/mL. The RBC recovery was 90% for AS-3 units and 89% for SAGM units. After 7 days of post-wash storage, the level of hemolysis of double-washed units was 0.4 ± 0.1%, and the residual volume of plasma was ≤ 2 µL.

Conclusions
This study shows that two sequential washes can reduce the IgA concentration of RBC units below the limit of 0.5 µg/mL, thereby complying with Canadian regulatory guidelines. Double-washed RBC units can be stored for up to 7 day post-wash when suspended in AS-3. A validation study is underway to confirm the routine use of the ACP 215 system to reduce the IgA content of RBC units.
53. PostThaw Quality of SAGM and AS-3 Leukoreduced Red Blood Cells after Deglycerolization and Suspension in AS-3 with an Automated Closed System

Scientific, Poster Presentation

Annie Jacques MSc, Héma-Québec, R&D
Hana Daoud MSc, Héma-Québec, R&D
Claudia Bédard BSc, Héma-Québec, R&D
Audrey Laforce-Lavoie MSc, Héma-Québec, R&D
Marie-Claire Chevrier MSc, Héma-Québec, Reference and Stem Cell Laboratory
Louis Thibault PhD, Héma-Québec, R&D

Purpose
Currently, deglycerolized rare red blood cell (RBC) units are processed using COBE 2991 cell processors. Thawed RBC units that are suspended without nutrient solution have a limited shelf life of 24 hours at 1-6°C. The ACP 215 automated closed system from Haemonetics allows for prolonged postglycerolization storage. In this study, the post-thaw quality of SAGM and AS-3 RBC units previously frozen on day 14 was investigated on day 7 following deglycerolization and suspension in AS-3 solution.

Methods
Five leukoreduced AS-3 and 5 leukoreduced SAGM RBCs were frozen with 40% glycerol on day 14 using the automated ACP 215. After at least 48 hours of storage at -80°C, frozen RBC units were deglycerolized with the ACP 215 and suspended in about 100 mL of AS-3 additive solution. The instrument was set with a standard hematocrit of 0.60 L/L. Thawed RBC units were stored at 1 to 6°C. Samples were collected on day 7 to study the effects of deglycerolization on the quality of RBCs (ATP, 2,3-DPG, free hemoglobin, lactate and potassium).

Results
After thawing, the percentage of recovery was comparable for both types of RBCs regardless of the nature of the nutrient solution used before freezing (85% vs. 88% for AS-3 and SAGM RBC units, respectively). The hemoglobin content was 46 ± 2 g vs. 39 ± 7 g for AS-3 and SAGM RBC units. After 7 days of storage, the mean level of hemolysis and ATP were also comparable for both types of RBCs (0.4 ± 0.1% vs. 0.5 ± 0.1% and 2.1 ± 0.5 µmol/g Hb vs. 1.9 ± 0.5 µmol/g Hb for AS-3 and SAG-M RBC units, respectively). No bacterial contamination was detected in these products.

Conclusions
These results show that AS-3 and SAGM RBCs can be stored for up to 14 days before glycerolization and deglycerolization with the ACP 215 system. Once suspended in AS-3, packed red blood cells can be stored for up to 7 days to 1 to 6°C. A validation study has been undertaken to confirm these results.
Quality of Red Blood Cell Units Washed with the Haemonetics ACP 215 Automated System and Stored in AS-3 Additive Solution

Scientific, Poster Presentation

Annie Jacques MSc, Héma-Québec, R&D
Hana Daoud MSc, Héma-Québec, R&D
Claudia Bédard BSc, Héma-Québec, R&D
Marie-Claire Chevrier MSc, Héma-Québec, Reference and Stem Cell Laboratory
Louis Thibault PhD, Héma-Québec, R&D

Purpose
Currently, washed red blood cells (RBCs) are washed with COBE 2991 and stored in saline solution for up to 24 hours to reduce the risk of bacterial contamination. The closed-system ACP 215 (Haemonetics) enables automated washing and 14-day post-wash storage in SAGM solution. We studied the quality parameters of SAGM and AS-3 RBC units washed at various storage times and stored suspended in AS-3 solution.

Methods
Leukoreduced AS-3 and SAGM RBC units (450 mL) were washed using the ACP 215 system after 7, 14, 21 or 28 days of storage and stored post-wash for up to 14 days (n=5 per arm). After washing, RBCs were stored in 80 mL of AS-3 solution. Product quality (volume, hematocrit, and hemolysis) and RBC metabolism (ATP, pH, lactate, potassium, and sodium) were analyzed before and after post-wash storage.

Results
Recovery was 96% for AS-3 RBCs and 90% for SAGM RBCs. Hemoglobin content in washed AS-3 RBC units was 55 ± 4 g/unit, and 44 ± 3 g/unit in SAGM RBC units. The hematocrit of AS-3 and SAGM RBC units was 0.54 ± 0.01 L/L and 0.49 ± 0.03 L/L, respectively. The percentage of hemolysis in RBC units washed after up to 14 days of storage and stored 7 days post-wash was approximately 0.5% for both SAGM and AS-3 RBC units. Immediately after washing, the average potassium level was 1.0 ± 0.4 mmol/L, and increased to 10.4 ± 1.3 mmol/L after 7 days of post-wash storage. The residual volume of plasma was 0.40 ± 0.14 mL in AS-3 RBC units and 0.13 ± 0.02 mL in SAGM RBC units.

Conclusions
Our data show that RBC units stored for up to 14 days before washing can efficiently be washed with the ACP 215 system and stored for up to 7 days post-wash when suspended in AS-3. The nutrient solution in which RBCs are stored before washing has little impact on their in vitro parameters. A validation study is underway to confirm the routine use of the ACP 215 system to wash blood for transfusion.
Method of predicting cryopreserved RBC quality while preserving units for transfusion

Scientific, Poster Presentation

Jayme Kurach MSc, Canadian Blood Services
Tracey Turner MLT, Canadian Blood Services
Adele Hansen BSc, Canadian Blood Services
Jason Acker PhD, MBA, Research and Development, Canadian Blood Services and Department of Laboratory Medicine and Pathology, University of Alberta

Background
Cryopreservation of RBCs is useful in maintaining an inventory of rare blood types. CBS uses the COBE 2991 to deglycerolize frozen RBCs in preparation for transfusion; which has been validated on normal RBC units to avoid unnecessary discard of rare blood units. However, these validation conditions do not consider that some rare blood types may be more susceptible to injury caused by cryopreservation.

Objective
To develop a method to predict the quality of rare blood types post cryopreservation while still allowing the RBC unit to be utilized for transfusion.

Method
Leukoreduced CPD-SAGM RBCs produced using the whole blood method were collected (n=6), ABO-matched and divided into two groups. Each group (n=3) was pooled, split and glycerolized according to standard CBS procedures. Before segments were made 6 Fenwal sample pouches were individually sterile docked, filled with 4 mL of glycerolized RBCs, and detached. The RBC units and sample pouches were frozen at -80 °C for ≥ 24 h. Each unit and its corresponding pouches were thawed and deglycerolized together. Units were deglycerolized using standard CBS procedures. Pouches were deglycerolized using a simulated COBE process in tubes. To simulate the COBE process in tubes each wash step was replicated using the same saline solutions (12 % NaCl, 1.6 % NaCl, and 0.9 % NaCl/0.2 % dextrose) to achieve similar glycerol concentration after each step. Both the units and pouch test samples were tested 24 h post-deglycerolization for hemolysis, extracellular potassium, and recovery.

Results
There was no statistical differences (p>0.05) between the units and corresponding pouches at 24 h post-deglycerolization for hemolysis (1.41 ± 0.19 %, 1.29 ± 0.22 % respectively) or recovery (77 ± 4 %, 76 ± 5 % respectively). Deglycerolized units (13 ± 2 mmol/L) had significantly higher (p<0.01) extracellular potassium than corresponding pouches (9 ± 1 mmol/L).

Conclusion
This method was able to obtain samples from glycerolized units without compromising unit integrity. The simulated method resulted in samples that were of comparable quality to their corresponding units. With further development this process could be used to predict the quality of frozen RBC units.
Red Cell Transport in Ambient Temperatures of 0°C and Greater

Scientific, Poster Presentation

Jennifer LeFrense BSc, MLT, Nova Scotia Provincial Blood Coordinating Program
Craig Jenkins BSc, MLT, Canadian Blood Services

Purpose
Nova Scotia redistributes blood components between hospitals as a means of continuously improving efficiency. Hospitals use the Canadian Blood Services (CBS) shipping containers which have been validated for the transport of red blood cells in ambient temperatures of 0°C or greater. J82 shipping containers are used with ice packs conditioned at temperatures between -8°C and -14°C. These conditioning temperatures posed some difficulty for several hospitals due to lack of freezers available in this temperature range.

Various attempts to validate a packing scheme using ice packs stored at -30°C were performed without success. The Nova Scotia Provincial Blood Coordinating Program (NSPBCP) liaised with CBS to seek a solution to obtain a validated RBC transport system which maintains red cells at a temperature between 1°C and 10°C for a maximum transit time of 24 hours as required by CSA standards.

Method
CBS’ Quality Monitoring Program provided information on the conditioning and behavior of the ice packs when subjected to different temperatures. Data suggested that removing the ice packs from current storage conditions until they reached the required conditioning temperature may help meet the necessary conditioning requirements. CBS QMP provided NSPBCP with temperature charts showing this ‘warming’ could be achieved in approximately 30 minutes.

The ice packs were removed from freezers and allowed to warm while resting at room temperature. An infrared thermometer was used to confirm the desired temperature was reached and packing continued according to CBS’ packing scheme. Using CBS temperature monitoring devices (TMDs), outdated red blood cells were shipped between facilities to validate that this method would sustain product temperatures between 1°C and 10°C.

Results
Trial shipments were successful with all shipping containers maintaining an internal temperature between 1°C and 10°C.

Conclusion
Allowing ice packs to warm followed by packing according to CBS’ validated packing scheme maintains red cell unit temperatures during shipping as required by CSA and CSTM standards. This approach supported one packing scheme that simplified verification at time of receipt as well as saving significant dollars as the need to purchase additional freezers was eliminated.
Monocytes are key players of the innate immune response and recent studies have shown that their differentiation and functions involve microRNAs which act through the inhibition of protein translation. Among them, miR-146a has been shown to suppress NF-κB and IRF3 activity, two key transcription factor involved in TLR4 signaling, and consequently, to inhibit their target genes including INF-β, IL-6 and TNF-α. Therefore, miR-146a could lead to anti-inflammatory effects similar to those reported for IVIg, a therapeutic preparation of polyclonal human IgG isolated from the plasma of thousands of healthy donors. Indeed, IVIg has been shown to abrogate pro-inflammatory cytokine secretion while increasing the expression of anti-inflammatory cytokines in monocytic cells. In this work, we evaluated whether miR-146a is involved in the anti-inflammatory effects of IVIg following LPS stimulation of human monocytes. Monocytes were obtained from the blood of healthy volunteers after informed consent and treated with LPS (1 μg/mL) or IVIg (15 mg/mL) alone or alternatively, pretreated with LPS followed by washing and addition of IVIg. The cells were incubated for 3, 6 and 12 hours prior to recovery and analysis of miR-146a, IRF3, IFN-β, TGF-β1 and IL-1Ra expression by qPCR while NF-κB and IRAK1 expression at 24 hours was measured by Western blotting. Our results show that addition of IVIg to LPS-pretreated monocytes induces a significant upregulation of miR-146a expression associated with a significant reduction in the expression of its target IRAK1 and consequently of NF-κB activation. Furthermore, the mRNA expression of IRF3 and IFN-β was reduced in LPS-pretreated monocytes following treatment with IVIg, whereas TGF-β1 and IL-1Ra expression was increased, thus skewing the balance between pro-inflammatory vs anti-inflammatory cytokine towards an anti-inflammatory condition. In conclusion, our data suggest that miR-146a is a mediator of some of the anti-inflammatory effects of IVIg in human monocytes. Our future work will address the possibility of using microRNA mimetics to reproduce these effects and could possibly lead to the development of synthetic IVIg substitutes.
Enhancing the Migration Ability of Cord Blood-derived Mesenchymal Stromal Cells for Tissue Repair

Scientific, Plenary Presentation

Leah Marquez-Curtis PhD, Canadian Blood Services
Yuan Qiu PhD, Canadian Blood Services and University of Alberta
Hilal Gul-Uludag PhD, University of Alberta
Loree Larratt MD, University of Alberta
A. Robert Turner MD, University of Alberta
Anna Janowska-Wieczorek MD, PhD, Canadian Blood Services and University of Alberta

Background
Cord blood (CB) is a source of hematopoietic stem/progenitor cells (HSPC) as well as mesenchymal stromal cells (MSCs). Their multi-lineage differentiation potential, immunomodulatory properties and ability to produce bioactive molecules make MSCs ideal for regenerative medicine and cellular therapies. MSCs migrate to sites of inflammation and tissue injury where the local concentration of the chemokine stromal cell-derived factor (SDF)-1 is elevated. The interaction between SDF-1 and its receptor CXCR4 plays an important role in cell trafficking, but because the surface expression of CXCR4 in MSCs is low, their recruitment to sites of tissue injury is limited. Previously we showed that C1q, the first component of the complement system, and valproic acid (VPA), a histone deacetylase inhibitor, increase the responsiveness of HSPC to SDF-1 (Transfusion, 2010, 2012; Stem Cells & Development, 2009). In this study we investigated whether these molecules increase SDF-1-directed migration of MSCs. We also examined whether transfection of CXCR4 into MSCs increases their migration towards SDF-1.

Methods
MSC cultures were established from the mononuclear cell fraction of CB and characterized by flow cytometry and in vitro differentiation. CXCR4 transfection was carried out using the cationic liposomal agent IBAfect. CXCR4 expression was evaluated by quantitative RT-PCR and flow cytometry, and cell migration was assessed using modified Boyden chambers.

Results
We found that C1q chemoattracts MSCs in a dose-dependent manner, reaching >5-fold increase in migration compared to media alone. Moreover, C1q increased CXCR4 surface expression in MSCs from 1.5% to 9.5%, and primed their chemoinvasion towards a low dose of SDF-1. VPA treatment of MSCs increased their CXCR4 gene expression by >40-fold and primed their chemoinvasion towards a low SDF-1 gradient by over 2-fold. Finally, IBAfect-mediated transfection increased CXCR4 transcript expression 10^5-fold, surface expression up to 40% and the number of MSCs migrating towards SDF-1 over 3-fold relative to those migrating towards media alone.

Conclusion
Our findings indicate that MSCs can be manipulated in vitro to enhance their in vivo recruitment to sites of injury where they can exert their full potential for tissue repair.
Genotyping Using the Polymerase Chain Reaction Methodology-Bioarray HEA BeadChip Assay

Scientific, Oral Presentation

Doris Neurath ART, MBA, EORLA, The Ottawa Hospital
Marc Pilon MLT, EORLA, The Ottawa Hospital
Melanie Tokessy MLT, EORLA, The Ottawa Hospital
Shauna Love MLT, EORLA, The Ottawa Hospital
Nancy Cober ART, EORLA, The Ottawa Hospital
Antonio Giulivi MD, The Ottawa Hospital

Background
The BioArray Solutions HEA BeadChip assay uses the proprietary Elongation-mediated Multiplexed Analysis of Polymorphisms (eMAP®) technology to identify the presence or absence of the selected alleles associated with a given phenotype. Each probe is covalently attached to a spectrally distinguishable bead type. A library of individual bead types contains all of the probes of interest, including positive, negative, and system controls.

Method
Polymerase Chain Reaction (PCR) with Immucor’s BioArray BeadChip microarray technology. Twenty-four polymorphisms associated with thirty-eight Human Erythrocyte Antigens and phenotypic variants are included in the BioArray Solutions HEA BeadChip Kit: C, c, E, e, VS, V, K, k, Jsa, Jsb, Kpa, Kpb, Fya, Fyb, Fyx, GATA, Jka, Jkb, M, N, S, s, U-, Uvar, Lua, Lub, Doa, Dob, Hy, Joa, LWa, LWb, Dia, Dib, Coa, Cob, SC1, SC2.

Results
The BeadChip assay was implemented in 2011, validation completed in 8 weeks using patient specimens with previously known phenotype or tested concurrently. We tested 27 patients for validation. Validation results obtained were compared with the serological phenotype results and discrepancies investigated. In total, we performed 132 more genotypes to date. The patients eligible for testing were selected based on diagnosis and difficulties with serological phenotype. These include: 45 warm autoimmune anemia, 29 sickle cell anemia, 16 multiple antibodies, 15 recently transfused and 9 BMT patients. The remaining 17 included Thalassemia patients, potential donor units and if antisera were not available. For discrepancies investigated the root cause was: serological phenotype performed on recently transfused patients or the antisera sensitivity was inadequate.

Summary / Conclusions
Our validation was successful and showed that genotyping using the BeadChip is a suitable modality where phenotyping is not possible. The accuracy and objectivity of genotype is a major benefit. Using genotyping where patients’ phenotype is invalid due to recent transfusions or positive DAGT is most valued. For a large tertiary care hospital this added technology increases the complexity in supporting patients with difficult serology and problematic compatibility. Identifying the presence or absence of antigens by PCR rather than phenotyping is increasingly useful. Our future plan is to include the perinatal cases.
MTS Gel Versus Tube Modified IAT for Eluates

Scientific, Poster Presentation

Lynne Oldfield MLT, Canadian Blood Services
Davide Au MLT, Canadian Blood Services
Liese Bolte MLT, Canadian Blood Services
Christopher Sumner MLT, Canadian Blood Services
Zofia Salomonde Friedberg MLT, Canadian Blood Services
Wendy Lau MD, Canadian Blood Services

Purpose
To validate the use of the MTS Gel IgG card technology (MTS Gel) for testing of eluates prepared by the Immucor ELU KIT Plus.

Method
Eluates were prepared and tested following the manufacturer's recommended Modified Antiglobulin Test (MAT). The same eluate was then tested in parallel by MTS Gel. A total of twenty eluates were tested, eighteen patient samples and two control samples. The first control was a known anti-Fya sample which was adsorbed onto known Fy(a+) cells and eluted. The second control was a known anti-D sample which was adsorbed onto D+ cells and eluted. Reactions were graded 0 to 4+.

Observation
In all twenty cases, the eluates reacted significantly more strongly in the MTS Gel assay than in the MAT. In seven cases, the eluate reacted 4+ by MTS Gel and 3+ by the MAT; in ten cases, the eluate reacted 3+ to 4+ in MTS Gel and 1+ to 2+ by MAT. In one case, the eluate reacted 2+ in MTS Gel and was non-reactive in MAT. The two eluates from the anti-Fya and anti-D control samples reacted 2+ in MTS Gel and weakly by MAT.

Discussion
The advantages of the MTS Gel IgG card technology are that it is easy to use, yields easy to read results, uses less sample, is more sensitive than the tube method and the cards can be stored for later review. The ELU Kit Plus does not recommend testing the eluate by MTS Gel, however in our lab we have demonstrated that the MTS Gel is a more sensitive test than the MAT for testing eluates. We have adopted the MTS Gel IgG card technology for testing eluates whenever we are presented with a limited volume of patient sample.
61. Alloimmunization Rates in Chronic Transfusion Patients in the Edmonton Zone

Scientific, Poster Presentation

Davita Ou, University of Alberta
Kristi Lew MLT, MSc, University of Alberta
Susan Nahiriak MD, FRCPC, Alberta Health Services
Gwen Clarke MD, FRCPC, Alberta Health Services

Purpose
Alloimmunization among patients receiving chronic transfusion therapy is a rising concern as these patients are in constant need of antigen matched donor units. Chronic transfusion patients are defined in our zone as those receiving a transfusion of red cells at least once every two months for a minimum of six months. The specificity of these antibodies and the time of identification (before or after starting chronic transfusions) are of particular concern as well as the frequency of antibody groups and whether antibody development can be related to any specific diagnosis.

Methods
A retrospective study was performed using data collected from the Laboratory Information System (LIS) to identify patients chronically transfused from January to December 2012. Additionally, the number and type of antibody each patient formed, date of antibody identification, the primary diagnosis, and demographic information such as age and gender were determined.

Results
A total of 254 chronic transfusion patients were identified, with 62 (24.4%) of these patients being alloimmunized. The most commonly occurring antibodies were the Rh, Kell, and Kidd blood group antibodies, which were found in 28, 18, and 14 patients respectively. Of these alloimmunized patients, 35 (56.5%) formed at least one antibody following the onset of their chronic transfusion therapy. The 254 patients received a total of 7269 red cell units with an average of 29 units each.

Conclusion
Despite efforts to supply chronic transfusion patients with Rh and Kell matched units, anti-E and anti-K are still the most commonly identified antibodies in this patient population. Antibodies first identified during chronic transfusion therapy belonged most commonly to the Rh, Kell, and Kidd blood group systems. The data suggests that further improvements can be made in providing these patients with antigen matched units in order to prevent the development of alloantibodies.
62. Relevant Coagulation Factors are Stable for 24 Hours in Thawed Cryoprecipitate Stored at Ambient Temperature

Scientific, Oral Presentation

William Sheffield PhD, Canadian Blood Services
Varsha Bhakta BSc, Canadian Blood Services
Craig Jenkins BSc, Canadian Blood Services

Background
Frozen plasma (FP) for transfusion can be further processed into cryoprecipitate by slow thawing, centrifugation and removal of the cryosupernatant plasma. Cryoprecipitate is contained in a small volume of 6 – 14 ml, and is enriched in large proteins such as fibrinogen, von Willebrand factor (VWF), FVIII, and FXIII. It is currently indicated for the management of patients requiring fibrinogen or FXIII supplementation. Current standards require cryoprecipitate to be maintained at 20-24°C once thawed, and infused within 4 hours, and to contain ≥ 150 mg fibrinogen activity per unit in ≥ 75% of units tested.

Objective
To determine if the period of cryoprecipitate storage could be extended from 4 to 24 hours to reduce product wastage at outdate.

Methods
Thirty-six cryoprecipitate units (18 type O, 18 type non-O) were thawed, diluted with saline and stored at ambient temperature for 24 hours. Samples were aseptically obtained at thaw (0), 4, and 24 hours post-thaw. Samples were tested for FVIII and fibrinogen activities using an automated coagulation analyzer, and for FXIII and VWF activities using commercially available kits.

Results
All units contained ≥ 150 mg of fibrinogen at thaw (range 158 – 543 mg/unit). At thaw, units contained 280 ± 100 mg of fibrinogen activity/unit, 80 ± 30 international units (IU) FXIII, 140 ± 40 IU FVIII, and 240 ± 90 IU VWF activities, respectively. No statistically significant reduction in mean coagulation factor activity was noted between 0 and 24 hours of storage. All factors showed a small but statistically significant increase between 0 and 4 hours of storage, ranging on average from 3.3 to 7.2 %. No statistically significant difference was noted between units stored for 4 versus 24 hours in fibrinogen, VWF, or FXIII activities; a small but statistically significant decline of -5.4 was noted in FVIII levels over this period.

Conclusion
Extending the storage time of cryoprecipitate to 24 hours post-thaw was not associated with a drop in either regulated (fibrinogen), indicated (FXIII), or historically important (FVIII, VWF) components of this plasma derivative. Small increases in factor recovery over time may have resulted from final dissolution of aggregated plasma proteins.
Dengue virus (DENV) is the most prevalent arboviral infection on the globe, annually infecting ~50 million people. Although DENV predominates in tropical regions, due to vacation travel, recent outbreaks in the southern United States and documented transfusion transmission Canadian Blood Services has listed it as an emerging pathogen. Four serotypes (DENV1-4) exist, manifesting from benign subclinical flu-like symptoms to severe life-threatening hemorrhagic fever and shock syndrome resulting in 500,000 hospitalizations per year and requiring extensive platelet and plasma replacement. Escalation to severe pathology likely occurs after infection by a second serotype introduces non-neutralizing cross-reactive antibodies that mediate infection of Fc-receptor bearing cells. To account for the thrombocytopenia typical of severe disease, earlier studies (Anderson et al, 1995) using washed platelets and semi-purified virus concluded that the dengue virus-platelet interaction requires this Fc-dependent mechanism. Since 105-106 DENV particles/ml of blood may exist in an asymptomatic blood donor without cross-reactive antibodies, we investigated the Fc-independent binding of purified DENV to purified platelets using sensitive qRT-PCR. Here, we present the first evidence that DENV1 and 2 bind directly to platelets independent of virus-specific antibody, further explaining the basis of platelet dysfunction during infection, but also implying that the virus may partition with platelets during blood cell fractionation. Binding at 37°C was saturable over the concentration range used for DENV1 and 2 at 4 and 3 viruses/platelet, respectively, which was confirmed antigenically. Fc had no effect on binding, which excludes a role for platelet Fc-receptors. After treatment of platelets with thrombin, enhanced binding indicated stimulus-induced expression of receptors, which may occur during pathology. Furthermore, pretreatment of platelets with purified DENV1 or 2 increased detection of P-selectin expression as measured by flow cytometry, suggesting that the virus can trigger platelet activation. Since the DENV genome consists of positive strand-RNA, replication by platelets was investigated. At 25°C, the temperature platelets are stored in the blood bank, the genome of DENV1-4 was replicated 5- to 7-fold after 7 days. At 37°C, only DENV2 and 3 genomes were replicated. These data suggest that platelets obtained from asymptomatic donors may harbor and enhance dengue virus during storage.
Antibacterial Activity of Cord Blood

Scientific, Poster Presentation

Louis Thibault PhD, Héma-Québec, R&D
Marie-Pierre Cayer MSc, Héma-Québec, R&D
Joanie Pichette, Héma-Québec, R&D
Diane Fournier, Héma-Québec, Public Cord Blood Bank
Gilles Delage MD, Héma-Québec, Medical Affairs
Marie-Claire Chevrier MSc, Héma-Québec, Public Cord Blood Bank

Purpose
Currently, no standards are available for microbial contamination testing in cord blood banks (CBBs). For instance, some organizations use cord blood plasma to inoculate culture bottles; others recover the leftover cell concentrate remaining in the bag or collect a small aliquot from the final unit before freezing. It is known that plasma might have an antimicrobial activity on some bacteria. In this work, we studied the antimicrobial activity of plasma extracted from cord blood units and adult whole blood.

Methods
The antimicrobial activity of plasma extracted from 60 cord blood units and 20 adult whole blood samples (controls) was analyzed using an inhibition test similar to that of an antibiogram. Blood agar dishes were first inoculated with Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Micrococcus luteus, Propionibacterium acnes, Bacteroides fragilis, Bacteroides uniformis, Klebsiella pneumoniae, Streptococcus agalactiae, or Enterococcus sp. Thirty µL of plasma or PBS (control) were next placed on 13 mm antibiotic assay disks deposited on the agar surface. Zones of inhibition were measured after an overnight incubation at 37°C. Aliquots of plasma showing an antibacterial activity were heated to 56°C for 30 minutes to inhibit complement proteins or treated with a β-lactamase enzyme to abolish penicillin activity.

Results
Significant antimicrobial activity was observed in 29% of cord blood samples, whereas none of the adult plasma samples inhibited the growth of tested bacteria. Only Gram-positive bacteria were growth-inhibited by cord blood plasma samples. The inhibitory activity was not destroyed by heating at 56°C, indicating that complement proteins are not involved. However, treatment of plasma samples with a β-lactamase enzyme abolished the antibacterial activity, confirming that antibiotics are present in these samples.

Conclusions
This study shows that antibiotics are often found in cord blood units. Consistent with this observation, antibiotic prophylaxis is frequently used to prevent infections in obstetrical procedures. Additional work will be required to better understand the impact of antibiotics on the efficacy of the sterility test done on each cord blood unit.
Purpose
Microbial contamination of umbilical cord blood (UCB) products represents a substantial risk of complications after transplantation to immunodeficient patients. Nowadays, sterility testing uses standard microbiological culture-based methods which are time-consuming and require high inoculum volumes. Recently, Patel and coworkers (Transfusion, 2012) have published on a new molecular strategy to detect microbial contaminations in platelet concentrates. Here, we have applied this qPCR assay to detect the presence of bacterial contaminants in UCB samples.

Methods
Cord blood samples were inoculated using four common UCB contaminants (E. coli, K. pneumonia, S. aureus and Enterococcus sp.) at 10, 100 and 500 colony-forming units (CFUs). DNA extractions were optimized using a commercial kit designed for isolation of bacterial DNA (Molzym). The qPCR assay was based on the amplification of a universal DNA sequence from the bacterial 16S ribosomal DNA. Mitochondrial DNA was co-amplified as an internal control to confirm the reliability of DNA extraction and amplification. We also compared the qPCR detection limits with our current bacterial contamination culture method (BacT/ALERT system).

Results
After optimizing the DNA extraction protocol and determining cut-off values for the qPCR assay, the average detection limit was about 300 CFUs per mL. No differences were observed between the detection of Gram-negative and Gram-positive bacteria. Despite efforts to optimize DNA extraction, the sensitivity and reproducibility of the qPCR assay seems to be affected by the presence of human DNA and inhibitors present in the UCB matrix. The total time for both DNA extraction and amplification was less than four hours, much shorter than the BacT/ALERT system, which may take several days to detect slow-growing microorganisms.

Conclusions
The qPCR method does not have the required sensitivity and efficiency to detect microbial contaminants in UCB samples. The BacT/ALERT system remains a more sensitive assay to detect bacterial contaminations in these samples. Further work is required to improve the sensitivity of the qPCR method.
Optimization of Whole Blood Centrifugation Process to Improve Plasma Recovery

Scientific, Poster Presentation

Louis Thibault PhD, Héma-Québec, R&D
Étienne Fisette MBA, Héma-Québec, Exploitation
Annie Jacques MSc, Héma-Québec, R&D
Jocelyne Dion, Héma-Québec, Quality Assurance
Bernard Renaud, Héma-Québec, Exploitation
Martine Richard PhD, Héma-Québec, R&D
Marie Joëlle de Grandmont MSc, Héma-Québec, R&D
Louis-Philippe Gagné BSc, Héma-Québec, Exploitation

Purpose
In recent years, Héma-Québec has introduced several changes in its blood processing operations. Automated extractors (MacoPharma) have now replaced manual extractors, thus improving the consistency and quality of blood products. The efficacy of these extractors is influenced by the settings applied during whole blood (WB) centrifugation prior to plasma extraction. Interestingly, our centrifugation settings have not been revised over the last 15 years. In this work, we have revised and validated a new centrifugation time to improve plasma recovery.

Methods
For centrifugation settings verification, WB (450 mL) was collected in CP2D using the Pall Medical Leukotrap WB collection set. Blood from 3 ABO-compatible donors was pooled and split to obtain 3 identical WB units. After filtration, WB was centrifuged for 5, 10 or 15 min. at 5 147 × g (4°C). Quality parameters of blood products were measured (volume, yield and hemolysis) to determine the optimal centrifugation time. Next, 63 WB units were centrifuged for 10 min. at 5 147 × g. Plasma expression and AS-3 addition to red blood cell (RBC) units were achieved using automated extractors. The amount of residual plasma in RBC units was determined, based on the ratio of IgG in RBC supernatants and their respective plasma. Results were compared to blood products (n=60) processed using our current centrifugation parameters (5 147 × g, 5 min.).

Results
By increasing centrifugation time from 5 to 10 minutes, the volume of plasma units increased from 263 ± 23 mL to 283 ± 16 mL, for an improved yield of 20 mL per bag (p < 0.05). Conversely, increasing the centrifugation time has reduced the residual volume of plasma in RBCs from 39 ± 12 mL to 20 ± 5 mL. The percentage of hemolysis in RBCs was comparable in both centrifugation conditions.

Conclusions
This work shows the importance of regularly reviewing the parameter settings applied to blood processing. An increase of only 5 minutes in the WB centrifugation time has little impact on operations, but significantly reduces the volume of residual plasma in RBCs. The average recovery of 20 mL of plasma per donation represents a potential gain of 4 000 liters of plasma annually.
Standardization of Hematocrit in COBE 2991 Deglycerolized SAGM RBCs

Tracey R. Turner MLT, Research and Development, Canadian Blood Services
Adele Hansen BSc, Research and Development, Canadian Blood Services
Jayme D.R. Kurach MSc, Research and Development, Canadian Blood Services
Jason P. Acker PhD, Research and Development, Canadian Blood Services and Department of Laboratory Medicine and Pathology, University of Alberta

Background
The COBE 2991 cell processor can be used to produce cryopreserved, deglycerolized and washed RBCs. Canadian standards require that the hematocrit (hct) in at least 90% of deglycerolized RBCs be ≤ 0.80 L/L. Historically, deglycerolized RBCs had an average hct of 0.80 ± 0.04 L/L, and a range of 0.71 L/L to 0.90 L/L, resulting in only 52% meeting the standard. The purpose of this study was to determine the optimal amount of saline/dextrose to suspend deglycerolized RBCs in order to reduce hcts.

Methods
Post-processing addition of saline/dextrose was used to reduce deglycerolized RBCs to the target hct range of 0.50 L/L to 0.70 L/L. Using historic quality control data for hct and volume of COBE 2991 deglycerolized RBCs, it was estimated that addition of 65 to 90 g (median 75 g) of saline/dextrose would result in a targeted mean hct of 0.60 L/L. A pool and split study was completed and 12 groups of 4 deglycerolized RBCs were used to examine four experimental conditions; suspension in 0 g (control), 65 g, 75 g, or 90 g of saline/dextrose. All RBCs were tested for weight, volume, hct, hemoglobin content, recovery, supernatant K+, hemolysis, and ATP concentration. Results: All resuspended RBCs were able to meet the hct standard of ≤ 0.80 L/L in at least 90% of units tested with a mean hematocrit of 0.66 ± 0.07 L/L. The addition of the median 75 g of saline/dextrose resulted in RBCs with mean hematocrit 0.62 ± 0.02 L/L, unit volume 286 ± 22 mL, hemoglobin content 51 ± 4 g/unit, recovery 92 ± 4%, hemolysis 0.48 ± 0.06% and a ATP concentration of 3.88 ± 0.50 μmol/g Hgb. The ATP concentrations for all resuspended RBCs were statistically higher than control RBCs (p<0.001).

Conclusion
The increase in ATP is likely due to the suspension solution containing dextrose that is known to preserve and stimulate ATP production. Deglycerolized RBCs suspended in a fixed mass of saline/dextrose had a more standardized hematocrit and were able to meet all standards with equivalent or improved in vitro quality.
One of the most effective interventions in clinical medicine has been the administration of Rh immune globulin (anti-D) to prevent immunization of the mother to the D antigen on the fetal red blood cells (RBCs); a phenomenon termed antibody mediated immune suppression (AMIS). The mechanism of action of how such antibodies can prevent an immune response remains unclear but several theories have been proposed. The most commonly accepted theory to explain how AMIS occurs in humans is by the rapid clearance of the antigen positive RBCs. Unfortunately mouse models to date have been unable to address this question because in these models the RBC (commonly sheep RBC) are xenogeneic to the host and the cells are rapidly cleared. To explore the role of RBC clearance in AMIS, we immunized naïve mice with murine transgenic RBCs expressing the HOD (Hen egg lysozyme (HEL), Ovalbumin, and human Duffyb) antigen in the presence or absence of 6 different monoclonal antibodies to selected portions of the HOD molecule. The isotype and specificity of these antibodies as well as their ability to sterically hinder the HOD molecule and cause accelerated RBC clearance were assessed. Mice immunized with HOD RBC made IgM and IgG reactive with the HEL portion of the molecule and 6/6 antibodies tested could inhibit the response. The HEL-specific monoclonals (4B7, IgG1; GD7, IgG2b; 2F4, IgG1) displayed minimal steric hindrance properties and did not accelerate the clearance of the HOD RBC. The 3 Duffy-specific antibodies (MIMA29, IgG1, CBC-512, IgG2a; K6, IgG2a) displayed no steric hindrance but all caused rapid clearance of HOD RBC. This is the first demonstration of AMIS in a full murine model. The model clearly demonstrates that AMIS can occur normally in the absence of steric hindrance and RBC clearance. The AMIS effect also appears to be independent of the IgG isotype and fine specificity of the AMIS inducing antibody.