Abstracts of the Joint Conference of the Canadian Society for Transfusion Medicine, Canadian Blood Services and Héma-Québec, Banff, Alberta, Canada, 21–24 April 2005

INTRODUCTION
The 2005 Joint Conference of the Canadian Society for Transfusion Medicine, Canadian Blood Services and Héma-Québec was held in the Banff Centre in Banff, Alberta, Canada in April 2005. With a theme of ‘Mountains, Moguls and Modern Advances in Transfusion Medicine’, the conference provided an opportunity for more than 350 Canadian and international scientists, physicians, nurses, technologists, educators and administrators to come together to discuss current and emerging issues in transfusion science and transfusion medicine. Dr Linda Pilarski, the Canada Research Chair in Biomedical Nanotechnology at the University of Alberta, gave the keynote presentation on recent advances in the integration of nanotechnology and microfluidic devices in diagnostic testing. The following abstracts of papers and posters presented at the Conference represent the diverse scope of transfusion medicine and the transfusion sciences in Canada.

INVITED ORAL PRESENTATIONS
Workshop A: SOP Development and Review

ABSTRACT NO.: 1
The importance of work process mapping
L. Berté
Quality Systems Consultant, Westminster, Colorado, USA.

All blood bank and transfusion service work is carried out as a series of work processes. Each work process needs to unfold in a defined sequence to ensure that pre-defined inputs lead to a successful output.

This program presents information about blood bank and transfusion service work processes and how to diagram them using flowcharts. Properly prepared flowcharts can subsequently be used to identify the written procedures needed to provide staff members with instructions about how to perform the activities in the process. The written procedures become the facility’s “SOPs”.

ABSTRACT NO.: 2
SOPs: Templates, tips and taboos
D. Evanovitch
Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario, Canada.

Templates Discussion of what must be in an SOP: facility identifier, title, references, pagination, purpose, etc. Some templates examples will be given and discussed.

Tips Discussion of standard language, use of glossaries, whether to include/not include IS steps, the importance of linking SOPs to process maps to avoid omission and duplication, implementing urgent changes quickly, etc.

Taboos Discussion of the ‘don’ts’ of SOPs: DON’T write on SOPs, post non-SOP referenced work instructions, have missing SOPs or SOP pages. Ensure that pertinent SOPs actually in the pertinent work area.

ABSTRACT NO.: 3
SOP development and review: Capital health experience
B. Luntz & T. Richardson
Capital Health, Edmonton, Alberta, Canada.

BACKGROUND After extensive restructuring in 1996, the transfusion service in the Capital Health (CH) region of Alberta identified the need for an efficient and effective Standard Operating Procedure (SOP) development and review process. Regionalization and the use of a common Laboratory Information System (LIS) created the need for joint development and review of standardized SOPs by 7 different hospitals under the direction of 2 Medical Directors.

METHODS The CH region has developed a series of tools to decrease administrative workload and delays in writing new SOPs and reviewing existing SOPs. Mapping the process has created a consistent model that has removed delays due to variation in practice. The use of a widely adopted word processor has allowed for flexibility in personnel responsible for writing the documents. Use of the existing network infrastructure in CH and an annual review schedule has allowed stakeholders to provide input at their convenience. Correlation of input by document editors has also been minimized by this practice. Reduction in administrative work-load has been realized through use of document control software. Tracking of version numbers for over 200 SOPs is now handled automatically by software. The use of competency questions and a topic index has increased user satisfaction.

CONCLUSIONS With the use of a set process and existing hospital network infrastructure, a SOP development and review system has been implemented in a large regional Transfusion Service.

Workshop B: Transplantation and Transfusion

ABSTRACT NO.: 4
Cryobiology and the preservation of stem cells
L. E. McGann
Edmonton Stem Cell Laboratory, Canadian Blood Services; Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada.

Recent developments utilizing transplantation of stem cells all depend critically on the number of functional cells transplanted, and this has revived interest in maximizing recovery and function during collection, processing and preservation of stem cells. As new sources of stem cells are being explored, it is becoming clear that some of these potentially valuable cells have low recovery using conventional approaches to cryopreservation. This presentation will outline the basic elements during cryopreservation procedures that affect cell viability, and describe how these are implemented in current procedures for cryopreservation of stem cells for transplantation. Even with the widespread use of cryopreserved
hematopoietic stem cells in autologous transplantation, there is morbidity and mortality associated with the use of the common cryoprotectant, dimethyl sulfoxide, which is not approved for human use. This presentation will describe the principles underlying current approaches to reduce or eliminate dimethyl sulfoxide in cryopreservation procedures.

ABSTRACT NO.: 5
Héma-Québec’s experience in the development of a public cord blood bank
G. Delage, L. Peltier, L. Richard & D. Roy
Héma-Québec, Québec, Canada.
In this presentation, the various aspects (legal, ethical, technical, regulatory, quality and economic) to consider in the development of a public cord blood bank will be described. The following areas of development will be described: donor information, recruitment and qualification, cord blood collection, volume reduction and cryopreservation, laboratory testing, information technology, coordination with partners and SOP validation. The costs associated with the development of such a bank will be outlined.

ABSTRACT NO.: 6
Unrelated stem cell transplantation
M. Goldman & B. Campbell
Canadian Blood Services, Ottawa, Ontario, Canada.
The use of unrelated donors for bone marrow and peripheral blood stem cell (PBSC) transplantation has increased over the last few years, with over 200 patients transplanted in 2004. The Canadian UBMDR is the sixth largest in the world. However, over 50% of marrow and PBSCs transplanted to Canadian patients come from international donors. A comparison will be made between various international registries. Challenges facing the Canadian registry will be discussed.

ABSTRACT NO.: 7
The Lifebank medical research donor program: A model for industry support of academic research
E. Stacey
Lifebank Cryogenics Corporation, Vancouver, British Columbia, Canada.
Hematopoietic progenitor cell use for research is rapidly increasing in Canada. However, accessing sustainable supplies of human cellular materials is often difficult. The purpose of the Medical Research Donor Program is to create an ethically acceptable and sustainable supply of hematopoietic progenitor cells for Canadian academic research. Traditional barriers to effective relationships between corporations and academia are being challenged. Adherence to strict policies of ethical practice are enabling improved corporate and academic relations. In support of academic research key variables impacting supply and access to human hematopoietic progenitor cells in Canada were studied. Canadians are willing to donate cord blood progenitor cells for academic research. Issues related to access of existing cord blood supplies were identified as the key impediment to academic utilization of these resources. We emphasize the principals of inherent value, complete disclosure and education in consent process design. A model consisting of a consent process encompassing a novel consent form and corporate due diligence was designed and implemented. We conclude that through the application of novel methods and attitudes an ethically acceptable and sustainable supply of human hematopoietic progenitor cells can be made available to Canadian academic researchers.

ABSTRACT NO.: 8
Transfusion support of stem cell transplant patients
J. Russell
Tom Baker Cancer Centre; Foothills Hospital, Calgary, Alberta, Canada.
Hematopoietic stem cell transplant (SCT) patients present particular challenges for transfusion support. All patients became pancytopenic after myeloablative conditioning and some may have had prolonged transfusion support and become refractory to platelets in particular. While most patients can be supported with relatively few blood products, the demands from a minority of patients can be high. Because these patients are particularly immunosuppressed, they are at higher risk from transmission of infection from blood products. Pre-transplant screening and modern filtration techniques can minimize these risks. Filtration has also helped to reduce the incidence of some transfusion reactions which are sometimes difficult to differentiate from other causes of fever in SCT patients. Transfusion related graft-versus-host disease, a potentially fatal disorder, can be prevented by irradiation of all blood products. Red cell transfusion may be particularly complicated by incompatibilities between donor and recipient. Allogeneic transplantation eventually results in partial or complete conversion to donor ABO type. However, intelligent management of transfusion products can enable support of patients, even across major ABO incompatibility barriers.

Workshop C: Donor and Recipient Management: A Nursing Perspective
ABSTRACT NO.: 9
Adverse reactions in donors: Identification and management
D. Benoit
Canadian Blood Services, Edmonton, Alberta, Canada.
All clinic staff observes donors for signs of reactions throughout the entire donation process. At CBS, we have several procedures to guide staff monitoring the donor to providing post donation care. The Donor Reaction and Incident Manual provide direction to staff to recognize, categorize, treat and document signs and symptoms. This has been an evolving process that includes moving from a two bed donor model to a one bed model. Keeping donor post-donation movement to a minimum assists in keeping reactions to a minimum. Reactions occur as early as registration to refreshment. Reactions are categorized into three main categories: light, moderate and severe. Reactions where the symptoms resolve quickly, in less than 15 minutes are categorized as light or mild. These also include reactions mild in nature with recovery time of greater than 15 minutes or, mild in nature and occur after the donation process is complete. When a donor experiences cardiovascular involvement with or without loss of consciousness taking about 30 minutes to fully recover, it is classified as a moderate reaction. Severe reactions include significant changes to vital signs, neurological signs (loss of consciousness greater than 30 seconds). These also take greater than 30 minutes to recover. Each of these adverse reaction categories has its own signs and symptoms as well as treatments. Occasionally, donor incidents also occur in conjunction with or without an adverse reaction. Incidents may be as minor as bandage adhesive dermatitis and venipuncture complications or serious as fractures and cardiovascular problems. All reactions/incidents are documented in detail on a Reaction/Incident Report. Donors are provided with instructions for care after leaving the clinic, including a follow-up call by one of our nurses. In addition to all of these reaction/incidents, donating by apheresis adds its’ own list of complications.

ABSTRACT NO.: 10
Apheresis blood donation – The process
C. Anderson
Canadian Blood Services, Calgary, Alberta, Canada.
Plasmapheresis and Platelethpheresis is the process whereby donor blood is separated into components using apheresis equipment that extract only the plasma or platelets respectively and return the remaining blood components to the donor. Prior to donation every Donor is assessed through a comprehensive screening process that includes: blood donor eligibility standards, pre-donation information pamphlet, questionnaire, interview, blood pressure and temperature measurements, confidential unit exclusion, verification of records of previous donations, and laboratory screening tests.
Donors may be temporarily, indefinitely, or permanently excluded from donating due to health history or test results. Apheresis procedures are generally well tolerated; however potential adverse reactions specific to this donor population include citrate and vasovagal reactions. The utilization of apheresis donations are effective in the treatment of patients experiencing: bleeding disorders, liver disease, serious burns, shock, and cancer/bone marrow therapy. Patients who receive multiple transfusions, sometimes produce antibodies. These patients require more extensive Human Leukocyte Antigen (HLA) matching in addition to ABO typing. Single treatment with platelet products collected during whole blood donation would require a minimum of 4 to 5 different donors. Because of the collection efficiency of plateletapheresis sufficient platelets can be collected from 1 donor. Plasmapheresis allows collection of 2 to 3 times the amount of plasma that is collected from a whole blood donation.

ABSTRACT NO.: 11
Therapeutic plasma exchange and stem cell apheresis
S. Selinger, J. Klassen, L. Coombs, L. Rothenburger, A. Hester & J. Merlin-Westcott
Foothills Hospital, Calgary, Alberta, Canada.

Therapeutic Plasma Exchange (TPE) is the separation of blood into its component parts using centrifugal force. Large volumes of plasma are removed and replaced using a plasma substitute such as albumin, cryoprecipitate, or fresh frozen plasma. Currently, Pentaspan is being investigated as a plasma replacement. Numerous controlled trials have shown Therapeutic Plasma Exchange to be effective in treating a number of autoimmune diseases such as Myasthenia Gravis, Acute Guillain-Barre, and Thrombotic Microangiopathy. TPE procedures must be repetitive, and may require the addition of immunosuppressive medications to prevent further antibody production. Medications such as prednisone, methylprednisolone, azathioprine, and cyclophosphamide are widely used for this purpose. Stem cell transplants are performed for patients with some types of tumors, for example, lymphoma. While drugs are available to eradicate the tumor, they would also destroy the patient’s own bone marrow. To rescue the patient from this eventuality, stem cells that can repopulate the destroyed bone marrow can be harvested (collected) using the Apheresis equipment (Stem Cell Apheresis). The cells are collected from the patient themselves or from a matched donor. This is usually accomplished in one procedure with few, if any side effects to the patient. Alternatively, a bone marrow harvest can require up to 200 needle aspirations necessitating a general anesthetic. Stem Cell Apheresis is performed for pediatric and adult patients in our centre.

ABSTRACT NO.: 12
Formation des infirmières en médecine transfusionnelle
G. Labonte
Hôpital Charles LeMoyne, Centre désigné des activités transfusionnelles, Montréal, Canada.

OBJECTIFS: Présentation de la démarche suivie depuis mars 2000 afin d’assurer la formation des infirmières dans notre région. Démonstration de l’élaboration des priorités dans un contexte de protection des patients.

MÉTHODES: L’évaluation des procédures dans les 10 centres hospitaliers de la région a démontré que plusieurs centres n’étaient pas à jour dans leur pratique transfusionnelle. Nous avons établi nos priorités de formation afin d’assurer la sécurité des patients. Comme aucun centre n’avait de procédure de prélèvements conforme aux normes de la médecine transfusionnelle, nous avons donc priorisé ce sujet. Un bref rappel antigènes-anticorps des systèmes ABO et Rh débutait cette formation afin de démontrer les conséquences d’une erreur de prélèvement. Toujours dans un souci de sécurité, la 2e formation portait sur les réactions transfusionnelles incluant l’enseignement des autres systèmes érythrocytaires. Par la suite, il a fallu former les infirmières sur le nouveau bordereau d’émission des produits sanguins qui est relié au système informatique provincial implanté dans toutes les banques de sang du Québec. Pour l’enseignement continu, un bulletin d’information est produit périodiquement afin de fournir de courtes informations de façon régulière.

RÉSULTATS: Depuis le début des formations, une nette amélioration a été notée. Par ailleurs, comme il est impossible de former toutes les infirmières compte tenu du grand nombre, mais aussi de la mobilité de celles-ci, il est important de s’entourer de personnes qui assurent le suivi de cet enseignement.

CONCLUSION: L’évaluation des connaissances permet de guider les besoins en formation et les audits en verifiant la compréhension.

Workshop D: Blood Conservation
ABSTRACT NO.: 13
Perioperative blood conservation
S. Tomlinson
Baxter BioSurgery, Calgary, Alberta, Canada.

FACt: bleeding can, and does, happen during surgery. Fact: blood products are a precious commodity so, anything that come be done to minimize need for blood products should be done. The presentation will cover a variety of hemostatics, glues and sealants used in surgery to obtain hemostasis, to prevent leakage, and to glue tissues together.

The information will include basics of wound healing process and then integrate techniques and products currently available in the operating room, their methods of action, and when one would expect to obtain the best results from each of these products and why. The goal is that the attendees will have a better understanding of methods of hemostasis incorporated during surgery and why.

ABSTRACT NO.: 14
Blood conservation techniques in the perioperative period
B. Muirhead
Health Sciences Centre, Winnipeg, Manitoba, Canada.

Blood Conservation Programs have had a definite effect on reducing the number of homologous blood transfusions. With the aid of a Nurse Coordinator, communication and strategies can be introduced between patient, surgeon, anesthesiologist and the blood supplier. Established blood conservation techniques include correction of anemia, iron therapy, erythropoetin, autologous predonation, intra-op donation, and cell salvage. Some of these techniques have become controversial, and vary from region to region. Cost effectiveness of these programs is difficult to establish, and the future of such programs is unclear. The most significant detriment to such a program continues to be the lack of physician education.

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ABSTRACT NO.: 16
Calgary health region’s approach to blood conservation
C. Gibson
Foothills Medical Centre, Calgary, Alberta, Canada.

The Perioperative Blood Conservation Program in the Calgary Health Region was conceived in January 2002 and began operation in July 2003. From initial multidisciplinary support and funding concerns through introduction of algorithm-based management of blood conservation, the PBCP continues to evolve. A brief review of this evolution along with an outline of new initiatives and challenges for the program will be presented.

ABSTRACT NO.: 16
Blood conservation – Transfusion triggers
S. Nahirmia
Department of Laboratory Medicine and Pathology, University of Alberta; Capital Health, Edmonton, Alberta, Canada.

This interactive workshop will allow for discussions of various “transfusion triggers” using a case-based format. Several different cases with various lab findings and a wide variety of perioperative and interoperative interventions will be presented in a step question based model. The goal is to provoke discussion of the various
factors which should be considered prior to transfusion of autologous or allogeneic products rather than hard and fast rules.

Workshop E: Advanced Techniques in Transfusion Medicine: Case Studies in Auto and Allo Immunity

ABSTRACT NO.: 17
Serological techniques
J. Ashdown
Canadian Blood Services, Edmonton, Alberta, Canada.
Through the use of case studies, the speaker will discuss techniques involving the treating of plasma or cells to aid an investigation. These techniques can be used to obtain autologous cells for pheno-typing, treating cells for exclusions and distinguishing IgG from IgM antibodies.

ABSTRACT NO.: 18
Advanced serological techniques – Case studies
H. Gaal
Department of Laboratory Medicine and Pathology, University of Alberta Hospital, Edmonton, Alberta, Canada.
Many types of serological problems are encountered in the transfusion medicine laboratory. Most of them are straightforward and easily resolved. However, some require more advanced techniques and a little detective work in order to provide the patient with a safe transfusion. The speaker will work through a number of serological case studies and will explain the techniques and tools used to resolve them. The cases will include a couple of examples of antibodies to high incidence antigens and a case involving an antibody secondary to passenger lymphocytes from a donor liver.

ABSTRACT NO.: 19
Warm antibodies – The dread of most transfusion medicine technologists
M. Phillips
Department of Laboratory Medicine and Pathology, University of Alberta Hospital, Edmonton, Alberta, Canada.
This workshop will cover not only the autologous adsorption method, but the homologous and differential adsorption methods. Several case studies demonstrating the PEG autoadsorption method will be reviewed.

ABSTRACT NO.: 20
Advanced serological techniques
M. Yazer
The Institute for Transfusion Medicine; Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.
In this session, after a brief description of a few of the important genes relevant to transfusion medicine, some cutting edge applications of blood group genetics will be described including the use of maternal plasma to perform fetal genotyping. In addition, the future of ABO blood group genetics will be described with a view to understanding the genes that encode hyperfunctional glycosyltransferases.

Workshop F: What’s in the Box?
Transportation of Blood Products in Canada

ABSTRACT NO.: 21
CBS transport box validation: Past, present and future
I. Croteau1 & J. P. Acker1,2
1Canadian Blood Services; 2Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada.
Since the beginning of centralized blood collection clinics, there has been a need to transport blood from donor to recipient. In 1968, in an effort to ensure blood product quality and in response to regulatory requirements, the Canadian Red Cross chose to validate its existing blood transport container. In 1996, a more rigorous validation was performed of the same container along with a newer container first used by the American Red Cross. Those containers continue to be used today by Canadian Blood Services (CBS) for all blood product and sample transportation. Recently, in response to client hospital requests for information, we decided to revisit the validation issue and perform another, more extensive validation on the existing CBS box for transportation of red blood cells. Our validation showed the boxes were suitable for temperature control within the operating procedures stated; however, it also brought to light certain deficiencies in design, such as poor temperature control when ambient temperature was below 10 °C. Packing technology has progressed significantly since the initial choice of the blood packing box. The availability of vacuum panel insulation and phase change materials, along with inexpensive continuous temperature monitoring devices, means that temperature-sensitive payloads can be transported longer, with better temperature control, and traceable temperature records, implying we are delivering a superior product to our customers. However, studies linking storage temperature with quality indicators such as hemolysis, 2,3-DPG and ATP levels, are needed to conclusively prove this assumption. At a time when CBS is under increased public scrutiny and regulatory requirements mandate continuous quality improvement, it makes sense to move towards using the best quality blood transport technology and practice.

ABSTRACT NO.: 22
Capital health blood recycle program
T. Richardson
Capital Health, Edmonton, Alberta, Canada.
Capital Health (CH) utilizes a number or processes to ensure effective blood conservation. One process is the Capital Health Blood Recycle Program. This program operates through the cooperation between transfusing facilities outside of the Capital Health region, and the CH Transfusion Service.

ABSTRACT NO.: 23
Le projet de transport inter établissements des produits sanguins (tips) de la province de Québec
J. Beaulieu
Centre régional de Santé et de Services Sociaux, Rimouski, Québec, Canada.
Durant l’année 2000, à la demande du Comité Consultatif National de Médecine transfusionnelle (CCNMT), le secrétariat du sang a participé à la création d’un comité de travail dont le mandat était d’établir des exigences relatives au transfert de produits sanguins labiles et stables entre les établissements. Le but de cette démarche était de minimiser les pertes de produits sanguins et de garantir la qualité du produit transféré. À la suite du rapport du comité de travail, le «Groupe de recherche sur le Transport Cargo Aérien de l’Université Laval du Québec» a procédé à la validation d’un système de transport des produits sanguins. La cible principale était de trouver une méthode d’emballage simple permettant de maintenir le produit à la température adéquate en fonction de la durée du transport plutôt que la température extérieure Durant le transport. Le débat des transferts de produits entre les établissements à l’aide de ce nouveau type d’emballage est en cours de réalisation.

ABSTRACT NO.: 24
Q: What’s in the box? A: Depends on who you ask
L. Denesiuk
Dynacare Kasper Medical Laboratories, Edmonton, Alberta, Canada.
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The Alberta Wide Transfusion Medicine Group is a network of Alberta professionals involved in Transfusion Medicine activities within the province. The group meets a few times a year via videoconference to share information.

Recently a subcommittee was formed to look at the feasibility of standardizing blood transport within the province. This presentation in the five-part workshop will discuss some of the following points: the genesis of the Alberta Wide Transfusion Medicine Group, the genesis of the subcommittee on blood transport, the results of an informal survey of current practice within Alberta, the recommendations on blood transport proposed by the subcommittee, and the next steps in the process.

ABSTRACT NO.: 25
What’s going on inside the box? Depends on what’s in there, how you packed it, what you packed it with and how long it’s in there! K. Biggins
Transfusion Medicine, Chinook Health Region Laboratory, Lethbridge, Alberta, Canada.
Due to changes in Canadian Blood Services policies, Health Regions in Alberta and across Canada are implementing their own blood boxes for the transport of blood components and products both within and outside their regions. Standards mandate that the temperature conditions inside these boxes, be monitored and maintained. This section of the “What’s in the Box” workshop will look at the CHR experience with respect to the following blood box transportation topics: What is the optimum mix of iepack(s) and/or Gelpack(s)? How long are optimum conditions maintained inside the blood box? What happens to the temperature conditions when things go wrong? Utilizing various DICKSON DataLoggers® to measure temperature conditions inside the box. Implementing a quality monitoring system for transportation of blood boxes. Recycling of blood components and products between the CHR and Foothills Medical Centre in Calgary.

Main Scientific Program
ABSTRACT NO.: 26
Microfluidic devices for cancer, infectious disease and pharmacogenetics
L. Pilarski1,2, G. Kaigala3, S. Adamia1,2, J. Chowdury1,2, R. Huskins1,2, J. Lauzon1,2, J. Preiksaitis4, A. R. Belch1,2 & C. J. Backhouse4
1Cross Cancer Institute; 2Departments of Oncology, 3Electrical and Computer Engineering, University of Alberta; 4Provincial Laboratory for Public Health, Edmonton, Alberta, Canada.
Microfluidic devices hold promise as automated, inexpensive methods that will change the practice of health care by aiding in the implementation of widespread and cost-effective delivery of otherwise difficult and expensive genetic testing for diseases such as cancer, for infectious agents and for detection of genetic polymorphisms that impact on disease susceptibility, drug metabolism and response to therapy. This is likely to translate to significantly improved health care. Microfluidic-based devices are photolithographically defined networks of microchannels whose versatility has led to terms such as “lab on a chip”. These platforms are able to process cells or other particles such as bacteria or viruses, and analyze their genomic profiles, individual genes, or chromosomes to inform medical decision making and the implementation of “personalized” medicine.

Routine analysis of genetic abnormalities in, for example, multiple myeloma, a deadly cancer of the immune system, requires assays of greater sensitivity, standardization and automation than are currently available. For example, in myeloma, aberrant intronic splicing of hyaluronan synthase 1 (HAS1), frequently expressed in MM B cells, is significantly correlated with poor outcome. Tests for this and other gene products not normally evaluated as part of cancer diagnosis and monitoring might provide valuable information for patient care. Although still under development, such testing is now feasible using microfluidic technology. PCR on a microfluidic chip effectively amplifies aberrantly spliced HAS1 transcripts, and PCR products are readily detectable when analyzed using capillary electrophoresis (CE) on chip. Myeloma is characterized by a distinct immunoglobulin gene rearrangement that provides a unique molecular signature for unequivocal identification of the myeloma clone. Using a CE chip, both genomic DNA and IgH VDJ transcripts amplified from individual cells are detectable on chip with as little as 0.001% of the amplified product from one cell. In kidney transplantation, BK nephropathy, due to high levels of reactivated virus is an important cause of graft dysfunction and failure. BK virus can be readily detected in both urine and plasma of patients at risk of this complication. BK virus is detected by PCR. Amplification of BK DNA from urine by on-chip PCR, followed by on chip CE, provides efficient PCR amplification and sensitive detection of BK amplicons comparable to that of conventional PCR thermocycling and DNA fragment analysis. This example provides a proof of concept confirming the future utility of microfluidic devices for detecting and containing spread of infectious agents such as e.g. influenza. Pharmacogenetics holds considerable promise for avoiding adverse drug events caused by inappropriate drug dosing, but is currently too difficult and expensive for routine clinic use. Single nucleotide polymorphisms can be detected by on chip PCR, restriction digestion of PCR products and analysis of restriction fragments by on chip CE. Microfluidic-based pharmacogenetic testing may help to make such testing routinely available to patients. These examples predict the feasibility of cost-effective screening strategies that could enable identification of high risk genetic profiles, of emerging infections threats as soon as they arise, and by identification of key genetic polymorphisms in at risk populations, could facilitate avoidance of adverse drug events. The development of miniaturized and automated diagnostic/monitoring tools is likely to have broad application for maintaining public health and safety as well as for more effective treatment of disease.

* Keynote Address

ABSTRACT NO.: 27
Buffy coat blood components
H. Hume
Canadian Blood Services, Ottawa, Ontario, Canada.
Canadian Blood Services will begin producing blood components using the buffy coat production method in Alberta and British Columbia in the summer of 2005 and will then have a gradual implementation to the other jurisdictions served by Canadian Blood Services in 2006. All labile blood components will be affected by this change in production methods. In this talk Dr. Hume will specifically address the following topics: the use of the additive solution SAG-M, in particular, for neonatal patients; the...
quality of platelets produced by the buffy coat production method; practical aspects of providing platelet transfusion support to patients using apheresis and buffy coat platelets; plasma protein levels in frozen components produced in this new production environment.

ABSTRACT NO.: 29
Overview of competency assessment of laboratory personnel
L. Bertel
Quality Systems Consultant, Westminster, Colorado, USA
This program distinguishes between education, training and competence assessment of laboratory personnel. The important differences between initial and ongoing competence assessment are reviewed and discussed. Information from a recently updated CLSI (formerly NCCLS) guideline about a model for training and competence assessment will be shared.

ABSTRACT NO.: 30
Alberta’s MLT continuing competence program
L. Hodgson
Alberta College of Medical Laboratory Technologists, Edmonton, Alberta, Canada
The College “Continuing Competence Program for Alberta MLTs” is presently in the process of being developed, with significant volunteer input through committee work and focus group sessions, to establish a Continuing Competence Program that is user friendly for all Medical Laboratory Technologists (MLTs) registered with the College. Through this informative session, discover the key steps in program development, the plan of action, where the College “is” in the “process” and what still needs to be done before the mandatory program implementation date of March 2007.

ABSTRACT NO.: 31
Competence assessment program for transfusion medicine service personnel
P. Courtney & L. R. Podlosky
Capital Health; University of Alberta, Edmonton, Alberta, Canada
PURPOSE To describe a competence assessment program (CAP) implemented by the Capital Health Transfusion Medicine Service, in order to meet requirements of regulatory and accreditation agencies.
METHODS Traces3™ LMS webClient, a web-based electronic learning manage-ment system, is a software application designed to assess knowledge and understanding of standard operating procedures (SOPs). Assessment of practical skills is fulfilled using results from proficiency sample testing. Samples are tested by each technologist on a rotational basis and performance is documented.
RESULTS Electronic tracking of competency decreases time required to evaluate results of individual competencies. The electronic learning manage-ment system provides immediate and direct feedback to learners. Staff have increased knowledge of procedures due to scheduled review and timelines for performing electronic competency assessment. Staff express increased confidence because they are more familiar with procedures. The results of the proficiency testing samples provide a broader reflection of an individual’s competence.
CONCLUSION An electronic learning management system is beneficial for learners due to ease of use and for assessors due to reporting capabilities. Testing proficiency samples in combination with electronic learning provide a balanced assessment of individual competence.

ABSTRACT NO.: 32
Galactosylation of GPIbα by an externally active endogenous β4Galactosyl transferase prolongs platelet survival
K. Hoffmeister
Hematology Division, Brigham & Women’s Hospital, Harvard Medical School, Boston, Massachusetts, USA
Cooling of platelets causes a clustering of the von Willebrand factor receptor (vWFR) complex on the platelet surface leading to platelet clearance. Clustered [βN-acetylglucosamine (βGlCNAC)] residues on the GPIbα subunit of the vWFR complex are recognized by the αβ3 integrin on macrophages, leading to phagocytosis of chilled platelets. Masking the exposed βGlCNAC residues with galactose via a 1,4 covalent linkage inhibits this interaction. Surprisingly, platelets have an externally associated β1,4 galactosyl-transferase (β4GalT) enzyme, which transfers galactose onto βGlCNAC residues on platelet GPIbα by the simple addition of its co-factor UDP-galactose. Washed platelets are as active as recombinant β4Gal-T1 in galactosylating exogenous substrates, benzyl-β-D-GlCNAC and ovalbumin. We have documented these phenomena for mouse platelets in vitro, and for human platelets in vitro. Upon transfusion, galactosylated chilled mouse platelets survive longer than RT-stored platelets. Importantly, platelet galactosylation has no impact on the GPIbα-vWF interaction, indicating that the hemostatic and clearance functions mediated by GPIbα are separable and can be manipulated independently, providing a practical approach to accommodate platelet cold storage. These findings revive the concept that platelets are “two-reactors”, designed to modify themselves and possibly other targets for physiological purposes other than adhesion, possibly platelet survival.

ABSTRACT NO.: 33
From Nuremberg to Krever – The principles of informed consent
J. Hari
Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada
Informed consent stems from the ethical principle of patient autonomy; the belief that people have the fundamental right to make their own health care decisions. The three elements essential to informed consent are: 1) Capacity, 2) Voluntariness, and 3) Disclosure. The process of obtaining informed consent requires discussion of material risks, which is what a reasonable person, under similar circumstances, would want to know. The idea that disclosure may cause unnecessary harm has never been a successful defence against the omission of certain risks. With respect to blood transfusions, informed consent should include the risks of a transfusion reaction, as well as the known infectious risks of blood. Unknown or theoretical risks should also be included as full disclosure helps preserve the patient/physician trust relationship, and assists the patient in understanding and evaluating the full implications of receiving the transfusion.
Implementation of informed consent is a complex task. Adequate information must be provided, the patient must have sufficient time to ask any questions or pursue alternative options, and there must be appropriate documentation of both the consent and the transfusions given. The best way to implement and regulate an adequate informed consent policy is not uniformly agreed upon.

ABSTRACT NO.: 34
Informed consent: Experience in Vancouver Island Health Authority and BC
B. Berry
Vancouver Island Health Authority, South Island, Victoria, British Columbia, Canada
Krever Inquiry made it clear that the Canadian public expected and had the right to an informed consent process prior to receiving blood transfusions prescribed by physicians. Undertaking this project in any given medical community can be complicated and requires consideration of many practical issues involving all participants in the transfusion process including recipients, physicians, nurses and laboratory staff. Simply a consent form with a witnessed patient signature does not satisfy the principles of informed consent. The process must involve a meaningful discussion between
a knowledgeable medical practitioner and the transfusion recipient with adequate information and capacity for questions considering of benefits, risks and alternatives. The process by which this was implemented in our health region in a practical and sustainable system within the provincial context will be reviewed. Recipient and physician perspectives prior to implementation will be reviewed and more recent recipient data with also be presented.

ABSTRACT NO.: 35
Informed consent for blood transfusion at Sick Kids
K. McShane
Department of Paediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Ontario, Canada.

The informed consent policy at the Hospital for Sick Children implemented in 1995 requires that the ordering physician or surgeon ensures that the benefits, risks and alternatives to blood transfusion are discussed with the patient and/or guardian before the transfusion is started. This discussion must be documented in the patient’s hospital record. Hospital rounds and in-services to introduce the consent policy were conducted from 1995 to 1997. In 1997, computer documentation of consent in the electronic charting system was added. A series of audits and educational interventions were performed in 1998, 2001 and 2002 to facilitate and monitor compliance with this policy. Using these techniques, overall consent rose from 57% to 62% to 74% in 1998, 2001 and 2002.

This presentation will review: informed consent for blood transfusion Sick Kids style where the physician, not the patient or parent, signs that consent was given; the effectiveness of the ‘audit and educate’ strategy and the value of one-on-one intervention versus group teaching sessions for increasing compliance with the consent policy; effectiveness of computer versus paper documentation; compliance with documentation of consent for blood components versus plasma products; additional interventions that were used to encourage documentation of consent; and future plans for increasing compliance further.

ABSTRACT NO.: 36
Mechanisms of action of intravenous immunoglobulin (IVIg)
A. H. Lazarus
Canadian Blood Services; Department of Laboratory Medicine and Pathobiology, St. Michael’s Hospital and the University of Toronto, Toronto, Ontario, Canada.

IVIg is prepared from large pools of plasma from healthy blood donors and the IgG is present predominantly in monomeric form. Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disease characterized by autoantibody-mediated platelet destruction. Platelets with associated IgG are targeted for destruction by phagocytic cells in the spleen. One of the treatments of choice for patients with ITP is infusion of large amounts of IVIg. Infusion of IVIg (or polyclonal anti-D) can reverse thrombocytopenia in patients with ITP commencing within hours of the administration of these products. Despite the extensive clinical use of IVIg in ITP as well as a variety of other autoimmune and inflammatory states, the mechanism of action of IVIg remains as yet incompletely understood. This talk will provide an overview of some of the major theories in how IVIg is thought to work in autoimmunity with an emphasis on ITP. The potential future replacement of IVIg (and anti-D) with highly effective monoclonal antibodies will also be discussed.

ABSTRACT NO.: 37
The use of IVIg in solid organ transplantation
P. Campbell
Histocompatibility Laboratory, University of Alberta Hospitals, Edmonton, Alberta, Canada.

Over the past 10–15 years the number of uses for IVIG has grown. Its use in solid organ transplantation has also evolved. In 1992 Dennis Glotz reported success with the use of high dose IVIG in reducing the levels of anti-HLA antibodies in dialysis patients awaiting transplantation. This enabled highly sensitized individuals to receive a crossmatch negative kidney transplant. This prompted further studies in Europe and North America. These studies coincided with the introduction of new solid phase technologies for anti-HLA antibody screening that enabled the Histocompatibility Laboratories to detect lower levels of HLA antibodies and define specificities. In addition the transplant community also came to accept that anti-HLA antibodies could cause graft rejection. The finding that C4d staining in the peritubular capillaries of the kidney transplant correlated strongly with the presence of donor specific antibody enabled transplant physicians to more accurately diagnose antibody mediated rejection and distinguish this from acute cellular rejection.

A number of centers have used either high dose IVIG 2 g/kg or low dose IVIG 0.1 g/kg and plasmapheresis to successfully treat humoral rejection for heart, lung and kidney transplant recipients.

More recently these protocols have been used to reduce antibodies and convert a positive T cell crossmatch to negative to allow transplantation to occur. This presentation will review the protocols used by these centers and the transplant outcomes for these recipients.

ABSTRACT NO.: 38
The use and misuse of IVIg in neurological diseases
T. E. Feasby
Capital Health Authority; Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada.

IVIg use has quadrupled in the last 10 years in Canada. The demand for IVIg exceeds current supply, resulting in foreign plasma being used to provide 2/3 of Canadian consumption. The cost for a typical treatment course now averages $10,000 and provincial governments have expressed concern regarding the escalating financial burden. Concerns have also been raised as to whether IVIg is appropriately utilized.

Several national consensus conferences have been held; at the June 2002 Canadian Blood Services conference, it was estimated that 20–25% of IVIg was off label and perhaps not indicated. National consensus guidelines are in development.

There is significant regional disparity of Canadian use. In 2001–2002, British Columbia used 0.07 g/capita while Alberta used 0.12 g/capita, with the Canadian mean 0.08 g/capita. It is unknown to what extent Alberta’s higher use is due to an increased rate of chronic treatment and/or reflects greater inappropriate use for incident cases. In 2002, BC implemented proactive management approach by developing an IVIg Management Program, including a reference handbook and centrally administered screening system.

This presentation will describe the CIHR-funded research project “The Appropriateness of IVIg”. It will measure IVIg use in two provinces with very different utilization profiles, Alberta and BC. The process involves finding and grading the evidence for IVIg use in 83 diagnoses in a variety of specialty areas. The RAND/UCLA method was used to develop appropriateness criteria for 340 scenarios (indications for IVIg use). The ratings of “appropriate”, “inappropriate” and “uncertain” will be used to evaluate the initial use of IVIg in both provinces during the calendar years of 2001 and 2003. Data will also be collected on chronic use. The chart review will include approximately 4000 charts at 100 + institutions throughout the 2 provinces.

This study will provide insight into: the appropriateness of initial IVIg use, variations in utilization, the effectiveness of the proactive management approach, and will help guide IVIg utilization management practices in Canada.

ABSTRACT NO.: 39
Getting it right – S&W initiatives to decrease the risk of ABO errors
A. Coovadia & A. Lima
Sunnybrook & Women’s College Health Sciences Centre, Toronto, Ontario, Canada.

Since the introduction of the MERS-TM 6 years ago, as part of our root cause analysis, we recognized the need to develop initiatives to address the gaps in our existing processes that were determined to
In the land of BSE
J. Saldanha
Roche Molecular Systems, Pleasanton, California, USA.

Bovine Spongiform Encephalitis (BSE) is a transmissible, neurodegenerative, fatal brain disease of cattle and is part of a group of diseases collectively called Transmissible Spongiform Encephalopathies (TSEs) which are characterized by spongy degeneration of the brain with severe and fatal neurological signs and symptoms. Several human TSEs exist, of which Creutzfeldt-Jacob disease (CJD) is the prototype human disease. CJD can either be associated with a hereditary disposition (5–10% of cases) or may occur in a sporadic form (85–90% of cases). BSE was first recognized in the UK in November 1986 with the appearance in cattle of a newly-recognized form of neurological disease. Between 1990 and 2002, over 180,000 cases of BSE were reported in the UK. Since 1989, cases have been reported in other European countries, and in Israel, Japan and Canada. In the mid 1990s, a new form of CJD, variant CJD (vCJD), which was mainly found in individuals less than 30 years old with no family history of CJD or genetic abnormality, was first recognised in the UK. The disease had a relatively longer duration of illness compared with CJD and was strongly linked to exposure, very probably through food, to BSE. As of 4th March, 2005, the total number of deaths from definite or probable vCJD in the UK is 149, plus 5 probable vCJD, living cases. From 1989, the UK implemented several measures to reduce the risk of transmission of vCJD. The theoretical risk of transmission of vCJD has led to strategies to reduce the risk of transmission of the disease by blood and blood products and include deferral of donors at risk from either vCJD or exposure to vCJD. Recently, two suspected cases of vCJD transmission by blood transfusion have been reported in the UK highlighting the possibility of this mode of transmission of the agent.

**ABSTRACT NO.: 40**

Bacterial detection in apheresis platelets: Experience of Canadian blood suppliers
M. Goldman and G. Delage
1Canadian Blood Services, Ottawa, Ontario; 2Héma-Québec, Montreal, Québec, Canada.

**Purpose** Bacterial contamination of platelets may lead to severe transfusion reactions. Challenges in the detection of bacteria in platelet components include: the small initial inocula, possible contamination at the time of sampling, different growth characteristics of various bacterial species, and the time necessary to obtain a positive result.

**Methods** Both Héma-Québec and CBS are performing aerobic cultures in apheresis platelets using the automated BacT/ALERT system.

**Results** Over 95% of apheresis platelets placed in inventory have had a culture performed. Of 28,852 units cultured to date, there were 31 initial positives (rate of 0.11%). Many of these appear to be machine false positives or contamination of the BacT/ALERT bottle or inoculation. There have been at least 3 true positives. There was a severe transfusion reaction due to Salmonella that was missed on culture (false negative).

**Conclusions** Introduction of universal bacterial testing for apheresis platelets in Canada has been accomplished with little or no inventory disruption. There has been an acceptable false positive rate, and a reassuringly low rate of actual bacterial contamination, compared to published data. However, the presence of a clinically significant false negative test highlights the need for improved testing methods.

**ABSTRACT NO.: 42**

The use of a bacteria detection system to evaluate bacterial contamination in platelet concentrates
G. Rock and D. Neurath
Department of Pathology and Laboratory Medicine, The Ottawa Hospital; University of Ottawa, Ottawa, Ontario, Canada.

**Background** Concern for bacterial contamination has limited the storage of platelets to five days. The use of a bacterial detection system to determine contamination would help to assure the safety of the product and may permit extension of the platelet shelf-life to seven days.

**Study designs and methods** For the first 12 months of study, only random donor platelets (RDPs) were assessed. Blood was collected into CP2D and leukoreduced at the local blood centre. Upon arrival at the hospital, a 2-3 mL aliquot was removed from each RDP and introduced into the Pall BDS pouch using a sterile docking device. The pouch was then incubated for 24 hours at 37 °C and the oxygen consumption measured. As an additional check, following pooling, an aliquot was removed for culture using the BacT/Alert system. Since 2003, we have tested both RDPs and apheresis platelets.

**Results** Over a 26-month period a total of 27,000 individual RDPs were tested. An additional 2,424 apheresis units were assessed during the past 14 months. The BDS system gave positive results in 15 RDP units and 14 apheresis units. Of these, 3 of 15 RDPs and 6 of 14 apheresis units were confirmed positive by culture. Only one of the six confirmed apheresis platelet had been assessed by the CBS prior to issue to us. In these nine units, the following organisms were identified: Propionibacterium acnes (2), Micrococcus species, Bacillus species, Coryneform bacterium (diphtheroids), Coagulase-negative Staphylococcus (3) and Staphylococcus aureus. The system is easy to use and takes less than 5 minutes to sample and read.

**Conclusions** The Pall BDS system permits evaluation of platelets for bacterial contamination. Testing enhances the quality assurance of the blood transfusion system.

**ABSTRACT NO.: 43**

Meeting 5.1.5.1 requirements in the transfusion laboratroy – Not that easy!
L. R. Podlesky, S. Nahirniak, & G. Clarke
Capital Health, University of Alberta and Dynacare Kasper Medical Laboratories, Edmonton, Alberta, Canada.

**Background** To prevent transfusion transmission of bacteria and comply with AABB standard 5.1.5.1, hospital blood banks in Canada must explore available testing options for random donor platelets (RDP). Methodologies that have been tested and found suitable by the AABB include blood culture systems, urine reagent strips (URS) to detect changes in glucose or pH, and staining.

**Purpose** To validate URS testing, sensitivity was assessed by inoculating individual sterile RDP collected in CLX bags with seven bacterial strains of known concentration. Correlations of colony count, culture, instrument and URS results for pH and glucose were performed. URS specificity testing involved comparing culture and URS test results of 30 sterile RDP daily over a five day period. URS turnaround times (TATs) were determined by bench technologists at two hospital sites.

**Results** Using cut-off values of pH <7 and glucose <28 mmol/l, culture and instrument readings were positive by day 0 and day 1 respectively. Most pH and glucose URS results were positive by day 2 post-inoculation. All sterile RDPs showed 100% URS specificity for pH testing (pH >7 throughout testing) while glucose values <28 mmol/l were obtained in 11% of tests performed, giving a 90% specificity rate. TAT results for URS manual and automated testing averaged 73 sec and 72 sec respectively per test.

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ABSTRACT NO.: 44
Biopreservation of blood cells
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The development of effective preservation and long-term storage techniques is a critical requirement for the successful clinical application of cell-based technologies. Long-term storage of blood and blood components is needed to ensure a readily available, safe supply of these products for transfusion medicine. Effective preservation procedures are required at various steps during the production of human blood products. Biopreservation is the process of preserving the integrity and functionality of cells, tissues and organ held outside the native environment for extended storage times. Biopreservation can be categorized into four different areas based on the techniques used to achieve biological stability and ensure a viable state following long-term storage. These include: in vitro culture, hypothermic storage, cryopreservation and desiccation. This presentation will review these techniques with an emphasis on the recent developments that have been made using these technologies for the biopreservation of blood products.

ABSTRACT NO.: 45
Cellular preservation in the dry state: Improvements and innovations
F. Tablin, M. Tang, W. Wolkers, N. M. Tsvetkova, Z. Torok & J. H. Crowe
Center for Biostabilization, University of California Davis, Davis, California, USA.

The Center is focused on long-term storage of platelets and red blood cells in the dry state using the non-reducing disaccharide trehalose. Trehalose loaded freeze-dried platelets can be directly rehydrated with good survival, (80-90%) and response to agonist. Biochemical analysis of intracellular pH regulation using the pH indicator dye BCECF-AM demonstrates that the pH of resting fresh cells and rehydrated cells was virtually identical (pH = 7.27). Both populations responded to decreasing concentrations of extracellular Na+ by decreasing their [pH]. This response could be blocked by the addition of a Na+/H+ antiporter inhibitor MIA. Fresh and freeze-dried platelets responded to the addition of thrombin by a brief initial acidification followed by an alkalization over virtually the same time frame. These data suggest that the freeze-dried rehydrated platelets have an intact pH regulation system. A method for freeze-drying red blood cells (RBCs) while maintaining a high degree of viability has important implications in transfusion and clinical medicine. RBCs, loaded with trehalose can be freeze-dried with hydroxyethyl starch, human serum albumin and trehalose. Rehydration of these erythrocytes results in about 60% survival. Strikingly, the freeze-dried rehydrated cells have preserved morphology, and exhibit high levels of ATP, 2,3-DPG and low levels of methemoglobin. Biochemical analysis demonstrates that the activities of superoxide dismutase, catalase and acetylcholine esterase in freeze-dried RBCs are very similar to those of fresh RBCs. Secondary structure of hemoglobin is also very similar to that of fresh hemoglobin, demonstrating that trehalose loading is critical for preservation of cellular proteins during lyophilization. These data provide an important step toward a stable erythrocyte product, which can prove invaluable for transfusion and clinical applications.

ABSTRACT NO.: 46
Red blood cell cryopreservation in Canada: Where we are and where we are going
J. Lecak, K. L. Scott & J. P. Acker
Canadian Blood Services; Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada.

A serendipitous discovery of the cryoprotective action of glycerol in 1949 has lead to the development of effective clinical techniques for red blood cell (RBC) cryopreservation. Current cryopreservation techniques remain labor-intensive, expensive, and therefore, logistically impractical for routine use. As a result, RBC cryopreservation is restricted for rare and unique blood phenotypes, as well as medically indicated autologous RBC units.

To assess the utilization of frozen blood, motivation for RBC cryopreservation and deglycerolization by the Canadian Red Cross/Canadian Blood Services (CRC/CBS) and its effect on the logistics of the Canadian blood supply, we examined the frozen blood documents from 1992 to 2002 at four CRC/CBS Centres that have been or currently are involved in the frozen blood program – Edmonton, Winnipeg, Vancouver, Ottawa, Toronto, and Halifax. The information collected included donor demographics, RBC unit processing information, as well as frozen blood utilization information.

The current status of the frozen blood program in Canada indicates that RBC freezing has a small effect on the logistics of blood supply. As frozen RBC are equally effective when transfused as RBC stored at 4 °C, and cryopreservation remains the only method for storing rare and unusual RBC phenotypes, we recommend that the current priority and management of the frozen blood inventory in Canada be re-evaluated. This presentation will also discuss the future directions of the frozen blood program in Canada, such as adoption of the Haemonetics ACP-21ST automated closed cell processor for RBC freezing and deglycerolization.

ABSTRACT NO.: 47
Platelet donor selection (PDS): A new tool for the selection of HLA/HPA compatible platelets
I. Bonacossa1, P. Nickerson2, & H. Hume3
1 'Canadian Blood Services, Winnipeg, Manitoba; 2 University of Manitoba, Winnipeg, Manitoba; 3 Canadian Blood Services, Ottawa, Ontario, Canada.

The management of immune refractoriness to random donor platelets, as well as of Neonatal Alloimmune Thrombocytopenia (NIT) have been or currently are involved in the frozen blood program. The Platelet Donor Selection (PDS) system was developed by Canadian Blood Services (CBS). This system allows the professional to perform searches across all centres to match patients' HLA and Platelet Specific Antigen (HPA) profiles and to combine HLA and HPA searches into a single search. In addition, PDS has been designed to take into account the recipients' HLA antibody specificity to define acceptable donors. ABO, Rh and CMV status can also be incorporated in the search. Donor searches are performed by two centres, Winnipeg and Toronto. The initial search list includes donors in the requesting center although, depending on donor availability, it can be expanded to include donors in other centres as well. PDS lists approximately 12,000 HLA type donors with a limited number of donors of rare HLA types, and 115 HPA-1a negative donors. The development of this model has allowed CBS to facilitate donor selection and improve hematopoietic support for the alloimmunized patient. To increase the number and broaden the ethnic diversity of the donor database in PDS are our future goals.

ABSTRACT NO.: 48
Platelet inventory management: From donation to distribution
S. Thibault
Héma-Québec, Saint-Laurent, Québec, Canada.

In 2002, Héma-Québec adopted an innovative inventory management strategy with a business objective to meet 50% of the Hospital demand in platelets with thrombapheresis by March
2005. Héma-Québec’s strategy was based on the following: development of infrastructure to realize the project; selection of equipment to improve performance; and registration of qualified blood donors to meet quantity and diversity.

On December 31st 2004, Héma-Québec met its objectives to supply 50% of platelets with thrombapheresis, to the great delight of its clients i.e. the hospitals. From a perspective of inventory management, some of the largest hospitals have analyzed and have decided to stock this product in an on-site blood bank to minimize the demand on emergency order and delivery of platelets. This new dynamic resulted in a reduction in urgent delivery of platelets without increasing the level of expired products in hospital.

Next year Héma-Québec’s goal is to increase to 65% the delivery of platelets with thrombapheresis to hospitals with a potential to reach up to 75%. To meet this objective, Héma-Québec aims at introducing a new donation technique such as the “double” thrombapheresis.

**ABSTRACT NO.: 49**

Efficacy and safety of rFVIIa in severe trauma patients – NovoNordisc J. Kortbeek1,2, J. Montano1,3, M. Z. Ratajczak4, J. Kortbeek1,2, J. Montano1,3, M. Z. Ratajczak4, J. Kortbeek1,2, J. Montano1,3, M. Z. Ratajczak4

**Department of Surgery and Critical Care, University of Calgary**: Surgery, Foothills Medical Centre, Calgary, Alberta, Canada.

**DESIGN** A multicentre, randomised, double-blind, parallel group, placebo controlled trial to evaluate the efficacy and safety of activated recombinant factor VII (rFVIIa/NovoSeven/Niastase) in the Treatment of Bleeding in Severely injured trauma subjects. Phase 2 efficacy and safety study with a planned 280 patients (140 in each arm), Randomized: 158 blunt and 143 penetrating. ITT population: All randomized patients receiving at least one dose, 143 blunt and 143 penetrating. Inclusion criteria: injury due to blunt and/or penetrating trauma, received 6 units of RBC within 4 hours of admission, received 8 units of RBC prior to dosing. Exclusion criteria: Glasgow Coma Score <8, pH < 7.0 or a base deficit >-15, gunshot wound to the head, injury sustained >12 h before randomisation. Primary endpoint: RBC’s from 1st dose to 48 hours. Secondary endpoints: death, blood products, surgeries, time to normalize INR temp & pH, ICU LOS, ventilator days and hospital LOS. Safety: SAEs, changes in coagulation parameters, 30 day survival, complications including MOF, ARDS and infections.

**RESULTS** Blunt Trauma: Significant reduction in PRBCs within 48 h; exploratory analysis excluding a patient who died within 48 hours demonstrated a significant reduction in massive transfusion (>20 units); non-significant differences in secondary endpoints except reduction of INR. Penetrating Trauma: no significant difference in primary or secondary endpoints except for time to normalization of INR; exploratory Intention to Treat analysis excluding deaths within 48 h demonstrated a non-significant 63%RR in need for massive transfusion.

**CONCLUSIONS** Trial results show that rFVIIa: reduced need for transfusion in Blunt trauma; potential to reduce complications/death in BLUNT trauma. No safety concerns identified: no difference in adverse events; no difference in thrombo-embolic events from placebo; apparent reduction in the incidence of 30 day morbidity warrants further study.

**ABSTRACT NO.: 50**

Recombinant activated factor VII utilization framework C. Moltzan

**Hematology/Blood Bank, St. Boniface General Hospital, Winnipeg, Manitoba, Canada.**

There has been an increase in the use of recombinant activated factor VII (rVIIa) in the last two years in the management of bleeding related to trauma and surgery. This has been related to case reports and clinical trials that have demonstrated that it can be safe and effective. However, there will be many situations where requests for rVIIa will not fit the best medical evidence that is available. Requests for rVIIa are often related to clinical situations where it is being used as a last resort. The National Technical Working Group on Blood and Blood Products (NTWG) has developed a framework for rVIIa utilization management to deal with such requests in a consistent manner. This will allow transfusion services to keep track of rVIIa use and it will allow the development of a registry to learn more about the situations in which rVIIa can be effective. The concepts of the utilization framework and the registry will be presented.

**ABSTRACT NO.: 51**

Malaria and other emerging blood-borne parasitic infections: The next century K. C. Kain

**Global Health Program, McLaughlin Center for Molecular Medicine, University of Toronto; Center for Travel and Tropical Medicine, Toronto, Ontario, Canada.**

Emerging infectious diseases represent a growing health, economic, and security threat for all countries. Canada, as the most culturally diverse nation on earth, is particularly susceptible to the globalization of infectious diseases as evidenced by SARS, BSE, and avian influenza. This presents unique challenges and opportunities for blood safety in Canada. This talk will explore emerging blood-borne parasitic threats and discuss advances in diagnosis, inactivation and management.

**ABSTRACT NO.: 52**

International issues in transfusion medicine R. Y. Dodd

**Holland Laboratory, American Red Cross, Rockville, Maryland, USA.**

Three categories of issues will be considered in this presentation. Firstly, variations attributable to geography and resource limitations. Not only is it more difficult to conduct transfusion medicine in areas with a low human development index (HDI), but such areas almost inevitably have very high incidence and prevalence rates for transfusion transmissible diseases and the population needs for transfusion are very different from those in areas with a high HDI. Secondly, the impact of human behavioral and sociological change has a profound impact upon the development and international movement of infectious disease. For example, modern agricultural practice has led to the appearance and distribution of bovine spongiform encephalopathy and its human form, variant CJD around the world. Additionally, within the past three years, a number of infections new to humans or to the continent have emerged in North America, probably as a result of rapid air transport. They include West Nile virus, SARS and monkeypox. Thirdly, a variety of regulatory systems exist around the world. Although there is little transport of blood components between countries, these variations nevertheless cause difficulties, particularly with the management of plasma for further manufacture. Not only do these issues cause problems directly, but they also generate difficulties in establishing consistent standards of practice and safety internationally.

**SUBMITTED ORAL PRESENTATIONS**

**ABSTRACT NO.: 53**

Microvesicles derived from activated platelets enhance the invasive potential of breast cancer cells A. Janowska-Wieczorek1,2, L. A. Marquez-Curtis2, J. Montano2 & M. Z. Ratajczakk

1Department of Medicine, University of Alberta; 2Canadian Blood Services, Edmonton, Alberta, Canada; 3University of Louisville, Louisville, Kentucky, USA.

There is increasing evidence that platelets contribute to cancer metastasis, and yet platelet concentrates (PC) are frequently transfused to cancer patients to treat post-chemotherapy thrombocytopenia. Recently, we reported that platelet-derived microvesicles
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(PMV) transfer various surface receptors/adhesion molecules to target cells and modulate their biological response. In this study we hypothesized that the interaction of PMV with breast cancer cells could increase their invasive potential. PMV isolated from outdated PC were pre-incubated with three human breast cancer cell lines (BT-479, MDA-MB-231, T47D) and the binding of PMV to these cells was evaluated by flow cytometry. The effects of PMV on adhesion to human umbilical vein endothelial cells (HUVEC), expression of matrix metalloproteinases (MMP, known to play a role in cancer progression), chemoinvasion, proliferation, and interactions of tumor cells with stroma were examined. We found that PMV (i) transfer platelet-derived CD41 to the surface of breast cancer cells and enhanced their adhesion to HUVEC; (ii) increase the proliferation of T47D cells; (iii) transfer CD62 and increase migration towards a-chemokine SDF-1, known to be upregulated at sites of metastasis; (iv) stimulate MT1-MMP in BT-479 cells and chemoinvasion across Matrigel; and (v) stimulate MMP-2 in co-cultures of MDA-MB-231 with stroma. In conclusion, PMV enhance the invasive potential of breast cancer cells in vitro; and because levels of PMV are known to be higher in old PC than in fresh ones, we recommend that cancer patients should preferably be transfused with fresh PC only.

ABSTRACT NO.: 54

Novel murine model of fetal and neonatal alloimmune thrombocytopenia demonstrates ivig ameliorates this disorder and downregulates pathogenic antibodies in both maternal and neonatal circulations

P. Chen1,2, R. G. Hynes4, A. H. Lazzara1,2,3, J. W. Semple1,2,3, C. M. Spring1,2,3, J. Freedman1,2,3 & H. Ni1,2,3

1Canadian Blood Services; 2St. Michael’s Hospital; 3Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; 4Howard Hughes Medical Institute and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.

Fetal and neonatal alloimmune thrombocytopenia (FNAITP) is a life-threatening bleeding disorder caused by maternal antibodies directed against fetal platelet antigens. The major risk of FNAITP is intracranial hemorrhage (ICH) in affected fetuses and neonates. The immunoreactive epitopes are primarily located in the extracellular regions of the platelet glycoprotein IIIa (b3 integrin). Currently, no animal model is available to investigate the pathogenesis of this disease and effective therapy is limited. Here, we established the first FNAITP animal model by breeding b3 integrin deficient (b3/-) female mice (both naïve and post-immunized) with wild-type (WT) male mice. We first demonstrated that b3/- mice, transfused with WT platelets, generated anti-b3 IgG1 and IgG2a antibodies. These antibodies are b3 integrin specific since they did not react with platelets from b3/- mice or red blood cells from WT mice and induced thrombocytopenia in WT mice. To establish the FNAITP model, the following experiments were performed: 1) Naïve female b3/- mice were bred with WT male mice; in contrast to human FNAITP, no bleeding disorders or maternal antibody was observed in the first and second deliveries. Bleeding disorders of pups and the maternal anti-platelet antibodies were detected in the third and the subsequent pregnancies. This difference between our mouse model and human clinical scenarios may be due to the short period of murine pregnancy. 2) Female b3/- mice transfused twice with WT platelets were bred with WT male mice; the female mice had moderate levels of anti-b3 antibody, which may be similar to women who have been pre-exposed to b3 integrin on sperm cells (pre-conceptual intercourse) or the fetus (previous pregnancies). We found anti-b3 integrin and platelet associated IgG in pup blood. Fetal platelet counts were significant decreased (P < 0.001) and bleeding disorders were observed (P < 0.05). 3) Female b3/- mice transfused four times with WT platelets were bred with WT male mice. The titer anti-b3 integrin antibodies in these mice were four times higher than mice transfused twice with WT platelets. No living pups were delivered from these mice. Two females had miscarriages and one female died during delivery after a 3 week pregnancy. Immediately autopsy showed 3 mature and 3 immature sized fetuses in utero. The mature sized fetuses exhibited either ICH, abdominal hemorrhage, or both. Thus, antibody titer correlated with the severity of FNAITP. 4) Action of intravenous immunoglobulin G (IVIG) in this disorder: we studied IVIG or albumin (control) administration in the female mice, which were transfused twice with WT platelets. We found that IVIG significantly ameliorated FNAITP in this model and downregulated pathogenic antibodies in both the maternal and fetal circulations.

ABSTRACT NO.: 55

Rational screening and optimization of a cytokine cocktail for in vitro megakaryocyte maturation and platelet production

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At present, platelets cannot be refrigerated, limiting their shelf life to 5 days and causing recurrent shortages. Their in vitro production would clearly contribute to facilitate their supply. Although progress has been made, so far, no efficient in vitro generation method has been proposed. A first step in this direction would certainly be the identification of an optimal culture medium. In this study, a two-step statistical procedure was followed to 1) identify the factors and their interactions that significantly affected megakaryocyte maturation and platelet production (the output variables, OV) and 2) optimize the selected cytokines concentration in order to maximize the OV. In the first step, a series of methods with incremental resolution were used: Placket and Burman, fractional factorial, and full factorial two-levels, experimental designs while in the second step a response surface method was performed. MK were obtained from human stem/progenitor cord blood cells cultured for 7 days. Day-7 MK were further cultured in the presence of different combinations of cytokines at various concentrations, dictated by the experimental design employed. Statistical analysis of the Placket and Burman experiments suggests that of the 13 factors tested, flt-3 ligand (FL), IL-8 and IL-9 combined with TPO, exerted the most significant positive effect on the total number of MKs produced. Moreover, a higher resolution analysis performed with two-level factorial designs revealed that in the presence of TPO, the three factors: SCF, IL-6 and IL-9 were significant stimulators of MK maturation, whereas FL had a positive effect only on the expansion of MK progenitors. In contrast, erythropoietin (EPO) and IL-8 were inhibitors of MK maturation. Finally, the response surface methodology allowed to find the optimal concentrations of TPO, SCF, IL-6 and IL-9, that maximized the OV and maintained a high purity of MK (~90%), using lower quantity of cytokines when compared to the best cytokine cocktail (TPO, SCF, FL, IL-6) previously reported.

ABSTRACT NO.: 56

Increased transforming growth factors and prostanoids characterizes cytokine production following Rh immune globulin prophylaxis

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Administration of anti-Rh(D) immunoglobulin to Rh-negative women is used to prevent immunologic sensitization of the mother to fetal Rh(D) antigen, thus preventing hemolytic disease of the newborn (HDN). Despite more than 40 years of use, the mechanism of this Rh immune prophylaxis remains completely unknown. In order to begin to elucidate how Rh immune prophylaxis prevents HDN, we examined immunomodulatory cytokine production following antenatal administration of Rh immune globulin in ten Rh-negative pregnant women. After informed consent, blood samples were obtained prior to and 48 hours after a standard dose
Platelet-poor plasma was separated and cytokine levels were determined (R&D Systems Analytical Testing Service). Seventeen cytokines were evaluated. IL-4, IL-5, IL-10, IL-13, IL-17, MIP-1α, GM-CSF, TNFβ and IFNγ remained below detection levels both pre and post analyses. IL-1αRII, IL-12p40, IL-16, and MCP-1 showed no significant changes while IL-1α showed a slight to moderate decrease in 7/10 women post administration of Rh immune globulin. In contrast, levels of TGFβ-1 and PGE2 rose dramatically in 7/10 and 5/10 women, respectively. One woman showed a >3-fold increase in TGFβ-1 and also an increase in TGFβ-2. TGFβ-1 and -2 and PGE2 are well known down-modulators of the immune response. These results indicate that Rh immune prophylaxis can induce high levels of the strongly immunosuppressive cytokines TGFβ and PGE2. These findings represent the first evidence for a possible mechanism of Rh immune prophylaxis.

**ABSTRACT NO.: 57**

Mix and match plasma proteins: Building a more specific thrombin-inhibiting blood product

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Many plasma proteins are serpins, related proteins that function by inhibiting different serine proteases. Z1-protease inhibitor (Z1-PI) inhibits elastase rapidly, thrombin (IIa) slowly, and activated protein C (APC) not at all. The M358R mutation of Z1-PI greatly enhances Z1-PI’s anti-IIa activity and confers anti-APC activity on Z1-PI. Another serpin, heparin cofactor II (HCII), specifically inhibits IIa and contains an acidic domain that binds IIa, exosite I. Purpose: To determine if fusion of this domain (residues 1–75 of HCII) to Z1-PI would selectively enhance Z1-PI’s anti-IIa activity by expressing two fusion proteins, HCIIζ75-Z1-PI (HAPI) and HAPI (M358R).

Methods: Wild-type Z1-PI, Z1-PI (M358R), and both fusion proteins were expressed in bacteria using recombinant DNA technology, purified, and characterized using enzyme kinetics.

Results: Addition of the HCII acidic domain increased the rate of inhibition of z-IIa by Z1-PI by 4.3-fold and that of Z1-PI (M358R) by 16-fold; the rate of APC inhibition by Z1-PI (M358R) was increased in HAPI (M358R) by only 1.3-fold. Reaction stoichiometries were unaltered for the HAPI proteins compared to their corresponding unfused Z1-PI proteins. Inhibition of γ-IIa, a form of IIa lacking exosite 1, was 11-fold greater in HAPI (M358R) than in Z1-PI (M358R).

Conclusions: The appended HCII domain increased IIa selectivity by both exosite I-dependent and exosite I-independent mechanisms. This strategy for increasing the specificity of recombinant serpins could lead to the development of novel therapeutic proteins that inhibit IIa and not APC.

**ABSTRACT NO.: 58**

Platelet serotonin and bleeding

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Inherited or therapy-induced serotonin deficiencies are known to cause bleeding problems. To test the hypothesis that serotonin is required for clot stability we investigated the fate of serotonin after its release from platelets. Using high-pressure liquid chromatography (HPLC) we found that most of the released serotonin is not free in solution, suggesting covalent linkage to a plasma protein. Immunoblotting of human plasma fibrinogen resulted in specific recognition of the alpha-subunit of fibrinogen by different monoclonal serotonin antibodies. Serotonin could be released from plasma fibrinogen by gentle hydrolysis and was subsequently identified by HPLC. A molar ratio of serotonin to fibrinogen of 2:1 was found. Fibrinogen isolated from thrombin-activated platelets had significantly more serotonin bound than fibrinogen isolated from ADP-stimulated platelets. In a purified system, FXIIIα crosslinked additional serotonin to the beta-subunit of fibrinogen as demonstrated by SDS-PAGE and Western blotting of the reaction product compared to unreacted plasma fibrinogen. Serotonin binding to fibrinogen was also verified by immunofluorescence microscopy of dually labeled platelet aggregates. Co-localization of the fluorescent labels of serotonin and fibrinogen confirmed their close proximity. Inhibition of serotonin release by selective serotonin reuptake inhibitors in vitro leads to the formation of loose aggregates. Together, these results suggest that serotonin release from platelets and covalent crosslinking to fibrinogen is required for the formation of stable clots. Increased availability of serotonin or serotonin agonists might enhance the hemostatic efficacy of blood products and reduce their demand.

**ABSTRACT NO.: 59**

The use of surveillance data to guide optimal West Nile screening of the blood donor population – A novel approach

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Purpose: Canadian Blood Services has performed blood donor screening for West Nile Virus (WNV) since July 2003, using Mini pools of 6 donor samples. Single unit (SUT), or individual donor testing provides enhanced sensitivity in the detection of WNV in blood donor samples. However, as technical, reagent and human resources preclude single unit testing all donations, Canadian Blood Services set up a risk assessment strategy, using surveillance data to target SUT to high risk areas during West Nile season, and therefore minimize the impact of West Nile on blood safety and supply.

Methods: A risk assessment system was set up, utilizing data on bird, mosquito and human case surveillance, obtained from the Canadian Cooperative Wildlife Health Centre, the Public Health Agency of Canada (formerly Health Canada) provincial public health reports, and donor testing data. In addition, public health laboratories and provincial public health authorities kept CBS personnel up to date on suspect and confirmed human cases in their jurisdiction. Risk levels were assigned to each health region across the country on a weekly basis, using the CBS database. Data on upcoming blood donor clinics by health region, with the expected number of donors per clinic, was matched with risk level from 0 to 4 (with 4 being the highest risk) and SUT assigned to clinics in areas of highest risk up to the anticipated testing capacity of the Calgary and Toronto Donor Testing Labs. All WNV nucleic acid testing (NAT) was performed using the Roche TaqScreen West Nile Virus Test Kit (investigational use). Screen positives undergo further testing using a second NAT, and antibody testing, at the National Testing Lab (CBS) in Ottawa.

Results: From August 2 to September 23, 2004, the Donor Testing Labs tested 20,523 donations by SUT and 115,986 donations by Mini pool, for a total of 136,509 WNV NAT tests. During this time, no positive donors were detected, although 4 donors had initial positive screen tests. These were negative for WNV by retesting, as well as on secondary NAT and serology for WNV (note that 14 positive donors were detected in 2003). No donor clinics were cancelled, and there were no reported cases of WNV transfusion transmission in Canada in 2004.

Conclusions: The risk assessment system combined with CBS data warehouse information on blood donor clinics by health region proved to be a nimble and responsive tool to guide allocation of SUT resources to regions of highest risk.
ABSTRACT NO.: 60
The blood donor screening questionnaire: Is it effective?
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The donor health assessment questionnaire (DHAQ) has been developed over more than 20 years with multiple additions to ensure safety for both the donor and the recipient, but it has never been re-evaluated in light of advances in cognitive science. The purpose of this study was to determine whether or not the format of the DHAQ questions was optimal to elicit thoughtful donor responses. In 2004, 252 donors were recruited from Ottawa and Hamilton and were asked to complete the DHAQ and participate in a short, scripted interview in which they were asked if they recalled being asked 15 specific items from the DHAQ. Participants included 139 males and 113 females; mean age 45.5 years (range: 17–69) and 98.4% were repeat blood donors with an average of 2 to 5 donations in the last 12 months. It was expected that if donors carefully read the questions and thought about their answers, most would remember being asked the questions immediately after completing the DHAQ, but of the 252 participants, only 137.9% remembered being asked all 15 items. Females were more likely to recall all questions correctly than males (29.7% vs. 15.0%). Of particular importance, participants were more likely to remember being asked items that were asked as an individual question (71.1%) compared with items asked as part of a list (26.1%) and it is noteworthy that many DHAQ questions include lists of items (up to 9 items in a list). These preliminary results suggest that blood donors’ ability to recall questions immediately after completing the DHAQ was remarkably low and that the format of the questions was not conducive to good comprehension. As the purpose of the DHAQ is ensuring safety for the donor and the recipient, revision of the DHAQ should focus not just on the addition of questions, but also on their format, with application of current cognitive science principles.

ABSTRACT NO.: 61
Informing blood donors about abnormal test results: … ongoing initiatives by Canadian Blood Services to improve process and outcome
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ABSTRACT NO.: 62
Potential impact on Quebec blood supply of excluding female parous plasma and platelet apheresis donors
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ABSTRACT NO.: 63
Levels of ADAMTS-13 in fresh, stored and SDP treated plasma
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ABSTRACT NO.: 64
Improving donor and physician communications and follow-up of positive donor TD test results are ongoing initiatives at CBS. Outcome evaluation of process changes is desirable.
processes 1 and 2. Cryosupernatant plasma from CBS was also assessed. Total protein, albumin, fibrinogen and immunoglobulins were also measured.

**RESULTS** The levels of both the 175 and 140 Kd subunits of von Willebrand Factor (VWF) were found to be constant at 1.38 and 1.35 OD units from 0 to 48 hours. There was some decrease at 72 hours. The Vitex SDP produced more of the 140 Kd subunit than did Octaplasm which gave essentially equivalent products of 175 and 140 Kd. In general, the solvent detergent treated plasma contained more (10%) protease activity than did normal human plasma. Cryosupernatant plasma demonstrated identical effects as did normal human plasma. The Vitex SDP contained slightly more albumin (41.8 vs 34.7) than FFP and a higher fibrinogen (4.3 vs 3.6 mg/mL). The total protein in the Vitex SDP was also higher at 66 mg/mL whereas that in FFP and Octaplasm was 56 mg/mL.

**CONCLUSION** ADAMTS-13 levels are not significantly decreased by storage of plasma at room temperature for up to 48 hours. Both cryosupernatant and SDP plasmas from two manufacturers also contained essentially normal levels of ADAMTS-13 and therefore could be used for treatment of patients with TTP.

**ABSTRACT NO.: 64**

Diagnosis of transfusion reactions (TR) in a pediatric intensive care unit (PICU)

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**OBJECTIVES** The diagnosis of TR may be difficult in critically-ill children because symptoms/signs associated with TR could also be due to the patient’s underlying disease. The objective of this study is to report discrepancies in the opinion of experts concerning the diagnosis of TR in a PICU.

**METHODS** All consecutive patients admitted to our tertiary care PICU, between February 2002 and February 2004, were followed prospectively and all transfusions were recorded. For each transfusion, the bedside nurse recorded the patient’s status during, and up to 4 hours after each transfusion. Any pre-defined signs/symptoms that were associated with the transfusion and that could evoke a TR were recorded. Three independent experts (FG, HH, JL) retrospectively reviewed all transfusion event reports, corresponding patient charts and laboratory data. Based on specific definitions, the presence of TR, as well as type, imputability and severity of TR were evaluated by each expert (judicating process). Presence of TR was confirmed by consensus of at least 2/3 experts.

**RESULTS** During the study period 2505 transfusions were given to 307 patients. Among all transfusions given, 51 events were reported by the bedside nurses. Each event was evaluated by the 3 experts. 40 events were considered TR by the experts. There was complete consensus (3/3) on the presence of TR in 24 events (47%) and partial consensus (2/3) in 27 events (53%). For the 40 TR evaluated, consensus on the type of TR, imputability and severity of TR are shown in the table.

| Criteria (N = 40 TR) | Complete consensus (3/3) | Partial consensus (2/3) | No consensus |
|---------------------|--------------------------|------------------------|-------------|
| Type of TR          | 15 (37.5%)               | 20 (50%)               | 5 (12.5%)   |
| Imputability        | 6 (15%)                  | 24 (60%)               | 10 (25%)    |
| Severity            | 15 (37.5%)               | 22 (55%)               | 3 (7.5%)    |

**CONCLUSION** Complete consensus of experts (3/3) on the presence of TR, as well as the type of TR, imputability and severity was present in less than 50% of events and the absence of consensus was frequently seen. We conclude that adjudicating the diagnosis of TR is difficult, even with specific definitions and with clinical judgment of experts. Immediate evaluation of each event, as well as specific definitions for critically-ill children could help diagnose TR more accurately in PICU.

**ABSTRACT NO.: 65**

Improvements to an autologous blood program

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**BACKGROUND** Autologous Blood Programs have been advocated by Justice Kreaver and are currently in place at many sites in the country.

**METHODS** To optimize our Autologous Blood Program, we have initiated a study comparing the non-autologous donation to a program using the Trima Accel machine, Gambro, BCT to permit automated collection of two red cell units with and without erythropoietin. Patients are randomized to three study arms.

**RESULTS** To date a total of 59 patients have had their blood collected using the Trima machine. Two units are taken at one sitting allowing us to perform double-unit collection a considerable time before surgery. This permits more time for the patients to recover their hemoglobin: a single Trima collection accompanied by erythropoietin resulted in an average pre-operative hemoglobin of 158 versus 108 in normal autologous patients. We have therefore decreased the standard pre-donation autologous requirement of three units of red cells to a single Trima collection. At $300 per autologous unit, this represents a cost savings of $18,000 in our 59 patients. When applied to the total autologous program at our hospital (450/year), this extrapolates to a more savings of over $100,000 when using combined collection approaches. 49/59 patients in this pilot study have received only autologous blood, again reducing the number of allogeneic units that would have been required. The need for only a single autologous donation cuts nursing time by 2/3 and also saves laboratory money, since only a single unit is tested for infectious diseases.

**CONCLUSION** When applied to an autologous blood program, the use of the Trima machine combined with erythropoietin in appropriate cases, results in a decrease in the number of units collected and a higher pre-operative hemoglobin. This program should see application in other sites.

**ABSTRACT NO.: 66**

Quality of components prepared from whole blood units by the PRP method after a hold of 24 hours at 20–24°C

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**PURPOSE** In the PRP method, whole blood (WB) must be processed within 6 hours after collection. The Buffy coat method allows a 24-hour hold (24-hH) of WB at RT before processing. Increasing the storage time of WB to 24 hours in the PRP method would be advantageous for component preparation logistics. Impacts of a 24-hH of WB on the quality of components prepared by the PRP method were evaluated.

**METHOD** 64 volunteers were enrolled in this study. Two ABO-identical donors were collected simultaneously and UB units were immediately pooled and split. The units were held either 6–8 hours or 22–24 hours at 20–24°C. The 24-hH units were rapidly cooled on 1,4 butanediol plates as in the BC method. Leucoreduced platelet concentrates (PC), red blood cells (RBC) and FFP were prepared by the PRP method and analyzed during storage.

**RESULTS** RBC prepared after an 8-hH or 24-hH were comparable in terms of weight, hemolysis, sodium, pH and ATP. Differences, observed immediately after processing in 24-hH units for 2,3-DPG (~36%) and lactate (~49%), were no longer apparent after 21 days. Residual leucocyte counts were 4.5 × higher (p < 0.01) in 24-hH RBC. For PC, results for glucose, ESC, HSR and pH were similar. After processing, lactate levels were higher (~53%) in the 24-hH PC but no differences were observed on days 5 and 7. CD62p activation was reduced (~9%) in the 24-hH PC. No differences were observed in FFP for vWF, FV, FVIII and Fg.

**CONCLUSIONS** The quality of blood products prepared by the PRP was not irreversibly affected by a hold of 24 hours of WB at

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20–24°C. The 24-hH of the BC method would also be acceptable with the PRP method.

**ABSTRACT NO.: 67**

Detection of bacterial contamination
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Canadian Blood Services Winnipeg implemented a project to detect bacterial contamination in whole blood platelet components. The BacT/ALERT 3D microbial detection system was implemented. The BacT/ALERT system functions by detecting the carbon dioxide emitted by microorganisms within the culture medium. The photodetectors will alarm if the sensor on the bottom of the bottle turns from dark to light due to the presence of carbon dioxide, thus indicating a positive culture. Samples for culture were obtained from whole blood platelets prior to issue. Platelets with segments of 23 cm long were used for culturing in pools of three to five. The segments were stripped three times to ensure the sample taken was representative of the unit, heat sealed and then separated from the main bag. The segments were then placed in a plastic rack and sterilized for 10 min in 70% isopropyl alcohol. The segments were then placed in a biological safety cabinet where the segments were vented on one end and 1–1.5 mL of sample from each segment was withdrawn by a syringe. The content of the segments were inoculated into the BacT bottle. The BacT bottle was then taken and incubated for 6 days. If no bacterial growth was present after 6 days, the BacT system displays a negative result. The bottle is then unloaded and a report was printed. If bacterial growth is detected, the system will alarm. The culture bottle and a component from each implicated donor was sent for gram stain and identification of the organism. In 9 weeks, 713 platelet pools were tested. Four positive pools were obtained. The first bacteria identified pool grew Ba culus filum. The platelets for the first positive culture were transfused. The patient did not become febrile. The second pool grew Staphylococcus epidermidis. None of the implicated components were transfused from the second positive pool. The third pool grew Staphylococcus hominis and Staphylococcus simulans and the patients’ blood cultures did not grow these organisms. Culture results on the final pool were pending at time of submission. Conclusion: The BacT/ALERT may be a useful method to detect contamination in whole blood platelets.

**ABSTRACT NO.: 68**

Traumatic and surgical bleeds: Non-protocol use of recombinant factor VIlia (rFVIlia)
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rFVIlia is approved for treatment of hemophilia and inhibitors to FVIII/FIX, and is currently being used with case reports of successful hemostasis in nonhemophilia patients with severe bleeding, including trauma and surgical bleeds.

**PURPOSE** To assess the successful hemostasis and survival of patients given rFVIlia for severe traumatic and post surgical bleeding and blood product use before and after rFVIlia.

**METHODS** A retrospective review of non-protocol rFVIlia use in regional tertiary care centres as requested by surgeons and ICU specialists for critically ill patients with intractable bleeding.

**RESULTS** 21 patients ages 17–77 (median 34) were given rFVIlia for bleeding; 14 (67%) achieved hemostasis and 8 (38%) survived.

| (Average) Acidosis | pRBC (u) | APACHE II | (Predicted mortality rate) |
|--------------------|----------|-----------|---------------------------|
| pH                 | Pre      | Post VIlia|                           |
| Survivors          | 7.25     | 16        | 6                         | 21.9 (43%) |
| Deceased           | 7.10     | 30        | 9                         | 36.3 (78%) |
| p value            | 0.05     | 0.02      | 0.45                      | 0.01       |

The mean APACHE score for the entire group was 35.2 with an estimated mortality risk of 77%. No difference was seen between the survivors and deceased patients in the use of platelets, fresh frozen plasma or cryoprecipitate before or after rFVIlia, in dose/kg of rFVIlia, time interval between injury and rFVIlia, platelet count or coagulation studies prior to rFVIlia.

**CONCLUSIONS** In this limited review of 21 patients with severe traumatic and surgical bleeds, rFVIlia use without a defined protocol was associated with hemostasis in 67% but only 38% survived to discharge. rFVIlia was used relatively late in the clinical course. Further study is needed to develop guidelines for timing for rFVIlia use in relation to injury, extent of prior blood product use, coagulopathy, and other variables in patient selection during surgical and trauma bleeds.

**POSTER PRESENTATIONS**

**ABSTRACT NO.: 69**

Efficient gene transfer into normal human B lymphocytes by Ad5/F35 chimeric adenoviral vectors
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The failure to efficiently introduce genes into normal cells, such as human B lymphocytes, limits the characterization of their impact on cellular growth, differentiation and survival. The development of adenovirus 5 vector to transfer genes in normal cells was promising but, like most expression systems, turn out to only poorly transduce genes into B lymphocytes. Nevertheless, many advantageous features of these recombinant adenovirus, such as transduction of either proliferating or quiescent cells, expression of transduce genes within hours and extremely high achievable titers; still make these as the most attractive gene delivery vectors. Recently, studies have shown that chimeric fiber-modified Ad5/F35 adenoviral vectors are able to efficiently transduce human haematopoietic progenitor cells. In this study, we compared gene transfers into human B lymphocytes using Ad5/F35 and conventional AdSâdenovirus. Peripheral blood B cells obtained from healthy individuals were cultured in vitro using CD40-CD154 system. Cells were transduced with replication-defective Ad5 and Ad5/F35, both containing a GFP reporter gene and efficiencies were monitored by flow cytometry. We show here that Ad5 infects about 3% of human B cells. In contrast, GFP expression was detected in up to 80% of human B cells 24 hours post-infection and could be detected for up to seven days post infection. Furthermore, Ad5/F35 transduction of lymphocytes did not result in impairment of proliferation nor viability. This ability to efficiently manipulate gene expression in normal B lymphocytes using adenovirus vectors open new avenues in the characterization of pathways affecting lymphocyte physiology.

**ABSTRACT NO.: 70**

Establishment of transplantable preleukemic myeloid lines that can be converted into AML-inducing cells
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**PURPOSE** We have previously demonstrated that the acute myeloid leukemia (AML)-associated fusion gene NUP98-HOXD13 (ND13) directly participates in the development of leukemia. However, the long latency and monoclonal nature of the diseases support the requirement for secondary mutations. Conversely, the HOX cofactor Meis1 was shown to strongly synergize with Hox or NUP98-HOX genes toward the rapid development of AML, suggesting that the combinations was sufficient for the induction of leukemia. Our primary objective was to establish a convenient model to identify such collaborating mutation(s) and/or for the study of leukemia progression.
We transduced fresh murine bone marrow (BM) cells with retroviral vectors (RV) to constitutively express ND13 or NUP98-HOXA10 (NA10). The BM cells were then expanded in liquid culture for the establishment of myeloid lines. These, with or without transduction with a Meis1 RV, were then characterized in vitro and in vivo (in mice).

The established lines (n = 6) consist of early myeloid progenitors with in vitro self-renewal capacity, short-term myeloid repopulating activity and low propensity for spontaneous leukemic conversion. Surprisingly, all lines were readily converted into AML-inducing cells upon ectopic Meis1 expression (survival median of 57 and 64 days for ND13 and NA10 lines). The leukemogenic synergistic activity of Meis1 reflected its ability to increase their self-renewal/proliferative capacity.

These results demonstrate that such fusion genes can establish a preleukemic condition, and that the preleukemic NUP98-HOXL cells faithfully replicate a “two hits model” of leukemogenesis.

Abstract No.: 71
Mesenchymal stem cells express homing receptors and tissue-specific markers: Implications for cellular therapies

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Clinical applications of human mesenchymal stem cells (MSC) are evolving rapidly in cell-based therapies such as tissue regeneration and as a support for hematopoietic stem cell (HSC) transplantation. The signals regulating MSC mobilization to injured sites have not been elucidated. We recently reported that stromal-derived factor (SDF)-1 and hepatocyte growth factor (HGF), known to regulate HSC homing to bone marrow (BM), occur in increased levels at sites of tissue damage. In this study we examined whether SDF-1 and HGF and their respective receptors, CXCR4 and c-met, mediate the migration of MSC. MSC cultures were established with mononuclear cells from BM and cord blood (CB) and the expression of CXCR4 and c-met and their function was evaluated. Moreover, we determined whether MSC express matrix metalloproteinases (MMPs), enzymes known to degrade basement membrane and facilitate mobilization and homing of HSC, as well as markers for cardiac, skeletal muscle and endothelial cells. We found that (i) CB and BM MSC express CXCR4 and c-met mRNA; (ii) these receptors are functional as MSC chemoinvasion towards gradients of SDF-1 or HGF increased for up to 3-fold compared with the control (media alone); (iii) CB and BM MSC express membrane type 1-MMP and MMP-2; and (iv) CB and BM MSC express early cardiac (Nkx2.5/Csx and GATA-4) and endothelial (VE-CAD) markers. These results indicate that the SDF-1-CXCR4 and HGF-c-met axes, together with MMP pathways, are important signaling mechanisms in MSC homing and recruitment to injured tissues.

Abstract No.: 72
Design and validation of a small scale perfusion bioreactor susceptible for the in vitro expansion of normal human cells

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The in vitro expansion of normal human cells is often required for cellular therapies and is most conveniently done in controlled bioreactors. Most available bioreactors are not suitable for this use because of the large size of the culture vessel. In this work, we have designed and validated a small scale perfusion bioreactor for use with hematopoietic stem cells.

The culture vessel is a modified 100 mL water-jacketed spinner flask (60 mL working volume) equipped with an acoustic cell retention device that can be operated at up to 1 L/day at a >90% cell separation efficiency. Oxygen, pH, temperature and level sensors were inserted through a custom-made head plate. For optimal flexibility, the control algorithms and process data acquisition were done on a PC using a custom modified LabVIEW program and analog/digital converters.

The custom bioreactor was validated by performing perfusion (up to 2 vol./day) runs with D5 hybridoma cells which were also cultured in a similarly perfused 3.5 L Celligen Plus bioreactor. The results showed that the 2 systems had almost identical performances in terms of viable cell yield (10,5–10,7 × 10^6 cells/mL perfused volume) and of antibody productivity (24,0–24,7 ± 0.5 µg/day*10^6 cells). The reliability of the small scale system was excellent with no failure over 3 weeks of continuous culture.

The small size, flexibility and reliability of the developed perfusion bioreactor will facilitate its use for the expansion of hematopoietic as well as other types of normal human cells.

Abstract No.: 73
Peripheral and cord blood CD34+ cells express early markers for heart and muscle: Potential for cellular therapy

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Some investigators support the concept of stem cell plasticity whereby bone marrow (BM) hematopoietic stem cells can differentiate into the cells of tissues other than blood. We, however, propose an alternative hypothesis whereby tissue-committed stem cells (TCSC) already exist in the BM, and can be mobilized into peripheral blood (PB) and chemoattracted to damaged organs during stress or injury. We recently reported (Leukemia 2004, 18:401) increased levels of TSCC in mobilized (mPB) and showed that these cells express CXCR4, the receptor for SDF-1. In this study we determined whether CD34+ cells from mPB and cord blood (CB) express early markers for TSCC such as heart (Nkx2.5/Csx, GATA-4 and MEF2-C) and skeletal muscle (Myo-D, Myf5 and myogenin). We found transcripts for the three cardiac markers and myogenin in all samples of mPB CD34+ cells tested; and Nkx2.5/Csx, GATA-4, myogenin and Myo-D in all the samples of CB CD34+ cells. We also found significantly higher expression of these markers in mPB CD34+ cells that migrated to an SDF-1 gradient, suggesting that chemotactic isolation may be employed for enrichment of TSCC expressing these markers. These results suggest that (i) mPB and CB could become a source of TSCC and (ii) that after their chemotactic enrichment these cells could be used for cell-based strategies for tissue regeneration.

Abstract No.: 74
Iron specific growth inhibition of Burkitt’s lymphoma cells in vitro due to homeostasis disruption by c-myc over-expression

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Burkitt’s lymphoma is an aggressive B-cell lymphoma resulting from the deregulation of c-myc expression. We have previously shown that cellular proliferation of Burkitt lymphoma cell lines such as Ramos and Raji is markedly reduced by iron (Habel et al., J Cell Physiol 2004; DOI 10.1002/jcp.20229). This effect is mediated by a cell cycle arrest in G2/M, whereas no effect could be observed in a cell line harboring amplified c-myc such as HL-60. Effects of iron on c-myc translocated cells might result from a disruption of iron homeostasis. Iron has been shown to induce c-myc expression and, as a transcription factor, c-Myc regulates genes involved in iron metabolism. Transient enhancement of c-myc expression by iron could lead to an increase of expression of genes involved in iron incorporation, conducting to an accumulation of intracellular free iron and consequently, to oxidative stress. Here we investigated whether cells with a high basal level of c-Myc were more likely to accumulate free iron. Basal level of c-Myc in...
Ramos cells is two-fold the level of HL-60. In Ramos cells, where c-Myc is expressed at high level, H-ferritine expression is down-regulated, transferrin receptor (CD71) expression is increased and Ferritin translation is blocked. These modifications in iron metabolism are probably caused by the strong basal expression of c-Myc and amplified by iron addition lead to a disruption of homeostasis and consequently to growth arrest.

**ABSTRACT NO.: 75**

Decrease of cyclin a expression triggers iron specific growth inhibition of Burkitt’s lymphoma cells in vitro
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Addition of ferric citrate to Burkitt lymphoma (BL) cell lines inhibits cell growth and leads to the accumulation of cells in G2/ M cell cycle phase. This specific effect of iron on BL cells appears to be related to predisposition of BL to oxidative stress, caused by the high basal level of c-Myc. Intracellular free iron, amplified by c-Myc over-expression, catalyzes the formation of highly reactive compounds such as cytotoxic hydroxyl radicals that cause damage to the macromolecular components of cells, including DNA and proteins, and thereby apoptosis. It has been reported that antiproliferative effect of reactive oxygen species (ROS) could result, at least in part, from the repression of cyclin A gene transcription. Cyclin A is a positive regulatory component of kinases required for the progression through S phase and for the transition between the G2 and M phases of the cell division cycle. Since iron addition to BL cells induced growth arrest and G2/M accumulation, we studied the effect of iron on cyclin A regulation in BL cells. Addition of iron to BL induces a decrease of cyclin A expression that precedes cell growth inhibition and G2/M accumulation. Finally, cyclin A mRNA stability seems unaltered by iron addition, suggesting that cyclin A could be transcriptionally repressed in response to iron treatment.

**ABSTRACT NO.: 76**

Phosphatidylserine distribution is highly sensitive to paraformaldehyde in platelets
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Previous studies reported that paraformaldehyde (PFA) fixation of platelets alters surface expression of antigens to varying degrees. Present experiments ask whether PFA modifies PS expression in human platelets. Results show that the asymmetric distribution of phosphatidylserine (PS) in platelets is especially sensitive to this fixative. It was found that PFA induced a dose- and time-dependent translocation of phosphatidylserine to the membrane surface as measured by annexin V binding and flow cytometry. The percent PS-positive cells increased from <5% to near 100%. Chelation of extracellular Ca<sup>2+</sup> with EGTA partially blocked this translocation. Spectrofluorimetric analysis of Fluo-3 loaded platelets indicates that PFA caused a concomitant elevation of intracellular Ca<sup>2+</sup> concentrations. The distruption of Ca<sup>2+</sup>-homeostasis in platelets was likely due to PFA-dependent decreases in internal ATP levels and a decline in Ca<sup>2+</sup>-ATPase pump activity. PS externalization may derive from Ca<sup>2+</sup>-activated randomization of membrane phospholipids and decreased transport of PS from the outer to the inner leaflets of the plasma membrane. In light of present results, PFA fixation is best avoided especially in studies involving platelet apoptosis or clearance.

**ABSTRACT NO.: 77**

Priming of CD34<sup>+</sup> stem/progenitor cell responsiveness to an SDF-1 gradient as a potential means for improving engraftment
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The means available to accelerate hematopoietic recovery after bone marrow (BM) or cord blood (CB) transplantation are limited. Moreover, it is not clear why hematopoietic stem/progenitor cells (HSPC) derived from mobilized peripheral blood engraft faster than those from BM. Recently we reported that platelet-derived microvesicles and the anaplythoxoizol complement C3a (elevated in leukapheresis products from patients mobilized with G-CSF), increase the chemotactic responses of BM HSPC to SDF-1, a chemoattractant for HSPC homing to BM. Hence we investigated whether supernatants obtained from leukapheresis products (SLP) collected from G-CSF-mobilized patients, as well as other components of SLP (thrombin, fibrinogen and fibronectin), enhance the chemotactic responses of HSPC to an SDF-1 gradient. We found that although SLP or their components alone do not induce chemotaxis, in combination with a low dose of SDF-1 they enhance it. They also induce secretion of matrix metalloproteinases important in migration of HSPC and increase incorporation of CXCR4, a receptor for SDF-1, into membrane lipid rafts. Based on these data we conclude that CXCR4 is primed by various molecules related to mobilization/leukapheresis allowing HSPC to better sense an SDF-1 gradient. We postulate that some of these molecules, used in recombinant form, could find clinical application for ex vivo priming of human HSPC before transplantation. Their action could enhance the homing of HSPC to BM (especially important for CB transplantation), and thus reduce the period of post-transplant neutropenia and thrombocytopenia.

**ABSTRACT NO.: 78**

Increased megakaryopoiesis in cultures of CD34-enriched cord blood cells maintained at 39 °C
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The future use in transplantation of ex vivo expanded hematopoietic stem and progenitors cells will facilitate the treatment of adult patients and speed up hematologic recovery. Culture of animal cells is routinely done at 37 °C. However there is previous clinical evidence suggesting that hematopoiesis may be more active in hyperthermic patients. We have therefore compared the effect of hyperthermia on the ex vivo expansion and differentiation of megakaryocytes (MK).

The cord blood-derived CD34<sup>+</sup> cells were cultured continuously at 37 °C or 39 °C for 14 days in cytokine conditions optimized for MK development. Compared to 37 °C, the cultures maintained at 39 °C produced significantly more total cells (4.9 fold) and total MKs (7 fold), and showed accelerated and enhanced MK maturation with increased yield of proplatelets and platelets (16.6 fold). Accordingly, the cells cultured at 39 °C contained an increased frequency of CFC-MK (8 fold) at day 14. Cultures done at 38 °C and 40 °C were less efficient. Control experiments showed that the culture of several cell lines was unaffected or inhibited by the 39 °C temperature.

These results reveal the unexpected resistance of hematopoietic cells to the deleterious effects of heat on cell physiology. The underlying molecular mechanisms remain to be identified but the observation will facilitate the ex vivo expansion of the progenitors of the MK and possibly other lineages.

**ABSTRACT NO.: 79**

Reactive oxygen species promote polyplidization of the megakaryocytic cell line M-07e
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Hematopoietic cells mature in the bone marrow under the control of a diversity of growth factors and the influence of various cell types producing superoxide and other reactive oxygen species...
significantly reduced cell proliferation without Plasma (250 mL) was processed in the Cryoseal FS.

\( Fc \)

E. L. G. Pryzdial receptors (Fc)

2005 Blackwell Publishing Ltd, treatments affected members of the

2. \( Fc \)

Cryoprecipitate is frequently combined with thrombocytope

3. \( Fc \)

mers represents a potentially promising approach to increase the
clearance of platelets in a thrombocytopenic mouse model more

prevent in vitro phagocytosis of opsonized red blood cells and

anti-human IgG (clone C5–1) and that C5–1/IVIg complexes also

nic mouse model. Surprisingly, IC prepared with C5–1 were highly

blood cells in vitro, and clearance of platelets in a thrombocytope-

parizations of human Fc fragments were at least 6 times more

monoclonal anti-human IgG (clone C5–1) and commercial pre-
tetramolecular immune complexes (IC) prepared using a mouse

participation of low affinity Fc

mechanisms by which IVIg can prevent platelet clearance in ITP

such as immune thrombocytopenic purpura (ITP). However, the

mechanisms by which IVIg can prevent platelet clearance in ITP

patients are not fully understood but are known to require the

of low affinity Fc\(_{g}\) receptors (Fc\(_{g}R\)) which interact

poorly with monomeric IgG. We recently showed that small-size
tetramolecular immune complexes (IC) prepared using a mouse
monoclonal anti-human IgG (clone C5–1) and commercial pre-
parations of human Fc fragments were at least 6 times more
efficient than IVIg to prevent phagocytosis of opsonized red
blood cells in vitro, and clearance of platelets in a thrombocytope-
nic mouse model. Surprisingly, IC prepared with C5–1 were highly
stable, even in presence of an excess of soluble IgG and did not
activate complement in vitro. Here we show that similar complexes
can be prepared simply by mixing IVIg (Gamunex, Bayer) and
anti-human IgG (clone C5–1) and that C5–1/IVIg complexes also
prevent in vitro phagocytosis of opsonized red blood cells and
clearance of platelets in a thrombocytopenic mouse model more
efficiently than IVIg. Thus, immune cross-linking of IgG monos-
mers represents a potentially promising approach to increase the
specific inhibitory activity of IVIg or to develop an IVIg-free sub-
stitute through the formation of IC with the autologous IgG of ITP
patients

This study was supported by a grant from the Bayer-CBS-HQ
Fund.

ABSTRACT NO.: 80

Increased phagocytosis inhibitory activity of IVIg following
immune cross-linking of IgG

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Intravenous immunoglobulins (IVIg) have immuno-modulatory
effects in vivo and are widely used to treat autoimmune diseases
such as immune thrombocytopenic purpura (ITP). However, the
mechanisms by which IVIg can prevent platelet clearance in ITP
patients are not fully understood but are known to require the
participation of low affinity Fc\(_{g}\) receptors (Fc\(_{g}R\)) which interact
poorly with monomeric IgG. We recently showed that small-size
tetramolecular immune complexes (IC) prepared using a mouse
monoclonal anti-human IgG (clone C5–1) and commercial pre-
parations of human Fc fragments were at least 6 times more
efficient than IVIg to prevent phagocytosis of opsonized red
blood cells in vitro, and clearance of platelets in a thrombocytope-
nic mouse model. Surprisingly, IC prepared with C5–1 were highly
stable, even in presence of an excess of soluble IgG and did not
activate complement in vitro. Here we show that similar complexes
can be prepared simply by mixing IVIg (Gamunex, Bayer) and
anti-human IgG (clone C5–1) and that C5–1/IVIg complexes also
prevent in vitro phagocytosis of opsonized red blood cells and
clearance of platelets in a thrombocytopenic mouse model more
efficiently than IVIg. Thus, immune cross-linking of IgG monos-
mers represents a potentially promising approach to increase the
specific inhibitory activity of IVIg or to develop an IVIg-free sub-
stitute through the formation of IC with the autologous IgG of ITP
patients

This study was supported by a grant from the CBS/HQ/Bayer
Partnership Fund to RB and RL.

ABSTRACT NO.: 81

High polyspecificity of autoantibodies present in intravenous
immunoglobulins (IVIg)

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Natural IgG serum antibodies are usually polyspecific and often
reactive with self structures (auto-IgG). The self-reactivity of auto-
IgG is inhibited in healthy individuals by anti-id IgM. We have
recently reported that the control of auto-IgG is impaired in serum
containing therapeutic amounts of IVIg since we could detect the
formation of autoimmune complexes (auto-IC) between IVIg-
derived auto-IgG and several soluble serum proteins. The hetero-
genesis of the auto-IC composition remained unclear since the
polyspecificity of the auto-IgG is unknown.

In the present work, we have characterized the polyspecificity of
auto-IgG reacting with serum proteins and from preparative west-
tern blot (WB) membranes into 4 sub-populations corresponding
to reactivity with serum proteins of different MW. Testing of the 4
auto-IgG sub-populations in secondary WB showed the predomin-
ant presence of polyspecific IgG reacting with proteins of all MW
with only a reduced proportion of monospecific IgG. This result
was confirmed in additional WB experiments in which the auto-
IgG purified by affinity chromatography on immobilized serum
proteins were found to react with auto-antigens expressed by sev-
eral types of human cells (kidney, liver, muscle . . .).

The high polyspecificity of the auto-IgG may be important for
the rapid formation of heterogeneous auto-IC during the infusion
of IVIg in plasma. These auto-IC may contribute to the mechan-
isms of therapeutic action of IVIg in some diseases.

This study is supported by a grant from the Bayer-CBS-HQ
Fund.

ABSTRACT NO.: 82

Thrombin enhances herpes simplex infection

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Our lab has previously shown that herpes simplex virus (HSV1)
can initiate and sustain clot formation directly, bypassing the
normal cell surface requirements. In addition to host-derived coa-
gulation initiators on the virus, we demonstrated that HSV1-
encoded glycoprotein C (gC) participates in coagulation enzyme
activation. The evolution of the HSV surface as a clotting agent
lead us to hypothesize that generation of thrombin is an advantage
to the virus. Consistent with our hypothesis, serum-free plaque
assays revealed that co-inoculation of the virus and human umbilici-
val vein endothelial cells with purified thrombin enhanced infection
by up to 400%, with half-maximal effects at approximately 20 nM
for all HSV strains studied. As a demonstration of specificity,
hirudin attenuated the effects of purified thrombin. The involve-
ment of the protease activated receptor 1 (PAR1) in this mechan-
ism was shown using a synthetic signaling analogue, TRAP. In the
absence of serum, TRAP resulted in increased infection compar-
able to that of purified thrombin. Approximately half the effect of
TRAP was observed at 10 uM, while a PAR4-activating peptide
did not have effect. The presence of serum, to allow in situ thrombin
generation, enhanced infectivity by as much as 20-fold. The
administration of hirudin attenuated infection by about 90% for a gC-
deficient strain, whereas the other viruses were only marginally or
insignificantly affected, supporting a role for thrombin generation
during infection and the participation of gC. Thus a hypercoagu-
able state may be a predictor of susceptibility to infection.

ABSTRACT NO.: 83

Preparation and characterization of human thrombin for use in a
fibrin glue

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Hospital, University of Ottawa, Ottawa, Ontario, Canada.

BACKGROUND Cryoprecipitate is frequently combined with throm-in to produce a fibrin sealant in order to enhance hemostasis
during surgical procedures. We evaluated the thrombin produced
from plasma using the Thrombin Processing Device (TPD).

METHODS Plasma (250 mL) was processed in the Cryoseal FS
System using the CP-3 disposable to produce cryoprecipitate by
automated freezing and thawing. Simultaneously, thrombin was
generated using the attached TPD. The cryoprecipitate and
thrombin were harvested after approximately 50 min, then frozen and stored at −80 °C until analysis. The products were assayed for total protein, fibrinogen, Factor VIII, von Willebrand Factor and thrombin activity. SDS gel electrophoresis was used to compare the thrombins. After combining the thrombin and cryoprecipitate, the rate of clot initiation and strength was measured using a thromboelastograph (TEG).

**Results** Cryoprecipitate was produced with a fibrinogen concentration of 22 ± 7.7 g/L (20 ± 2% recovery), Factor VIII activity was 14.2 ± 4.0 U/mL, von Willebrand Factor 19.9 ± 5.2 U/mL. The separate thrombin product had a concentration of 64.3 ± 16.7 U/mL thrombin and total protein of 0.39 ± 0.19 with SDS gel electrophoresis showing many bands with a major band at 37 kD. Commercial human thrombin showed similar bands. The TEG curves from autologous cryoprecipitate and TPD-produced thrombin were compared to those from standard cryoprecipitate and commercial human thrombin. R values (time to clot initiation) were somewhat slower but the maximum strength (MA) of the clot was greater with the TPD-produced thrombin.

**Conclusions** Human thrombin can be produced during automated cryoprecipitate production. This thrombin is in sufficient concentration to initiate clotting and crosslinking of cryoprecipitate to produce an entirely autologous fibrin glue.

**ABSTRACT NO.: 84**

Regionalization of budgets for blood products in Québec, 2003–2005

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The Ministère de la Santé et des Services Sociaux du Québec has put in place measures to ensure a new method of financing for blood products in Québec, as of April 2003. The objective of this presentation is to report on activities and follow-up indicators of the 2003–2005 experimentation phase of the Québec project on the regionalization of budgets for the provision of blood products.

**Methods** The Act respecting Héma-Québec and the Hemovigilance Committee (L.R.Q., c-H-1.1), guiding principles, approaches and structural and systemic mechanisms (DGSP, 2004) have supported the decision-making and implementation of project activities. Follow-up indicators were defined through the use of scientific literature and expert analyses and applied to years 1999 to 2003 and subsequent years.

**Results** Since April 1, 2003, Québec hospitals have been receiving invoices from Héma-Québec as well as government budgets for the provision of blood products. These are virtual invoices and budgets for the experimentation phase. In particular, the hospitals do not have to pay these invoices. Hospitals follow up on expenditures and management targets of blood products, as defined by the Ministère de la Santé et des Services Sociaux. The annual utilization growth of packed red blood cells was 5% for 1999–2003 and 1% for 2003–2004; the expiration rate of packed red blood cells in Québec hospitals was 4% for 2001–2003 and 3% for 2003–2004.

**Conclusion** The increase in the cost of blood products in Canada as well as elsewhere poses a threat to public health systems (Wilson, 2003; Ness, 2003). Optimizing use, evaluating the costs of safety measures and benefits derived, and reviewing the financing methods of blood products could contribute to reducing the increasing costs of blood products. However, studies are needed in order to measure the real impact of financing methods in this regard.

**ABSTRACT NO.: 85**

Implementation of a national inventory at CBS

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Canadian Blood Services provides blood to Canadian hospitals outside the province of Québec. The 2002/2003 Order/Fill rate for hospitals nationally was 90.4% with a range by centre from 73.8% to 100.0%. The 2003/2004 Order/Fill rate for hospitals nationally was 96.4% with a range by centre from 92.25% to 99.5%. To ensure equal service to all hospitals in Canada the concept of a National Inventory was implemented. Daily calls with key personnel in Production were implemented to discuss sites that had a deficit or abundance of a blood component. The complete implementation of MAK Progesa allowed for all inventories of blood components to be viewed on line. A spreadsheet was developed to capture the volume of ABO/Rh red cells available and predetermined levels of hospital requirements (4 Days on Hand). A summary of all inventory movement is captured daily. Weekly reports are completed to track national volumes of frozen inventory, LRF platelet production and collection of apheresis plasma and platelets. Weekly a summary of Order/Fill rates for all components and products are distributed to Production Managers to ensure correct information for service to hospitals. The national inventory also captures potential risks for shortfalls in collections and contingency planning in the case of a service disruption. Order/Fill rates for the past 9 months have hit 98.6% nationally with a centre range of 97.9% to 99.8%. Integral to this increase order fill rate has been an overall rise in collections at CBS. The National Inventory will be used to monitor increase blood order/fill rates to hospitals and set the groundwork for a National Production plan.

**ABSTRACT NO.: 86**

Canadian Blood Services (CBS) blood issues – Using the CBS data warehouse to provide information to improve practices

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**Background** The CBS Data Warehouse is a powerful information management system designed to help CBS monitor, analyze and evaluate key business intelligence. Its goal is to help CBS stakeholders understand data available in the organization that can be used to support improvements in performance and effectiveness.

**Methods** The Data Warehouse was used to provide data on blood components issued by CBS to hospitals on a per capita basis for nine provinces.

**Results** As shown below there is considerable variation in the number of units of labile products issued per year by CBS to hospitals in different provinces.

Units issued by CBS to hospitals per ‘000 population

|          | RBCs | Platelets | FFP | Cryo |
|----------|------|-----------|-----|------|
| BC       | 27   | 17        | 7   | 1    |
| AB       | 31   | 19        | 8   | 3    |
| SK       | 36   | 22        | 6   | 2    |
| MB       | 37   | 27        | 7   | 1    |
| ON       | 31   | 20        | 9   | 2    |
| NB       | 31   | 21        | 7   | 1    |
| NS       | 34   | 17        | 12  | 2    |
| PEI      | 28   | 5         | 3   | 0    |
| NF       | 35   | 17        | 11  | 2    |

**Conclusions** The CBS Data Warehouse allows comparisons of issuing patterns between provinces & could be extended to analyze specific hospital issues. This may allow the identification of factors which influence transfusion practice and of opportunities for improving inventory management. The addition of data on final disposition would further improve understanding of utilization patterns.

**ABSTRACT NO.: 87**

Requests for phenotyped red blood cells received by Canadian Blood Services, Toronto regional office

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**Background** Canadian Blood Services (CBS) is asked with increasing frequency to provide blood phenotyped for antigens beyond ABO...
blood group and the RhD antigen. To better understand the nature of these requests, a retrospective review was conducted.

**METHODS** Records of immunophenotyping investigations conducted at the Toronto regional office were reviewed from June 1st to November 29th, 2004. Estimated population frequencies of the antigens tested for were taken from standard reference texts.

**RESULTS** 580 requests for a total of 1607 units of phenotyped blood were received. 261 requests were from community hospitals, 270 from academic hospitals and 46 were from external CBS regional offices. The median estimated population frequency of each phenotype request was 0.66% (IQR 0.21–3.2%) for community hospitals, 0.01% (IQR 0.24–2.39%) for academic hospitals and 0.22% (IQR 0.19–0.57%) for external requests from other CBS centres. When adjusted for the number of units requested with each phenotype request, the median estimated screening requirement was 304 units (IQR 85–998) for community hospitals, 299 (IQR 94–1078) for academic hospitals and 664 units (IQR 287–1579) for other CBS centres.

**CONCLUSIONS** Academic and community hospitals within the Toronto area have similar needs for phenotyped blood. Requests from other CBS centres, while less frequent, are proportionately more labour intensive.

**ABSTRACT NO.: 88**

Blood, sweat and tears

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The poster supplies comprehensive information in an easy to read format displaying actual products with their associated price identified on the item. The cost of blood products in Canada is not readily available or accessible to physicians and other healthcare providers. This often results in products being ordered without cost/true expense being considered. To optimize appropriate usage and minimize wastage, costs were calculated. A poster was developed that follows a unit of red blood cells from collection to administration of the same unit – "vein to vein" tracking. The poster information was developed from a collated survey conducted with Blood Transfusion Services within Nova Scotia. Actual product costs were based on data obtained from Canadian Blood Services for 2002–2003. Health Care Professionals/Hospital based costs were derived from workload information and averaging the cost of each based on the 2002–2003 collective bargaining agreements. The poster content reflects known and associated hidden costs. These together provide an overall total which can be applied to any institution regardless of size. Additional information and Internet links are identified on the poster.

**ABSTRACT NO.: 89**

Development of an on-line hospital blood inventory month-end data collection and reporting system

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**PURPOSE** To enhance patient safety, there is a need to develop a standardized approach for tracking blood product utilization and transfusion practices. A pilot project was developed to facilitate the monthly collection of blood utilization patterns from 26 Ontario hospital transfusion laboratories.

**METHODS** An on-line data entry system was developed to produce an automated system to collect and report blood product inventory. The objectives of the pilot phase were: to launch the line system at the hospital level for entry of month-end blood inventory data; to pilot test the functionality of the system; to provide hospitals/CBS with easily accessible inventory histories in an electronic format; and to ascertain if automated summary reports would be helpful to hospitals. The on-line system went live on August 23, 2004 and hospitals were asked to begin entering data prospectively and to back-enter from April 1, 2004 to capture four months of retrospective data.

**RESULTS** 100% of hospitals complied with online data entry. Follow-up phone calls were made to any sites that had not logged on by the deadline (2 sites needed more time to train staff; 3 sites had difficulty entering the website address, therefore the URL address was emailed to them as a link). The on-line data was validated against hard-copy inventory reports. All errors were logged and reviewed for verification, corrections were made online, and letters were sent to inform hospitals of the discrepancies and changes made. Thirty-five discrepancies were noted from 19 hospitals: data not entered into the system (22); data entered in wrong row (8); incorrect numbers entered (3). Whole Blood entered as Red Blood Concentrates or Red Blood Concentrates entered as Whole Blood (2); and one value entered online that was not entered on the hard copy (1).

**CONCLUSION** The pilot phase was successfully implemented with all hospital sites complying with retrospective and prospective data collection.

**ABSTRACT NO.: 90**

Survey results from 26 hospitals using a new on-line hospital blood inventory month-end data collection and reporting system

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**PURPOSE** An on-line project was conducted to facilitate the monthly collection of blood utilization patterns from 26 Ontario hospital transfusion laboratories. On completion of the pilot phase, a survey was sent to participating hospitals to obtain their input about their experience using the on-line system.

**METHODS** The survey consisted of three sections: Section 1: Current Means of Completing CBS Month-End Reports (six questions); Section 2: Using the On-line Hospital Entry System (14 questions); and Section 3: Useful Applications for Hospital Entry System Data (10 questions).

**RESULTS** Highlights from each section are outlined below. Section 1: 17/26 hospitals utilize a combination of manual counting and LIS reports to compile month-end inventory data; 22/26 hospitals have designated staff responsible for this task with 20/26 hospitals indicating they expect 1–3 staff to access the on-line system. Section 2: 17/26 hospitals feel the on-line system is very user friendly while 7 feel the system is OK or somewhat user friendly; 20/26 hospitals rate the instruction manual as very clear while 6 hospitals feel it is OK or somewhat clear; 23/26 hospitals feel that no changes are required to the manual. Section 3: 24/26 hospitals feel that automatic summary reports would be useful at their hospitals with 19/26 indicating that they prefer bar graphs for graphical displays in reports. 13/26 hospitals feel that the inventory data should be shared amongst hospitals in an open and transparent manner, although 9 hospitals do not want information sharing and 4 are unsure. 15/26 hospitals feel that it would be useful to have on-line access to the blood inventories of other hospitals to assist in blood re-distribution during a blood shortage.

**CONCLUSION** Survey results from 26 hospitals using the on-line hospital blood inventory system demonstrate that hospitals were very enthusiastic with excellent compliance. It is feasible to utilize this system for monitoring and reporting blood product inventory.

**ABSTRACT NO.: 91**

Transfusion medicine laboratory – Vancouver Island Health Authority – South: A model in progress towards lean production in transfusion medicine

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**INTRODUCTION** Lean production is about doing more with less: less inventory, less space, less labour and less money. It is a concept inspired by Kaizen – the Japanese strategy of continuous
improvement. TML for VIHA-S is developing a model for lean production as a means to address the problems that health care organizations are facing.

The Model Regionalization of The Royal Jubilee, Victoria General, Saanich Peninsula and Lady Minto Hospitals into the Vancouver Island Health Authority – South Island supplies the groundwork for lean production. A common computer system for the Transfusion Medicine Laboratory enables us to work as a single unit, performing blood grouping, antibody screens and cross matches at any site that are valid within the region. The Computer Assisted Crossmatch and the distance Computer Assisted Crossmatch is possible with the computer upgrade to Cerner Millennium. The Ortho ProVac™ Automated instrument for pre-transfusion testing opens up the possibility for centralized and technologists’ time. A partnership with the Canadian Blood Services, the Provincial Blood Coordinating Office and other Quality organizations that follow Best Practice Principles supports lean production. A Regional Quality Assurance Coordinator position and a Regional Nursing Coordinator position are instrumental in developing a Quality System that supports the concept that “Quality is Free”. Doing something right the first time minimizes the costs incurred with rework and scrap. ‘Poka Yoke – mistake-proofing’ – a Japanese phrase which indicates prevention over correction – is a concept that TML demonstrates through occurrence management initiatives, competency assessments and continuous improvement.

Conclusion This presentation highlights the components of lean production that are in place in the Transfusion Medicine Laboratory at the Vancouver Island Health Authority – South Island. It also illustrates how lean production is being incorporated into plans for the future and the implementation of the new Health Canada Standards. The title states that this is a model in progress. We have taken the initiative and are well on our way to making this model a reality. A Quality System and Lean Production is a journey not a destination, therefore, this model will always be a project in progress.

ABSTRACT NO.: 92
Interhospital shipping box – Ice pack storage temperature validation study phase I

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Community hospitals in British Columbia routinely ship short dated RBCs to larger hospitals to minimize wastage. Shipping container validation is required to ensure the temperature is maintained between 1–10 °C. The CBS shipping validation is based on ice packs stored at −11 ± 3 °C. Many hospital laboratories do not have −11 °C freezers; colder freezers, ranging from −20 °C to −40 °C, are frequently in use for ice pack storage. Purpose: Phase I is a fact-finding study to see if ice packs stored in a colder freezer could adversely affect the internal box temperature.

Methods Two units of RBCs were packaged in CBS containers following established procedure. A TempTale™ monitor was placed between the RBCs for 24 hours at an ambient temperature of 21 °C. Six packing configurations were tested: one ice pack stored at −20 °C, −30 °C and −40 °C; two ice packs stored at −20 °C, −30 °C and −40 °C.

Results A total of 43 tests were done, all six configurations showed at least one test with the internal box temperature below 1 °C. The colder the ice pack storage temperature, the higher the number of tests below 1 °C.

Conclusions The storage temperature of ice packs could adversely affect the internal box temperature. Ice packs stored at −30 °C and −40 °C are not suitable for interhospital shipping boxes. Phase II study, currently underway, is to develop alternative blood shipment guidance and policy to assist hospitals with ice packs stored at −20 °C freezers.
2003, CBS submitted a proposal to Health Canada to have the upper age limit removed for regular blood donors. A modified proposal was accepted in August 2004 and implemented on December 1, 2004.

Methods The change in criteria was publicized in a press release on December 13, 2004, to be followed by letters sent nationally to potentially eligible donors. To be assessed for eligibility, donors must first contact CBS. If they have donated in the last 2 years before turning 71, a medical enquiry is initiated and sent to their Family Doctor (FMD). The medical enquiry is returned to CBS with an assessment of the donor’s fitness to donate. The CBS Medical Director reviews the medical enquiry and donors are notified of the outcome. Acceptable donors must be assessed annually for fitness to donate and go through the usual health screening process at the time of donation. A national database has been created to collect data pertaining to these donors.

Results In December 2004, the Ottawa Centre initiated enquiries on 22 donors. All had donated at least 25 times with many having many more donations. Three donors were found fit by their FMD; 1 donor was deferred by the CBS Medical Director. As of January 17, 2005, 44 donors over 71 had donated nationally. More information will be available as data is collected on these donors.

Conclusion Preliminary results indicate an enthusiastic response from donors who have demonstrated a commitment to donating blood on a regular basis.

ABSTRACT NO.: 96

More molecular typing of blood groups
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Héma-Québec, Ste-Foy, Québec, Canada

Purpose Molecular typing of blood groups is playing an increasing role in blood banks where serology techniques are powerless. This molecular diagnostic tool has been already applied to several situations including hemolytic disease of the newborn, polymers(trans) fused patients, maternal plasma and many more. In the past, our group implemented assays for Rh(D), Rh(C/c), Rh(E/e), K/k, Fya/Fyb and Jka/Jkb. We here described assays developed for N, s, Duffy GATA box and RHD pseudogene.

Methods These molecular assays are done by PCR (Polymerase Chain Reaction) as the others. Specific primers were designed based on published data and DNA sequences. Each assay includes an internal control to avoid false-negative reactions. Results are visualized on agarose gel electrophoresis.

Results Amplification conditions were established by using DNA samples of known phenotype. In the case of N, s and s, 100% concordance was observed between genotype and phenotype. The Duffy GATA box and the RHD pseudogene assays caused a different problem, since no known samples were available to us. Dr. M. Reid (New York) and Dr. G. Daniels (Bristol) provided us with RHD pseudogene samples. To test the Duffy GATA box, we tested old clinical DNA samples kept for future reference. Two families were found positive for the Duffy GATA box and one, positive for the RHD pseudogene.

Conclusion The N, s and s assays will be used on a regular basis by Héma-Québec’s Hospital Services. The more specialized assays, Duffy GATA box and RHD pseudogene, should be used depending of the patient’s ethnic origin and clinical history. We are now in the process of developing an assay for the M.

ABSTRACT NO.: 97

Process improvement team (PIT) used to develop bedside containers for use at mobile blood donor clinics in the region of central Ontario
C. Buchanan & R. Naiman
Central Ontario Region, Canada.

Background The bedside supply carts (used for materials required during blood collection) were in violation of Health and Safety specifications. They exceeded height restrictions and were unstable. Due to wear and tear and the fragile nature of the cases they were being held together by duct tape. The cases were designed to alleviate this problem and reduce the cost of replacing broken equipment. Shakers, used to mix the blood donation, will also be housed in these cases. This will allow the drivers to transport 6 shakers at once instead of the existing process of one at a time. These cases will also alleviate the need for separate shaker cases which are prone to cracking. Therefore, the prototype design actually combines the current use of supply carts and individual shaker cases into one error-proofed sturdy container.

Project The first steps were meetings consisting of brainstorming the issues. The group was made up of key players with ownership of this issue, specifically collections and transport, and a facilitator. From there the team determined safety and user requirements. The design was drafted and submitted to the CBS National Health and Safety manager and the SR. Collection manager. Once consensus was achieved, prototypes were built. These containers were piloted on one truck that serviced the entire region. Front line staff provided positive feedback.

Results As a result of the team’s efforts, a vastly improved, user friendly safe multi-functional container will become a part of the clinic operation.

ABSTRACT NO.: 98

Validation of an in-house NAT assay for the detection of West Nile virus in tissue donor blood
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To comply with regulatory agencies, the tissue and organ donor banks must take measures to prevent WNv transmission through transplantation. However, the nucleic acid test (NAT) assays developed by companies to test blood samples from live donors have not been tested and validated for cadaveric blood specimens. During the summer 2003, prior to the availability of a commercial test, Hémquébec has developed an in-house NAT assay for the detection of WNv in all blood donors. This in-house assay was modified and validated for cadaveric blood specimens to fulfill the regulatory requirements. The modifications include a change from mini-pool to a single-donor format and an internal control was added to eliminate the false-negative results. To reduce the inhibition effect and the viscosity of these specimens, samples were diluted 1/3 in saline before extraction. To improve the sensitivity, all samples, including controls, were tested in triplicates. To perform the assay, fresh or frozen cadaveric specimens (serum or plasma) were spiked with various quantities of inactivated WNv standard provided by Health Canada. The validation protocol included the specificity, limit of detection (LOD), precision assays (repeatability, inter-analyst, different day and inter-apparatus), robustness and stability of the specimens kept at 4 °C and −35 °C. The LOD was established at 300 c/mL in cadaveric blood specimens compared to 100 c/mL in blood from live donors. The expected results were obtained in all precision assays. The plasma or serum samples can be kept up to 48 hours at 2-8 °C before analysis or frozen at −35 °C without affecting the viral detection. In summary, the validation show that the in-house NAT assay can be efficiently used for the detection of WNv in cadaveric specimens.

ABSTRACT NO.: 99

Bacterial contamination of CBS apheresis platelets
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Bacterial contamination was the earliest recognized infectious complication of transfusion therapy with an estimated occurrence of 1:1000 to 1:5000 platelet units. To limit the initial inoculation of bacteria into the collected blood product, CBS implemented the sample diversion pouch and subsequently a phased implementation of bacterial detection of apheresis platelets beginning in March 2004. One hundred percent of apheresis platelets produced are

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tested by culture using the bioMerieux BacT/ALERT platform. Following a 24 h post-collection hold to allow time for sufficient organism growth to reach detectable levels, an aerobic culture bottle is inoculated with ~4 mL of apheresis platelet sample and incubated at 35°C for six days or until positive. Once identified as positive the culture bottle is sent for external confirmatory testing and speciation. In 2004, 15,703 apheresis platelets were collected in sites with testing in place, of which 15,516 (98.8%) were tested with 16 positive BacT/ALERT cultures. Seven were confirmed positive on retest. Organisms detected included B. subtilis, B. cereus, B. megaterium, Staph. epidermidis, Staph. hominis, Staph. capitis, Actinobacillus ureae, and Sphingomonas paucimobilis. To date there have been 3 repeats performed on retrieved product initially positive by BacT/ALERT. All had no growth on retest. No retrieved product has tested positive, and it was not determined whether non-viable bacteria were present in the product. In addition, the incomplete product retrieval precludes confirmation of the source of bacteria: donor or laboratory. Thus, in testing to date, the rate of bacterial contamination of apheresis platelet products is less than 1:2000 but a precise number cannot yet be determined.

**ABSTRACT NO.: 100**

Validation of a digital pH meter for bacterial detection testing of platelet concentrates

J. Torrance, L. Harrison, D. Deokharan, & J. Samsoondar

**Credit Valley Hospital, Mississauga, Ontario, Canada.**

**Purpose:** To validate the use of a digital pH meter for implementation of routine bacterial testing of random donor platelet concentrates (PLT).

**Background:** The pH of a random donor platelet concentrate is influenced by the donor and the anticoagulant used in donor collection. The presence of bacteria will cause the pH to decrease due to consumption of glucose and production of organic acids.

**Methods:** IQ125 pH meter requires one drop of liquid for measurement of fluid pH. The meter was tested for precision and accuracy using liquids of various pH’s. Fifty random donor PLT of various ages were measured for pH and cultured for bacteria. Ten units of random donor PLT were spiked with bacteria at a concentration of 3–5 × 10⁷ CFU (E. coli, S. epI). The pH was measured at 12-18 hour intervals. Staff evaluated the method for ease of performance and assessed the possible delay in the provision of product.

**Results:** The IQ125 pH meter achieved acceptable results for precision and accuracy. All culture negative units achieved a pH of 7.0 or higher. A higher percentage of 3 day and 5 day units were tested to ensure the normal range would encompass both average and older units. All 5 units spiked with E. Coli showed decreased pH below 7.0 within 24 hours of injection. Four of the 5 units spiked with S. epI had a pH below 7.0 within 48 hours of injection. Most units received from the blood supplier are at least 48 hours old. Staff reported positively on the ease of performance once the stripping of segments was mastered. Testing of pH would add approximately 5 minutes to the provision of blood products for transfusion.

**Conclusions:** pH measurement provides adequate surrogate testing for the presence of bacterial contamination of random donor platelet concentrates. The acceptable threshold will be set at 7.0. All random donor platelet concentrates measuring less than 7.0 pH will be quarantined to avoid transfusion and sent for culture. Implementation of testing will not adversely affect product availability.

**ABSTRACT NO.: 101**

Causes of discordant results in transfusion medicine external quality assessment (EQA) surveys

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**Quality Management Program-Laboratory Services (QMP-LS), Toronto, Ontario, Canada.**

**Purpose:** For many years, QMP-LS has corresponded with laboratories regarding unexpected or discordant findings associated with EQA surveys. In 2003 laboratories were provided with problem-solving guidelines for investigating discordant results. The objective was to provide assistance in cause analysis and identification of corrective action and to track laboratory responses to discordant findings and summarize performance issues.

**Methods:** Laboratories reporting unacceptable results were provided with an investigation template that they were asked to complete and return to QMP-LS. The responses were reviewed and categorized.

**Results:** 176 laboratories participated in 4 transfusion medicine EQA surveys, each consisting of 4 samples. There were 249 discordant results associated with the following tests: ABO (49%), Rh (38%), direct antiglobulin (6%), crossmatch (3%), phenotyping (2%), antibody detection (1%) and antibody identification (1%). Contributing causes of discordant results were classified as 71% technical (attributable to actions of staff), 15% methodological (problem in test system or SOP), 9% clerical (transcription errors), 4% material (problem with EQA samples) and 1% random (unknown). The root cause for the discordant result was frequently not identified in participant responses. Most corrective actions were appropriate and included training of staff and rewriting of procedures.

**Conclusions:** Application of a problem-solving process for the investigation of discordant findings in EQA surveys provides information that can assist laboratories in identifying opportunities for improvement and developing processes for root cause analysis and corrective action. QMP-LS continues to collect this information and will assess its usefulness for inclusion in cumulative laboratory performance history.

**ABSTRACT NO.: 102**

Effectiveness of a network1 of Ontario transfusion coordinators: ONTraC

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**Purpose:** Although often life-saving, blood transfusions are associated with significant risk to the patient and escalating costs to the blood system and hospital. Transfusions are often given unnecessarily. Blood conservation represents the use of alternatives to transfusion. The ONTraC program attempts to enhance transfusion practice outside the blood transfusion laboratory, promote blood conservation in surgery patients, and reduce allogeneic red cell use.

**Methods:** In the first such large scale program, funding was obtained from the MOHLTC for a Transfusion Coordinator in 23 Ontario hospitals selected based on blood utilization and pro- gram. At specific time periods, detailed anonymized information was collected in a defined number of all consecutive patients admitted for the three designated surgical procedures (knee arthroplasty, abdominal aortic aneurysm (AAA) and coronary artery bypass graft (CABG) surgery).

**Results:** Considerable inter-institutional variation was observed in the proportion of patients and amount of blood transfused. With increasing education and conservation measures, at the 12

1Guelph General Hospital, Hamilton Health Sciences Centre, Hospital for Sick Children, Kingston General Hospital, LakeRidge Health, London Health Science Centre, Mt Sinai Hospital, Niagara Health System, North Bay General Hospital, Peterborough Regional Health Centre, Sault Area Hospitals, Scarborough General Hospital, St. Joseph’s Health Centre, St. Mary’s General Hospital, St. Michael’s Hospital, Sudbury Regional Hospital, Sunnybrook & Women’s College Health Sciences Centre, The Ottawa Hospital, Toronto East General Hospital, Trillium Health Centre, University Health Network, Windsor Regional Hospital.
month analysis, most, although not all, hospitals had decreased use of allogeneic blood and there was an overall 24% reduction in blood use in patients undergoing knee surgery, 14% in AAA and 23% in CABG. Patients who did not receive allogeneic transfusions had significantly lower postoperative infection rates and length of stay. Preliminary 18 month analysis indicates even greater reduction in allogeneic transfusion.

Conclusions The ONTraC coordinators have become leaders locally, nationally and internationally in blood conservation. The reduction in allogeneic transfusion associated with the implementation of the ONTraC program represents important savings in costs associated with blood components, hospital stay and work in transfusion laboratories and nursing units, as well as enhancing patient satisfaction and safety.

ABSTRACT NO.: 103

Manitoba Hepatitis C blood recipient notification project
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Manitoba compiled with one of Justice Krever’s recommendation, and launched the Hepatitis C Blood Recipient Notification Project on May 14, 2001. The primary objective of the project was to identify and notify Manitobans who received blood transfusions prior to April 1992. The poster presentation presents the description of the project’s process and evaluation of Manitoba’s response to the project. The project was a provincial general lookback where notification letters informed Manitobans that they may have been exposed to hepatitis C virus through the blood supply, and encouraged them to seek testing. A public information campaign was employed, recommending Manitobans to obtain a test, even if no letter was received. The project office monitored three main activities. These activities include the number of notification letters delivered, the number of hepatitis C virus tests performed, and the number of telephone inquiries received. The overall system response has been very good. A strong and positive response rate of 88.76% was achieved from both letter recipients and public information campaign. Several recommendations identified in the Krever Report and the project were implemented after completion of the project.

ABSTRACT NO.: 104

Adverse transfusion reactions reported to the national surveillance system in Canada, 2001–2003
M. Cator, N. McCombie, P. Robillard, M. Miron, A. Giulivi, & the Members of the National Transfusion Transmitted Injuries Surveillance System Working Group

Blood Safety Surveillance and Health Care Acquired Infections Division, Public Health Agency of Canada, Ottawa, Ontario, Canada.

Purpose of the Investigation To describe the adverse transfusion reactions (ATRs) reported to the Transfusion Transmitted Injuries Surveillance System (TTISS) for the period April 1, 2001 to December 31, 2003.

Methods ATRs collected at participating hospitals are investigated and reported to a provincial/territorial office according to standardized definitions. Non-nominal data are transferred as per agreement to the Public Health Agency of Canada. After a review of these reactions, cases meeting TTISS requirements are analyzed.

Results As of December 31, 2003, a total of 380 definite, probable or possible ATRs were analyzed. Of these, 90% were related to blood components and 10% to plasma derivatives. Reactions associated with blood components were: major allergic/anaphylactic (35%), circulatory overload (14%), TRALI (12%), acute hemolytic (12%), bacterial contamination (11%), ABO incompatibility (6%), and others (10%). Reactions associated with plasma derivatives were major allergic/anaphylactic (41%), aseptic meningitis (22%), hypotensive reaction (11%) and others (26%). Overall 3 cases of death were definitely associated with transfusion: 1 bacterial contamination, 1 post transfusion purpura and 1 TRALI.

Conclusion Major allergic/anaphylactic reactions are the most frequent ATR reported to TTISS both for blood components and plasma derivatives. TTISS is still an evolving surveillance system with more sites participating year after year. Denominator data on products transfused will be available for calculation of rates of adverse transfusion reactions.

ABSTRACT NO.: 105

Results of adverse event reporting using MERS-TM in a multi-site transfusion service
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Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario, Canada.

Purpose and Methods In 2002 our institution piloted the use of the adverse Medical Event Reporting System for Transfusion Medicine (MERS-TM). Pilot experience led to a decision to implement MERS-TM at our 4 sites. Commencing in January 2003, adverse event data was collected both manually on paper and entered on-line. A parent database had also been established to examine both the compilation of the data, and at each site’s results separately. Prior to data entry, there was careful review at each site. The resulting output allowed for the necessary clinical follow-up to occur in a timely fashion and corrective actions to be taken if required.

Results and Outcomes MERS-TM allows for the collection of adverse event data into various categories relating to workflow. Results from 5 of the 13 workflow categories for 2003 and 2004 were as follows:

| Year | Product Check-in | Product Request | Order Entry | Sample Collection | Sample Testing |
|------|------------------|----------------|-------------|-------------------|----------------|
| 2003 | 57               | 111            | 43          | 651               | 71             |
| 2004 | 115              | 174            | 27          | 625               | 84             |

Event reports across the 4 sites averaged 115 per month. Impact of any interventions could be followed and trended. Thus, the impact of new implementations, like MAK Progesa at Canadian Blood Services, could be monitored. Reports can be derived from the system to assess specific clinical areas at any site, as well as summarizing event data over a period of time. Sample collection errors represented the category with the highest number of events. Most were minor mislabeling events. Sample rejection practice are rigorously followed in Transfusion Medicine, compared to practice in other clinical laboratories, which tend to accept samples that may be imperfectly labeled. Results are reported quarterly to a MERS review committee. Number and severity of events, as well as available corrective actions arising from such events, are considered. Recommendations for future actions are thus generated.

Conclusions Over the past 2 years MERS-TM has provided information to help analyze adverse events occurring at 4 acute care sites. These data have resulted in corrective action being taken, which will hopefully lead to prevention of recurrences.

ABSTRACT NO.: 106

Manitoba adverse event reporting system (AERS): Analysis of first complete year of data
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Provincial Blood Programs Coordinating Office, Winnipeg, Manitoba, Canada.

The Manitoba AERS is part of the national Transfusion Transmitted Injuries Surveillance System sponsored by Health Canada.

Objectives (1) Qualitative and quantitative analysis of the first year of data; (2) monitoring changes in the type and magnitude of known risks; (3) assessing the magnitude of new transfusion
ABSTRACT NO.: 107

West Nile virus reporting between public health and Canadian Blood Services ... Improving blood safety through more closely integrated surveillance

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PURPOSE Timely, relevant data that links health determinants and events to outcomes are essential qualities of an effective, integrated surveillance system for evaluating blood safety and best blood banking and transfusion practices. Canadian Blood Services, BC Centre for Disease Control, the Canadian Network for Public Health Intelligence and the BC Ministry of Health Services are partners in a project to harness the potential of enhanced data integration as a tool for strengthening blood safety surveillance in BC and as a model for Canada.

METHODS Strong stakeholder partnerships and anonymized data linkage (ADL) processes are viewed as key enabling factors of an integrated surveillance system, particularly in addressing relevant protection of privacy issues.

RESULTS During the initial project phase, completed Dec 2004, an ADL process was developed and evaluated, successfully demonstrating how ADL can interface between different agency databases to achieve virtual real-time linkage of infectious disease data relevant to blood safety. A second phase, underway in Jan 2005, will evaluate the process in an operational setting using West Nile virus as an indicator blood-borne pathogen, while directly addressing statutory and agency requirements for privacy and confidentiality of information.

CONCLUSIONS ADL is potentially a powerful tool to achieve a vision of an effective, integrated, blood safety surveillance system in Canada.

ABSTRACT NO.: 108

Using anonymized data linkage to advance state-of-the-art surveillance of Canada’s blood system

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ABSTRACT NO.: 109

The Québec hemovigilance committee: An important partner in blood safety

D. Page, P. Robillard, G. Delage, C. Poulin & the Québec Hemovigilance Committee members

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The Québec Hemovigilance Committee, instituted in 1998, comprises members of different backgrounds (hematologists, epidemiologist, microbiologist, public health and population representatives) has the mandate to advise the Québec Health Ministry on blood transfusion safety in the province. To do so, it analyses the results from adverse transfusion reaction surveillance and monitors emergent blood-borne pathogens. Between 2001 and 2003, a total of 4 562 adverse transfusion events reported to the Québec surveillance system were analysed. Most were minor or had no sequelae for the patients. However more than 4% were life-threatening and 10 resulted in death. Incidence of major reactions for blood components were:

| Incidence ratios per unit transfused | 2001 | 2002 | 2003 |
|-------------------------------------|------|------|------|
| Allergic reaction                  | 1:9 168 | 1:6 295 | 1:9 207 |
| ABO Inc                            | 1:30 561 | 1:36 194 | 1:40 280 |
| Acute Hemolytic                    | 1:39 293 | 1:28 955 | 1:21 483 |
| Bacterial Cont                      | 1:34 381 | 1:20 682 | 1:35 804 |
| TRALI                              | 1:68 763 | 1:36 194 | 1:53 704 |

Regarding emergent blood-borne pathogens, West Nile Virus and variant Creutzfeldt-Jakob Disease received much attention from the Committee, which gave advice on various safety measures proposed by Héma-Québec before their implementation. In the period 2001–2003, the Committee made specific recommendations...
to the Quebec Health Minister with respect to informed consent for transfusion, prevention measures for transfusion-transmitted West Nile Virus, mandatory accreditation of blood banks and transfusion medicine services and impact of new payment mechanism for blood products on the traceability of coagulation factors. The Committee noted that the most serious risks of transfusion lie within hospitals, particularly risks related to incorrect patient identification. Strong preventive measures within hospital settings are paramount to further improve blood safety.

ABSTRACT NO.: 110

Transfusion specimen collection errors in Nova Scotia Ve Thanhbui
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The province of Nova Scotia is divided into nine (9) District Health Authorities (DHA) with a total of 41 hospital facilities ranging from large acute care tertiary/teaching facilities to small community based facilities.

PURPOSE OF INVESTIGATION To identify events occurring during the collection of blood Transfusion specimens used in pre-transfusion testing.

METHODS A survey was conducted to determine hospital blood collection policies for transfusion specimen collection and identify those health care professionals responsible for specimen collection. A quantitative data collection tool was developed which captured events, collector involved and total number of transfusion specimens collected and the study was conducted between April 5, 2004 and June 30, 2004.

RESULTS Data obtained represents >85% of transfusions that occurred in Nova Scotia between April 5, 2004 and June 30, 2004. Two demographic categories were defined: A) facilities with specimen collectors from multiple disciplines (blood collector, nursing and physicians) and B) facilities with specimen collectors from one discipline (laboratory). During the collection period 370 events were observed out of 12,265 transfusion specimens collected. Total rate of events equals 3.0% of which 63.0% were related to nursing, 36.5% were related to blood collectors and 0.5% were related to physicians. Within the A) category of facilities 2.8% of events were observed of which 80.6% were related to nursing, 19.4% related to blood collectors and 0% related to physicians. Within the B) category of facilities 3.2% of events were observed of which 43.1% were related to nursing, 55.7% related to laboratory and 1.1% related to physicians.

CONCLUSIONS Transfusion specimen collection events occur in all categories of facilities and by all health professionals responsible for procurement of specimens. Perhaps the development of a standardized provincial process and procedure for the collection of blood Transfusion specimens and standardized training mechanisms would help to reduce most of these events. This standardized approach should include root cause analysis to detect flaws in the current collection policies and develop strategies to reduce the impact of human error.

ABSTRACT NO.: 111

A web-based competency assessment program for transfusion medicine (eCAS-TMT)
G. Rock, D. Neurath, D. Van Klaveren, M. Osman & members of the Ottawa Council of the Transfusion Ontario Program The Ottawa Hospital, NEXTMOVE Inc., Ottawa, Ontario, Canada.

BACKGROUND Transfusion medicine laboratories require highly skilled technical staff in order to carry out their duties. Recently the OLA program has introduced a requirement to determine the competency of technologists. At present there are few approaches that would permit such assessment in a cost effective and standardized fashion. We have designed and developed a web-based, online computerized system that provides competency assessment of technologists involved in Transfusion Medicine.

METHODS The Ottawa Council of the Transfusion Ontario Program (Funded by the MOHLT) developed questions for use in a competency assessment program. Nextmove Inc. partnered and designed a web-based format to present, administer and manage questions within the framework of a competency assessment system (CAS) specifically for transfusion medicine technologists. The system covers all areas of Transfusion Medicine in a comprehensive fashion. It has three levels of difficulty: basic, advanced and clinical and addresses skills in 14 major topics including ABO, Rh antibody screening, cross match techniques, component use, special investigative procedures and quality control. The program groups the questions into theory, bench practice & results interpretation. Technologists from the Ottawa area hospitals participated in a pilot to assess the program and provide baseline information on the competency-testing program (eCAS-TMT) and their knowledge of Transfusion Medicine.

RESULTS The pilot group was found to be heterogeneous with regard to their knowledge of transfusion medicine technology with considerable difference in the breadth of knowledge of technologists from small hospitals and large referral hospitals. The pre-pilot evaluation questionnaire showed that the organization of the test and usefulness of the material was assessed as “very good”. The difficulty level and the relevance to the current job were rated as “good”. There were no ratings in the “unsatisfactory” category. Content coverage was very satisfactory. No additional topics were recommended.

DISCUSSION AND CONCLUSION These findings indicate the potential and utility of a computerized, standardized testing format that will allow technologists at any hospital to ascertain their level of competency and be able to address areas of deficiency. The system is expected to provide supervisors and medical directors with information they could use to fulfill requirements for laboratory licensing (OLA, in Ontario) and will permit establishing a common baseline for competency assessment in the province of Ontario and potentially, throughout Canada.
ABSTRACT NO.: 113
A standard operating procedure for the validation and evaluation of the ProVue automated MTS gel test system
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Transfusion Medicine Laboratory, Royal Jubilee Hospital, Vancouver Island Health Authority – South Island, Victoria, British Columbia, Canada.

Purpose: The introduction of the Ortho ProVue™ Automated MTS Gel System has presented Transfusion Medicine Laboratories with a new challenge. Health Canada Standards, Canadian Accreditation Agencies and the American Association of Blood Banks require that certain conditions be met before a new procedure can be used for in vitro diagnostic testing. A standard operating procedure for the validation and method evaluation was our first step in satisfying these requirements. The SOP included a system description, purpose & objectives, risk assessment, responsibilities, validation procedures, acceptance criteria, approval signatures and supporting documentation.

Standard Operating Procedure The Purpose of the validation plan was to identify the instrument functions and tests specific to the intended use of the instrument in Transfusion Medicine Laboratory for the Vancouver Island Health Authority – South Island. The Process included a flow chart and six separate case studies that enabled the technologist to accomplish several requirements at once. These included installation qualification, operational qualification, and product qualification. The method evaluation plan compared the Tube and Peg method with the Ortho ProVue automated MTS-Gel method and the ProVue™ Automated MTS-Gel method. Method equivalency and operational efficiencies were carried out for donor group confirmation, type and screen, crossmatch, and antibody identification. Results and conclusions of these activities were appended to the approved validation SOP along with limitations, approval signatures, supporting documentation and implementation time lines. Documentation also included any discrepant results and corrective actions. The Medical Director carried out a final review and approval of the validation plan, results and corrective action.

Conclusion The laboratory went live with the ProVue Automation system within four weeks of installation. Having a standard operating procedure in place for the validation and method evaluation before installation was the main factor in determining our success.

ABSTRACT NO.: 114
Taking the Ortho ProVue™ to the next step
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Blood Transfusion Laboratory, Lakeridge Health Corporation, Oshawa, Ontario, Canada.

Purpose: To manage increasing workload in the face of budget restrictions it is important to make maximum use of the available technology. A computer interface can greatly enhance an automated test system in the Blood Transfusion Laboratory (BTL). We will share our experience and the impact on the BTL of the automated and interfaced Ortho ProVue™.

Methods The data presented was based on the Type and Screen (T/S) procedure which constitutes our major workload. All shifts were included, with batch size representative of that most efficient for each shift. Timing studies were performed to determine the impact of the interface with respect to efficiency, turnaround time (TAT) and value added technologist time (VATT).

Results Result entry with the interface was accomplished in 1–2 min irrespective of batch size. By comparison manual entry required an average of 2 min per sample of hands on time and required a second technologist to verify that there were no transcription errors. While the ProVue™ has had a significant impact on VATT, the use of the interface has ensured fast and accurate reporting of results.

Conclusions The Ortho ProVue™ has provided our Blood Transfusion Laboratory with walk away capabilities that have resulted in significant improvement to workflow. Implementation of the interface has resulted in additional improvement in technologist time management and sample TAT, especially off hours. Automation of result entry using the interface has provided a fast, accurate and efficient process that has truly taken the Ortho ProVue™ to the next step.

ABSTRACT NO.: 115
Comparison of the tube method to the ID-MTS gel and Diamed gel technique for direct antiglobulin testing
D. Harris
Department of Laboratories, Joseph Brant Memorial Hospital, Burlington Ontario, Canada.

Purpose: To compare the two techniques to determine the feasibility of changing from traditional tube technique to the Gel technology for the Direct Antiglobulin Test (DAT). Comparison to include sensitivity, ease of use, standardization and cost.

Method Fifty patient’s red cell samples (adult and cord) for DAT testing were being analyzed using both techniques. The study will include thirty-five positives BS (Ortho ID-MTS Broad Spectrum) and fifteen negatives. * Positive samples are followed up with complement and IgG reagents (Diamed DC Screening II). All procedures are completed and graded as per manufacturer’s instructions.

* Study 15 Negatives and 20 Positives at time of submission.

| Negative DAT | Total # | Tube | Gel |
|--------------|---------|------|-----|
| 15           | 15      | 15   | 14**|

**1 discordant result in gel -showed IgG, C3d and control positive

| Positive DAT (either one/other or both) |
|----------------------------------------|
| BS | IgG Pos | C3d Pos | Reagent Control |
|----|---------|---------|----------------|
| Tube | 20 | 13 | 7 | 20 |
| Gel | 20 | 13 | 5*** | 20 |

***2 were very weak in tube, negative in Gel (more study on this to be done)

Preliminary Results On average the Gel reactions were stronger than the tube and the complement reagent control cells showed stronger reactions in Gel than in the tube. Technologists favour the standardized method, ease of use and reading of the Gel cards.

Conclusion Considering the preliminary results, feedback from technologists and cost of converting to Gel, our laboratory will be looking to switch techniques.

ABSTRACT NO.: 116
Comparison of identical automated technology: Immucor ROSYS versus Immucor Galileo
R. Haynes
Health Sciences Centre, St. John’s, Newfoundland and Labrador, Canada.

Purpose: To compare hands on technologists time and total testing time for various batch sizes for Blood Group and Antibody Screen between the Immucor Rosys and the Immucor Galileo automated blood bank systems.

Method Different sample batch sizes were tested using Immucor Rosys and Immucor Galileo blood bank systems. A total of 204 samples were tested.
The Immucor Galileo system increased the available technologist time in all batch sizes and provided more efficient use of the technologist time. The Immucor Galileo system reduced the total testing time in all batch sizes with greater than 10 samples. The Immucor Rosys system had a shorter total testing time in the batch size of 10 samples. Time for reporting results was not included. An interface with our LIS is not installed at this time.

### Table 1.

| Batch size | Rosys Tech. Time | Rosys Total Time | Galileo Tech. Time | Galileo Total Time | Difference in Time |
|------------|------------------|------------------|--------------------|--------------------|-------------------|
| 10         | 14.3             | 40.2             | 2.4                | 46.0              | 11.9 – 5.8        |
| 22         | 17.5             | 57.0             | 6.1                | 51.2              | 11.4 – 5.8        |
| 34         | 28.4             | 78.3             | 15.0               | 65.0              | 13.4 – 5.8        |
| 46         | 24.3             | 82.2             | 13.9               | 68.5              | 10.4 – 3.7        |
| 92         | 49.3             | 139.3            | 20.5               | 100.1             | 28.8 – 18.2       |

### ABSTRACT NO.: 117

Supporting a massively transfused trauma case with blood product when blood group is unknown

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Hamilton Regional Laboratory Medicine Program, Hamilton Health Sciences and St. Joseph’s Healthcare, Hamilton, Ontario, Canada.

In December 2004 at 1500 hrs, the trauma centre received a 53-year-old male with both femoral arteries severed. He had received 8 units of O Rh negative (neg) Red Blood Cells (RBCs) before reaching the trauma centre. Upon arrival a Transfusion Medicine (TM) sample was not obtained on this patient. According to established procedures at our hospital, when a blood group is unknown and no sample is available to establish the patient’s blood group, O Rh neg RBCs and AB FFP are provided. After transfusion of several O Rh neg units, the patient was switched to group O Rh pos blood to conserve O Rh neg stock.

The issue of a TM sample was addressed at 2100 hrs when the Technical Specialist (TS) on call was contacted about the use of an alternative group of FFP, due to rapid depletion of AB FFP supply. At this point the patient had received 40 units of RBCs, 30 units of FFP, 39 units of platelets, 20 units of cryoprecipitate and 1 dose of rVIIa.

The TS recommended a TM sample to be obtained ASAP. Faxed results from the referring institution indicated the patient was A Rh negative, with a negative antibody screen. Our institution had no historical data on this patient. The TM sample had an anomalous grouping; nonreactive with anti-A, anti-B and B cells; weak reactions with A1 cells; strongly reactive (+) with anti-D. The CBC sample collected at 1638 hrs was retrieved from the Core lab and grouped as follows: weak results with anti-A, nonreactive with anti-B and A1 cells, 2+ with B cells, and 2+ with anti-D. This was not a valid TM sample since it was missing the necessary requirement of a phlebotomist’s name on the sample. The patient stabilized; the need for blood products subsided. If the need continued, TM’s recommendation to the hematologist would have been to select group A FFP based on the CBC and the outside institution’s grouping. All nonconforming steps were appropriately documented. This case illustrates the importance of a balance between following SOPs and making appropriate clinical and laboratory decisions to provide effective patient care even when it results in nonconformances.

### ABSTRACT NO.: 118

New cause of ABO discrepancy: ABO incompatible heart transplant

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Problem: A 3 year-old boy presented to the University of Alberta Hospital for routine ABO blood group determination and isohemagglutinin titer as part of a workup for cardiac transplantation. The blood grouping revealed a questionable ABO blood group (Table 1). The possibilities included a weak but undetectable subgroup of A (ie. a forward discrepancy) or a reverse discrepancy with diminished anti-A production in a group O individual.

![Table 1.](https://example.com/table1.png)

### ABSTRACT NO.: 119

Systematic review of the effectiveness of FFP and coagulation factor concentrates in reversing oral anticoagulants

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Hemorrhages are a serious side effect associated with oral anticoagulants (OACs), which may require urgent reversal with FFP or coagulation factor concentrates. This review examined the effectiveness of FFP and coagulation factor concentrates in reversing OACs.

Methods: RCTs and prospective observational studies were identified through a systematic literature search of Medline, Embase and Cochrane Databases. Results: Eleven studies (4 RCTs, 6 prospective cohort studies, 1 mixed study design) met the inclusion criteria. The interventions...
included FFP (n = 4), pathogen reduced FFP (n = 2), prothrombin complex concentrates (PCC) (n = 6) and recombinant activated factor VII (rFVIIa) (n = 2). The laboratory outcomes included INR (n = 8), PT (n = 6), aPTT (n = 4), coagulation factor assays (n = 8). Only 4 studies reported clinical bleeding outcomes. The quality of the included studies was low. All 4 products improved the coagulation test results. The mean post-transfusion INR was lower with rFVIIa (<1.5) and PCC (1.1 to 1.3) as compared to FFP (2.3). In the 4 studies evaluating bleeding, PCC and rVIIa were subjectively effective in nearly all patients; FFP was effective in less than 50% of patients in 1 study. There was no clear association between any of the products and an increased thrombotic complications.

**Conclusion** For patients on OACs, FFP, PCC and rVIIa are all effective in lowering the INR, but the reduction with FFP appears to be less. Due to the low quality of the studies, no conclusions can be drawn regarding the relative clinical effectiveness of FFP, PCC or rFVIIa for the treatment of bleeding.

**ABSTRACT NO.: 120**

Therapy of TTP: The role of ADAMTS-13

G. Rock, D. Anderson, W. Clark, P. Leblond, D. Palmer, M. Sterbich, D. Sutton, G. Wells & members of the Canadian Apheresis Group and CAAN

**Canadian Apheresis Group, Ottawa, Ontario, Canada.**

**BACKGROUND** TTP is an uncommon disease. However, without intervention with plasma therapy it has a greater than 85% mortality. In 1991 the Canadian Apheresis Group (CAG) reported the results of a randomized prospective clinical trial which showed that plasma exchange was preferable to plasma infusion in the treatment of this disorder. Subsequently, our pilot study (BJH, 1996), supported the concept that cryosupernatant plasma (CSP) was preferable to fresh frozen plasma (FFP) in the treatment of both previously treated and untreated patients. We then initiated a large scale (n = 236) randomized trial to compare CSP with FFP; with patients from nine centres across Canada. A total of fifty patients entered the trial (26 CSP and 24 FFP) then the study was stopped as a new trial comparing CSP with Solvent Detergent Plasma (SDP) was initiated. In all patients the levels of vWF and ADAMTS-13 and the inhibitor to the enzyme were measured before and after therapy.

**RESULTS** Based on the number of patients entered there were no differences in survival or death rates between CSP and FFP. Platelet counts rose significantly in both groups by day nine. At entry the vWF multimers were normal in all patients studied; vWF levels ranged from 1.1 to 3.95 IU/mL decreasing significantly following 10 days of therapy with lower levels achieved in patients receiving CSP. ADAMTS-13 levels showed large variations ranging from 10 to 100% activity. At entry, 53% of the FFP group and 68% of the CSP had levels of less than 50% of controls. By day 9 (end cycle), 89% (FFP) and 67% (CSP) had levels greater than 50% of the controls. One patient with a 100% inhibitor level at one month had a platelet count at six months of 247,000.

Antibodies to glycoprotein IV were found in 26 of 35 samples tested. Antibodies to both the ADAMTS-13 and GP IV were present in 16 of these 35 patients.

**CONCLUSION** Data are insufficient to determine the appropriate replacement fluid for patients with TTP. Only by recruiting large numbers of patients will definitive answers be obtained. However, it is clear from the laboratory data that there is heterogeneity of ADAMTS-13 levels and these values do not correlate well as predictors of the disease. It is becoming increasingly apparent that TTP, like HIT is an autoimmune disorder.

**ABSTRACT NO.: 121**

Platelet aggregation independent of von Willebrand factor and fibrinogen: Novel mechanism of thrombosis and hemostasis

H. Yang, A. Rehemat, P. Chen, J. Freedman, D. D. Wagner, & H. Ni

Platelet aggregation and adhesion at the site of vascular injury play an important role in the process of thrombus formation, as well as arrest of bleeding. Fibrinogen (Fg) and von Willebrand factor (vWF) are the two key molecules that mediate platelet adhesion and aggregation. Fg is required for platelet aggregation, which has been documented for more than four decades. However, we found that platelet aggregation and thrombus formation still occurred in mice lacking both vWF and Fg (vWF/Fg/-/-) (Ni et al, JCI 106:385–392, 2000) but not in mice lacking GPIIbIIIa (b3 integrin) (P. Yuen, H. Ni, Q. Xiao, D. Wagner, and R.O. Hynes, unpublished data). This indicates that other alternative ligands of b3 integrin from plasma or platelet granules are capable of mediating platelet adhesion and aggregation independent of both vWF and Fg. To investigate vWF and Fg independent platelet aggregation in vitro, we studied platelet aggregation in platelet rich plasma (PRP) of vWF/-/-, Fg/-/-, and vWF/Fg/-/- mice. Consistent with current theory, no platelet aggregation was found in either Fg/-/- or vWF/Fg/-/- PRP. Since no fibrin formation occurred in Fg/-/- or vWF/Fg/-/- plasma, we studied Fg/-/- and vWF/Fg/-/- platelet aggregation in non-anti-coagulated Platelet Poor Plasma (PPP). To our surprise, robust aggregation occurred in both Fg/-/- and vWF/Fg/-/- PPP after ADP treatment. However, this vWF- and Fg-independent (vWF/Fg/-/-) aggregation can be completely inhibited by high doses of thrombin inhibitors such as hirudin (250 IU/mL), hirudin (50 mg/mL) and PPACK (120 mM). This suggests that ADP may induce thrombin generation, which plays a key role in vWF/Fg/-/- platelet aggregation in vitro. To distinguish the contribution of plasma protein(s) from platelet granule proteins, we used gel-filtered vWF/Fg/-/- platelets and induced aggregation with thrombin. Platelet aggregation was observed in PIPES buffer, but was less robust than that in vWF/Fg/-/- PPP. This indicated that proteins from both platelet granules and plasma contributed to this robust vWF/Fg/-/- aggregation in vitro. We further studied thrombosis in vWF/Fg/-/- mice in a real-time intravital microscopy model after hirudin injection (3 mg/kg). Consistent with our in vitro platelet aggregation data, thrombus formation was completely inhibited. In summary, this is the first observation that in vitro platelet aggregation occurs in absence of both vWF and Fg. We found that thrombin is critical and both plasma and platelet granule proteins contribute to this novel mechanism of platelet aggregation.

**ABSTRACT NO.: 122**

Recombinant FVIIa use by the QEII health sciences centre

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**BACKGROUND AND PURPOSE** Although recombinant FVIIa (rFVIIa) is licensed in Canada for hemophilia A/B patients with inhibitors, it has been increasingly used for various "compassionate" clinical situations. Using criteria developed by the National Technical Working Group (NTWG) for guiding physicians in screening these requests, specific data was retrospectively collected on each patient that received rFVIIa over a one year period at the QEII Health Sciences Centre, Halifax, Nova Scotia. This data is reviewed to decide whether implementation of a screening mechanism is warranted for compassionate use of rFVIIa.

**METHODS** From January 2004 to December 2004, retrospective chart and Information System reviews were completed on all patients receiving rFVIIa. The patients were separated into either "licensed" or "compassionate" categories. If rFVIIa was compassionate, the patients were classified as one of the following: Jehovah Witness who is bleeding, massive perioperative bleeding or "other".

**RESULTS** Twenty-one patients received rFVIIa using a total of 133.20 mg. One hundred percent was for "compassionate"
requests. Fifty-two percent of the patients were classified as massive perioperative bleeding (however, only 55% fulfilled the criteria required by the NTWG), 5% percent of the patients were Jehovah Witnesses and 43% of the patients were categorized as “other”. The patients in the “other” group used 42% of the rFVIIa. The dose varied between 1.2 to 9.6 mg per administration. Out of all treated patients, 62% expired (46% of those within 48 hours of administration).

Conclusions The use of rFVIIa for compassionate request is variable. Implementation of a specific request form for rFVIIa will assist physicians in decision making for compassionate use of this product.

abstract no.: 123
The effect of patient controlled analgesia on co-administrated filtered packed red cells.
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Introduction Patient controlled analgesia (PCA) is a very effective pain control method. It is usually used in peri-operative settings and in cancer patients. Because many of these patients have poor venous access, the question has been raised as to the possibility of administrating opioids in the same line as that used for red cell concentrate (RCC) transfusion. The literature on this approach is not extensive, but is generally cautionary.

Methods RCC (average 39 days) were used in this study. To determine the effect of morphine, Dilaudid and Demerol on the cells, we used several approaches: 1. Continuous low dose opioid (Demerol, 20 mg/mL, Dilaudid, 0.4 mg/mL and morphine, 2 mg/mL) infused with blood at a 1:240 (Demerol) or 1:120 (Dilaudid and morphine) ratio with a single bolus; or 2. Multiple boluses added to the continuous infusion (at ratios of 1:4 for Demerol, 1:2 for Dilaudid and 1:2 for morphine); 3. In vitro assessment of blood 2 hours after adding different quantities of the opioids to give ratios of 1:2 to 1:1 of drug to blood. All the results were compared to those obtained using similar amounts of normal saline as control. Samples were assayed for Hb, MCV, plasma Hb, potassium, and LDH. A peripheral smear was also made.

Results addition of each drug as a single or multiple bolus in the background of continuous infusion showed the same effects as those seen with saline with no effect on MCV and a dilutional effect on the other measures. In vitro exposure of drugs to blood at 1:4 and 1:2 ratios showed minimal effect although there was an increase in MCV at 1:10 and at 1:2 Demerol. At very high ratio (1:1), Demerol produced significant RBC swelling (MCV 120 fL) and hemolysis of 4.5% (at 2:1 ratio increased to 9.2%). The other two opioids (morphine and Dilaudid) showed no effect with results similar to normal saline.

Conclusion Morphine, Dilaudid and Demerol given as a bolus in the IV line have the same effects as those seen with saline with the standard single bolus flow rate of 1.5. However, when mixed directly with the blood for more than 1 hour, Demerol caused increasing RBC swelling and hemolysis. Based on these data the drugs can safely be given as a PCA bolus to the delivery line during blood transfusion, but Demerol should not be added directly to the RCC bag.

abstract no.: 124
Questionnaire to assess knowledge of transfusion practice in a community hospital.
J. Cartwright, & L. Harrison
Credit Valley Hospital, Mississauga, Ontario, Canada.

Purpose A nursing questionnaire was developed to determine current knowledge and practice of the transfusion process.

Methods A “Red Cell Administration/Transfusion Questionnaire” was randomized distributed to 100 nurses on all wards of the hospital in December of 2004. Multiple choice questions focused on pre-transfusion steps, patient identification, vitals, use of gloves and transfusion reactions. Respondents were asked to select all answers that they felt were correct. Of the 36 possible choices, 22 were considered correct and 14 were considered incorrect choices.

Results A total of 71 responses were received: 38 from nurses working on units that frequently transfuse while 33 responses were from nurses working on units that infrequently transfuse. An average of 71% chose the correct answers while an average of 36% chose the incorrect answers.

Conclusion The following subjects need to be emphasized during future educational initiatives to ensure compliance to transfusion procedures:

- accurate patient identification at the bedside before beginning transfusion. Process to follow if any discrepancies are found
- when to obtain baseline vitals
- recognition of adverse reactions, including less obvious symptoms, such as back pain and/or headache
- differentiating between mild allergic, mild febrile and major transfusion reactions
- reinforcing the appropriate steps to be taken in response to an adverse reaction.

abstract no.: 125
Preadmission testing practices for elective surgical procedures.
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Purpose Same day admission for elective surgical procedures requires appropriate blood work be done in advance of admission. There are very few guidelines, supported by studies that define the time limit between drawing a sample for pre-transfusion testing and the surgical procedure. Validation of the 6-week window used at Kingston General Hospital (KGH), was the purpose of this study.

Methods Blood Banks across Canada were randomly surveyed with respect to preadmission testing (PAT) practices including cross-matching techniques, temperature, length of storage, and procedures when antibodies were detected. At KGH samples are frozen for up to 42 days. Plasma in which antibodies were initially detected were reviewed at the time of transfusion for antibody identification, strength of reactions, and compared to the initial findings.

Results Information from 11 hospitals suggests there is wide variation in practice with storage times from 2–8 weeks. The majority store plasma at 4 ºC for 4 weeks and may freeze samples if antibodies are present. Eighteen paired plasma samples at KGH frozen from 6–38 days showed no change in antibody detection or reaction strength. A larger number of paired samples will be analyzed at defined time points over 42 days.

Conclusions Preadmission testing protocols for elective surgery in patients who have not been transfused or pregnant in the previous 3 months vary across Canada. Frozen storage of plasma up to 38 days appears safe. Extended storage at –20 ºC and 4 ºC and up to 56 days will be presented.

abstract no.: 126
Paediatric transfusion: A sample of clinical practices in Canadian hospitals.
K. H. Luke1, R. Berger2, J. Chisholm2, S. E. l. Saadany3, M. Afzal3, A. Giulivi3, & G. Rock2
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Background Currently there are no Canadian Paediatric Transfusion Practice Guidelines. The Transfusion Ontario Program in Ottawa developed a questionnaire on Paediatric Transfusion Practices and it was distributed to paediatric and general hospitals with paediatric practices across Canada.
METHOD The questionnaire included questions on transfusion of packed cells and platelets for neonates (<4 months) and paediatrics (>4 months).

RESULTS Seven children’s and 21 general hospitals with paediatric practices completed the questionnaire. Sample data revealed the following: (a) a haemoglobin transfusion trigger for transfusion is not widely used – only in 25% of hospitals; (b) the attending physician usually decides on transfusion based on clinical conditions; (c) in a stable patient, there is general acceptance among respondents (62%) of a low haemoglobin of 50–60 g/L for paediatrics and 80–90 g/L for neonates, before transfusion; (d) in 80% of hospitals, a platelet count below 10 to 20 × 10^9/L was required before platelet transfusion in non-bleeding and stable patients; (e) plasma-reduced platelets were available in 61% of hospitals and was the standard product in 48% of hospitals; (f) CMV negative and irradiated blood was required in 60% of hospitals for neonates and 54% for oncology patients; (g) 29% of hospitals used washed packed cells for neonates.

DISCUSSION Results confirmed considerable variables in clinical practices of paediatric transfusion across Canadian hospitals. Some practices are outdated in reference to current standards. National guidelines for paediatric transfusion practices are necessary for hospitals to standardize and improve practices with the potential for cost savings.

ABSTRACT NO.: 127

Paediatric transfusion practices: Blood bank associated – a sampling of Canadian hospitals

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BACKGROUND Currently there is very little Canadian data on Blood Bank associated pediatric transfusion practices; to gather information in this area the Transfusion Ontario Program in Ottawa developed a questionnaire on Paediatric Transfusion Practices that was distributed across Canada to both paediatric and adult hospitals with paediatric services.

METHOD Questions pertaining to Type & Screen; age of red cells for transfusion and the use of washed red cells; reduced volume platelets; CMV negative products and interest in EBV/HHV tested products were addressed. The patient population was identified as neonate (<4 mths) and paed (≥4 months).

RESULTS Type & Screen – 100% of responding hospitals used T&S as an alternative to crossmatching; 82% identified the utilization of the T&S order as high to moderate. Crossmatching (XM) – 64% of hospitals did not have a policy to discontinue XM in the presence of massive transfusion. Age of Red Cells for Top-up Transfusions to Neonates – 36% of respondents required blood that was <7 days and 14% would accept blood <5 days or up to expiry with AS-3 removed. CMV – CMV negative/irradiated products were required by 60% of hospitals for Neonates and 54% for Oncology patients. 61% would not accept leucodepleted for seronegative products. EBV/HHV – 4% of hospitals would like to see EBV testing of products, this rose to 68% for HHV testing. Plasma Reduced Platelets – was available in 61% of hospitals and was the standard product in 48%.

DISCUSSION The responses confirm that in several areas paediatric transfusion practices vary considerably across Canada. Pediatric and Adult hospital responses were similar. A Canadian national guideline for Paediatric Practice would be a valuable tool for hospitals to assist in standardizing and simplifying practices.

ABSTRACT NO.: 128

Novel method for screening for the presence of Hb S

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Various transfusion agencies mandate that a method be available for screening for the presence of Hb S in blood to be transfused into specific patient groups. Although the Hb S solubility test may be used for this purpose, the test is subjective, difficult to interpret and in commercial kits the reagents have short dating once reconstituted.

The Bio-Rad VARIANT II HbA1c method, although developed for the quantification of HbA1c, detects the presence and identification of hemoglobin variants. Figure 1(a) and 1(b) show chromatograms of patients with Hb A and Hb S respectively. In a 12 month period, using 14 different lot numbers of reagents/cartridges on three VARIANT II analyzers, the mean retention time of Hb S was established as 1.9666 ± 0.0007 minutes. The mean % of Hb S in patient samples was established as 41.53 ± 3.14. Other hemoglobin variants elute with similar retention times to Hb S but could be distinguished from Hb S by characteristic retention time and % of hemoglobin variant. Each analysis is completed in 3 minutes and is therefore suitable for STAT screening.

The Bio-Rad VARIANT II HbA1c method offers a novel method for screening units of blood for the presence of Hb S.

**Fig. 1.** (Abstract 128) Chromatograms of patients with Hb A (a) and Hb S (b).

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