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Molecular Basis and Investigation of Blood Group Genes

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There are 284 blood group antigens recognized by the ISBT Committee on Terminology for Red Cell Surface Antigens. Of these, 244 antigens are included in one of 29 blood groups systems. The rest are included in collections of similar antigens, or in a series of either low incidence or high incidence antigens. The biochemical work of the middle of the last century revealed these antigens to be carried on different glycoproteins and glycolipids on the surface of the red blood cell (RBC). Their usefulness as protein markers recognized by highly specific immune antibodies permitted functional studies of many of these membrane components. In the last twenty years, the genes encoding all but one of the 29 blood group systems have been sequenced, and the molecular basis identified. These studies have shown that the majority of blood group antigens are encoded by simple single nucleotide polymorphisms within the coding sequence of the gene. Where gene families exist such as at the Rh and MNS loci, high nucleotide identity between related genes has resulted in the formation of hybrid genes that encode novel antigens and create unusual phenotypes. Mutations and other alterations in the non-coding regions of genes are also responsible for creating blood group antigen diversity. The molecular identity of blood groups has many different applications. From a clinical perspective, molecular assays can be used to determine likely RBC phenotype in situations where it is difficult or undesirable to obtain RBCs. It also provides a model for the study of diversity within humans and enabled scientists to look at ancestral genes. These studies not only tell more about who we are but also map the influences of different external factors such as disease, on our evolution.

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KODE™CAE: Creating the World's First ABO Analytical Control System

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Aim: To engineer controllable expression ABO glycolipids onto the surface of group O red blood cells to create weak expression ABO cells for blood grouping sensitivity controls.

Methods and Results: The natural phenomenon by which glycolipids can insert into red blood cells underpinned the development of this technology. Blood group A and B glycolipids were constructed with a novel linker molecule that confers aqueous solubility, and a carbohydrate glycotope design of generic specificity. Extensive research was carried out to determine the conditions which facilitate controlled engineering of the red blood cell membrane to express specific levels of A and/or B blood group antigen. Cells expressing low levels of antigen were able to be constructed with KODE™ technology, providing for the first time a reliable supply of cells, consistently expressing antigens suitable as analytical sensitivity controls. Comprehensive internal comparative testing and a large external field trial were performed to analyse KODE™ cell stability and performance against a range of monoclonal antibodies and technologies. These trials clearly demonstrated that the KODE™ constructed cells are stable and performed in the same manner as naturally occurring ABO weak cells. Furthermore they demonstrated a wide range of blood grouping result errors and discrepancies with blood grouping in Australasia, thus highlighting the need for an ABO quality control system in Immunohaematology laboratories

Conclusion: KODE™ technology enables production of the first ABO blood grouping sensitivity control cells that can be precisely manufactured in large volumes. They have been incorporated into a process control system that consistently tests and challenges blood grouping performance.

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Emerging Infections: WNV and Beyond

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Aim: To discuss emerging infections that impact blood safety and to outline approaches to their identification and interventions to reduce their impact.
Emerging infections are defined as those that have increased in frequency over the past 20 years. They include novel agents, such as HIV and vCJD, those that have been imported into areas where they were not previously endemic, like *T. cruzi* and West Nile virus (WNV) and those whose geographic range is expanding, like *Babesia* spp and malaria. In addition, aggressive new therapies lead to a population of patients that is much more susceptible to serious outcomes from infection with normally benign agents such as CMV and the B19 parvovirus (erythrovirus). Each emerging agent must be evaluated for its potential impact upon blood safety, including issues of public perception. Where appropriate, interventions must be designed and implemented and such interventions should ideally be continuously evaluated for efficacy. Key examples that will be discussed are the explosive outbreak of WNV in the US, where nucleic acid testing was rapidly developed and implemented, and vCJD, where preventative measures were implemented even before it became apparent that the disease was transmissible by transfusion. Other infections that will be discussed include *T. cruzi*,(the agent of Chagas' disease), babesiosis, malaria, SARS and dengue. A variety of actual or potential interventions will be discussed.

Transfusion Transmitted Infection

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Lessons Learned From SARS Epidemic

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In the period of March – July 2003, Hong Kong had experienced an epidemic of Severe Acute Respiratory Syndrome (SARS), which started out as an outbreak of community acquired pneumonias of unknown aetiology. It seeded and spread rapidly throughout hospital patients and healthcare workers, with high morbidity and mortality. The outbreak has led to a boom in the art and science of infection control and health protection. Precautionary measures, such as frequent cleansing of utilities, personal hygiene and protective gears have become important. The provision of public healthcare services was re-prioritized. The pattern of blood usage changed. The demand for blood (↓12.8%), as well as the availability of donations (↓16.9%), dropped during the period. With concerted effort of medical and scientific experts, the culprit, which later identified as a novel pathogen, SARS-associated coronavirus (SARS-CoV) transmitted through droplets person-to-person, was isolated. Brief viraemia in patients was documented in anecdotal reports. Still, not much was known about the biology of the virus, and its impacts in relation to transfusion.

Despite the advancement in testing blood borne viral infections, there are still limitations in detecting window period donations and "new" pathogens. Blood centres have to rely on the use of health history enquiry in donor screening to alleviate the risk of transfusion transmitted infections caused by novel pathogens, of which very limited knowledge are known. SARS-CoV is a typical example of one of these emerging pathogens. During the SARS period, the HKRCBTS implemented a donor deferral policy to ensure blood safety. The application of healthcare informatics to capture SARS or suspected SARS patient database for screening donors/donations was useful.

No one knows when, or where, it will re-emerge. Therefore, it is important to carry on further research for the understanding of its pathogenesis, laboratory diagnosis and its role in transfusion, and continuous stringent global surveillance.

Sponsored by CSL Bioplasma
ANZSBT / ASTH Joint Symposium: *Bloody Problems*

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Pre-emptive Management of Challenging Surgical and Critical Bleeding Scenarios

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Three clinical case scenarios will be presented to illustrate management of challenging critical bleeding:
I. Case 1: A Jehovah's Witness with an unanticipated blood loss of 8500 ml at reoperative hip surgery. Teaching and discussion points will include:

1) How low can you go?
2) Lifesaving "matters of conscience" blood products and perisurgical interventions useful in the management of Jehovah's Witness patients.
3) Use of erythropoietin and darbepoietin in the perisurgical setting.
4) The Oregon Health & Science University "Transfusion Blood Refusal" form as a way to guide clinical discussion and management with Jehovah's Witness patients; how we handle transfusion of minors.

II. Case 2: A 6 ½ year old boy with exsanguinating pulmonary hemorrhage post bone marrow transplantation despite correction of platelet count, PT INR, PTT and fibrinogen. Teaching and discussion points will include:

1) Off-label use of recombinant factor VIIa in control of refractory critical bleeding.
2) Limitations of current standard coagulation testing and potential value of the thromboelastogram (TEG) in monitoring coagulopathy and response to rVIIa.

III. Case 3: The CABG patient from hell and his massive transfusions x 2: the first massive occurred in the context of 4 vessel post-operative graft occlusion and cardiogenic shock suspected due to heparin-induced thrombocytopenia (HIT). The patient had had an intraaortic balloon pump placed preoperatively and was undergoing his third cardiopulmonary bypass (CPB) procedure in as many days for BiVAD placement as a bridge to cardiac transplantation. Anticoagulation with the direct thrombin inhibitor (DTI) argatroban was used and post-operative bleeding was hard to control. The second massive transfusion occurred in the context of his heart transplant 12 weeks later. Teaching and discussion points will include:

1) Coagulopathy of cardiopulmonary bypass,
2) Monitoring the degree of anticoagulation on DTIs and treating bleeding due to DTIs.
3) Treating bleeding on antiplatelet agents.

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Attempting to Apply Logic to Chaos in Perioperative Bleeding: An Anaesthetist's Perspective

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The idealized view of surgery and anaesthesia working in concert is sometimes stressed when dealing with unstable coagulopathic patients. The clinical trigger to consider the presence of a coagulopathy is the onset of non-surgically controllable bleeding. You see it in the surgical field, and may remain the best predictor of the need to administer haemostatic agents. While earlier consensus documents considered this event predictable by modeling blood loss, and factor level decline[1], we now recognize confounding variables such as shock, hypothermia, metabolic disturbances and fibrinolysis confuse the picture to such an extent they are often irrelevant[2]. The monitoring of coagulopathy by traditional coagulation screens also lacks validation in non surgical bleeding, and is usually so delayed in response time to become irrelevant to appropriate management.

Attaining euvolaemia is possible with large volume dedicated infusion systems but in a patient with massive mediator release and comorbidities may not achieve adequate tissue oxygen delivery, and inotropic support may be needed. Evidence based transfusion triggers are still lacking, but the degree of insult means while adequate haemoglobin replacement is possible, correction of the coagulopathy in the presence of ongoing loss, hypothermia and acidosis is not. Active warming methods are more advanced and effective, with forced air warming, infra red radiant heaters, and extracorporeal circuits available. Management of coagulopathy is by component replacement. Delays occur because of slow coagulation test turnaround times, and the place of newer point of care monitors and thrombelastography needs evaluation, and shows promise[3]. Delay in delivery of blood components due to processing time may worsen the coagulopathy. Factor VIIa probably has a unique place in the management of coagulopathy in these patients, but studies are needed to define dosage and time of dose[4]. The management of acidosis and hyperlactaemia are fundamentally improved by better perfusion, but short term correction may be indicated to reinforce coagulation factor function[5].

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Your Body, Your Choice

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Many authorities have identified the need for change in transfusion practice. The precautionary principle, unsustainable increasing (and currently underestimated) direct and indirect costs of blood, chronic blood shortages, donor deferrals, loss of altruism, wide variations in transfusion practice and growing knowledge of transfusion limitations and possible adverse outcomes necessitates a paradigm shift in transfusion practice and the management of anaemia and critical bleeding. Historically, changing medical practice in a sustained manner has been challenging. A recent editorial suggested changes in transfusion practice would require "a cultural shift among clinicians, managers, and policy makers."

Informed and empowered patients can be important drivers for change in transfusion practice. Surveys in Australia, United States, Canada and Europe reveal consumers, including lay public and health professionals, would prefer alternatives to donor blood transfusions[1].

A force for change in recent years has been the international evolution of comprehensive, patient-centred, blood conservation / management programs. These programs are effecting significant reductions in blood usage (42 – 95%) along with positive patient and fiscal outcomes. Strategies include managing the patient's own blood by a multidisciplinary, peri-event approach, utilising individualised patient assessment and work-up, strategies to minimise blood loss, optimise red cell mass and a greater understanding of anaemia and its appropriate management. Transfusion decisions are based on individualised patient-specific clinical and physiological factors. Decisions also need to consider patient values and choices.

Bloodless surgery, initially developed as a response to patient request, has metamorphosed into the global approach of comprehensive patient blood management – a cultural shift addressing "bloody problems" that can take transfusion medicine into the 21st century.[2] The AABC is working to establish such programs in Australia.

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ANZSBT / ASTH joint symposium: *Bloody Problems* (contd)

38
Cost Effectiveness of Transfusion Therapy vs. Blood Conservation Therapies

Axel Hofmann, ME

To date governments, hospital administrators and clinicians have grossly underestimated the cost of blood transfusions and transfusion related processes. This may be attributable to a lack of comprehensive and precise cost analyses in this clinical field. In Europe blood components and blood products already account for the biggest proportion of therapeutics purchased by hospitals, but they represent only a *fraction* of the overall transfusion related cost. The total cost might be in the vicinity of *20 billion USD for the European Union* alone. Many different activities and services within a hospital are directly related to transfusions such as receiving, controlling and appropriate storage of RBCs, managing and administering the internal blood supply, transfusion preparation, including extensive laboratory work with numerous regular and irregular tests, non-productive labor time/stand-by time, administration and monitoring of transfusion, treatment of immediate adverse effects, reporting and documenting, cleaning, hemovigilance and long-term outcomes-tracking. In addition, several of these cost elements have a constant tendency to increase disproportionately high, compared to public health expenditures.

From this perspective, blood conservation strategies ("bloodless medicine") and patient blood management are becoming increasingly important. If accurate cost data can prove a more favorable cost effectiveness ratio of blood conservation therapies compared to transfusion therapies, then it is more likely that the paradigm will shift towards a new gold standard for maintaining patient's tissue oxygenation.

The challenge to measure the total cost of these competing therapies can be best met by using 'Process Cost Analysis' or 'Activity Based Costing'. This methodology enables the capturing of actual resource consumption in terms of labor, materials, third party services and capital for each single process step of a therapy. Such costing models, to compare blood conservation strategies and transfusion therapies, are currently being developed.

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Removal of Infective Prions by the Chromatographic Processes of Plasma-derived Albumin and Immunoglobulin

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Aim: To demonstrate, using a validated scaled-down laboratory model, that the chromatographic processes for the manufacture of plasma-derived albumin and immunoglobulin (including IVIg) are capable of removing prions should they be present in the plasma pool.
Methods: Two sets of studies have been conducted to evaluate the capacity of several albumin and immunoglobulin process steps to remove the abnormal isoform of scrapie prion (PrP^{sc}) and scrapie infectivity. Studies were conducted on validated scaled-down models of the manufacturing process steps examined. The first set of studies was conducted using hamster-adapted scrapie agent strain 263K and used Western blot technology. The second set of studies was conducted using mouse-adapted scrapie agent strain ME7 for bioassays utilising C57 Black mice.
Results: Infectivity studies on the upstream delipidation process step that is common for both the albumin and immunoglobulin methods of manufacture demonstrated a logarithmic reduction factor (LRF) of at least 2.40 logs.
Substantial LRFs were also demonstrated across the anion and cation ion-exchange steps of the albumin and immunoglobulin processes with both PrP^{sc} and scrapie infectivity.
Infectivity removal studies on the cold ethanol and depth filtration steps of the CSL intramuscular immunoglobulin (IMIG) process demonstrated a LRF of at least 5.63.
The studies conducted by CSL also demonstrated that infectious scrapie on chromtographic resin following the load-eluation steps is removed to below the level of detection from the resins by the cleaning regime used in the manufacturing plant of solvent-detergent and NaOH treatment.
Conclusions: These studies provide assurance that the chromatographic plasma fractionation processes utilised by CSL Bioplasma to manufacture plasma-derived albumin and immunoglobulin possess substantial capacity to remove putative prions during fractionation.

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Increased Red Cell Susceptibility to Apoptotic Change is Associated with Duration of Packed Cell Storage

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Red cell apoptosis is characterized by loss of lipid asymmetry and cleavage of trans-membrane and cytoskeletal proteins by proteases such as caspase 3. Re-exposure of stored red cells to physiological concentrations of calcium and glucose when transfused may provide some of the signals required for initiation of apoptosis in susceptible cells.
Aim: Whether red cells subjected to prolonged storage at 4°C show increased rates of red cell apoptosis following transfusion has not been studied. In this study we measured susceptibility to apoptotic change after various periods of storage.
Method: Flow cytometric methods were used to examine changes in annexin V binding to exposed phosphatidylserine, eosin-5'-maleimide binding to band 3 and expression of an adhesion receptor ICAM 4 (CD242, LW protein). Data were analysed using paired tests for non-parametric variables.
Results: After 30 minutes of exposure to physiological levels of calcium and glucose we found that the proportion of red cells binding annexin V increased with duration of red cell storage. On day zero 0.25% of calcium and glucose exposed red cells showed annexin V binding which increased 12.6 fold to 3.16% of red cells after 42 days storage at 4°C. These changes were enhanced by 1uM ionomycin; on day zero 6.38% of red cells showed annexin V binding which increased 3.3 fold to 21.19% of red cells after 42 days storage at 4°C. Monoclonal antibody binding to ICAM 4 (LW protein) on red cells exposed to calcium and glucose was reduced with increased duration of red cell storage. Eosin-5'-maleimide binding also varied under these conditions.
Conclusions: Our findings provide some insight into the factors that may influence differences in survival of transfused red cells related to period of storage. Interventions which minimize these changes, and may improve the outcome of transfusion with red cells after prolonged storage, will be the basis of future study.

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Cryoprecipitate Audit Within Six Centres in New Zealand

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New Zealand Blood Service

Introduction: An audit of apheresis cryprecipitate transfusions and patients not receiving cryoprecipitate was conducted in 6 centres, covering approximately 84% of the country's cryoprecipitate use.
Method: Transfusion Nurses Specialists prospectively collected data on cryoprecipitate transfusion episodes for 11 weeks. Where necessary, data was collected retrospectively to make a total of 30 episodes per centre. During each centre's study period, data on patients with fibrinogen levels below 1.0g/L who did not receive cryoprecipitate was also collected (non-recipients). Two medical assessors reviewed each episode for clinical appropriateness.
Results: All centres except one captured at least 30 episodes of cryoprecipitate transfusion. Cryoprecipitate used for fibrin glue (7) and as part of paediatric cardiac bypass surgery protocol (22) were excluded leaving 152 episodes (316 units). Non-recipients involved 86 patients (134 episodes). The mean pre-transfusion fibrinogen level was 1.3g/L with 46% below 1.0g/L and 26% above 1.5g/l. The median fibrinogen concentration of non-recipients was 0.7 g/L. The median number of units transfused per episode was 2. 68% of episodes had the right dose (0.5 to 1.5 u/30kg bodyweight) with underdosing in 24% and overdosing in 8%. Weight-adjusted dose and pre-transfusion fibrinogen level showed no association ($r=0.19$, $p=0.434$). Underdosing was associated with more episodes per 48 hours compared with the correct dosing (2.5 vs 1.1) ($p<0.005$). 18% of cryoprecipitate episodes were considered inappropriate. 27% of non-recipients' episodes were considered inappropriate (cryoprecipitate indicated). Non-recipients showed a similar pattern of fibrinogen levels to recipients.
Discussion: Underdosing was more common than overdosing and required more cryoprecipitate transfusions than when the right dose was given. Education is needed regarding the correct dose, the importance of checking pre-transfusion fibrinogen levels and ensuring systems are available for monitoring fibrinogen levels in massive transfusion. It is recommended that Blood Banks ask for the patient's weight before issuing cryoprecipitate.

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Identification of Proteins that Accumulate in the Supernatant of Platelet Concentrates During Storage

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Platelet transfusion has been implicated in adverse reactions. Soluble factors, including plasma proteins and bioactive molecules released from platelets during storage, are likely to play a critical role. Comprehensive maps of the proteins present in platelet concentrates (PCs) are not available. The aim of this study was to identify the soluble proteins that accumulate in the supernatant of PCs during storage by using a multifaceted proteomics approach.
Irradiated, prestorage leucocyte-filtered pooled buffy-coat PCs in additive solution (T-sol, Baxter) were prepared and stored according to standard blood bank procedures. Samples were collected at days 1, 3, 5, 6 and 7. Platelet membrane integrity was monitored by release of Annexin-V and platelet activation was determined by expression of CD62P. Proteins in the supernatant of PCs were identified by two-dimensional (2D) gel electrophoresis and mass spectrometry, and cytokine antibody microarrays. Cytokines and bioactive molecules were quantitated by ELISAs.
2D-gel maps of PC supernatant showed a number of plasma-derived proteins that appear to undergo alterations and degradation during storage. Several platelet-derived bioactive molecules, such as RANTES and derivatives of platelet basic protein (β-thromboglobulin, CTAP-III and NAP-2) accumulate to high levels. A number of proteins that have not previously been examined in the transfusion setting also accumulate during PC storage. Levels of leucocyte-derived cytokines (IL-1β, IL-6, IL-8, TNF-α) remained relatively low.
These results provide an expanded view of the proteins that accumulate in the supernatant during storage of PCs and may lead to a greater understanding of the factors that contribute to adverse transfusion reactions and strategies to improve transfusion outcome.

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Storage Related Increase in Red Blood Cell (RBC) Adhesion to Vascular Endothelium: Effects of Bacterial and Inflammatory Activation

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Physical and biochemical changes that occur to RBCs during storage may increase their adhesion to vascular endothelium thereby potentially impeding blood flow and decreasing oxygen supply in transfusion recipients. Although blood is often required during trauma or surgery where infection, inflammation and/or tissue damage is prevalent, it is unknown whether prior activation of vascular endothelium further affects RBC-endothelial cell (EC) interactions. The aim of this study was to determine whether prior activation of ECs affects adhesion of stored RBCs under conditions of continuous flow *in vitro*.

Human umbilical vein ECs were grown to confluence on gelatin-coated coverslips. Non-leucocyte-reduced, buffy-coat-reduced and leucocyte-filtered RBC products were prepared according to standard blood bank procedures. RBC samples were collected at weekly time points until product expiry. RBCs and ECs were incubated with saline, endotoxin (250ng/ml) or TNF-□ (5ng/ml) for 4 hours. RBCs were then perfused across the EC monolayer using a parallel flow chamber mounted to an inverted microscope. Perfusion of RBCs was controlled for shear stress and temperature. RBC-EC interactions were recorded using a digital camera attached to the microscope. Activation of ECs was confirmed by immunohistochemical assay using E-selectin and VCAM-1.
Adherance of RBCs to unactivated and activated EC layers increased with product storage time. RBCs from products containing leucocytes were significantly more adherent to the unactivated and activated EC layers in the later stages of storage than RBCs from leucocyte-reduced products. Significantly increased numbers of RBCs adhered to the activated EC layers at Day 1 of storage compared to the unactivated EC layer. Interestingly, a response of this magnitude was not seen at other time point during storage in our model system.

These results may lead to greater understanding of the interaction of transfused RBCs with recipient endothelium during inflammation and sepsis and the biological consequences of this adherence.

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TRALI: What Do We Know and What Can We Do About It?

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Transfusion-related acute lung injury (TRALI) has been the leading cause of transfusion-related deaths reported to the United States Food and Drug Administration (FDA) for the past 4 consecutive years, and is being increasingly recognized as a leading cause of transfusion-related morbidity and mortality worldwide. It is likely that TRALI is both under-recognized and under-reported--a situation complicated by the fact that there is no definitive laboratory test to confirm the diagnosis, and that, until very recently, there has not even been an international consensus definition of TRALI. In consequence, much of our knowledge about the incidence, epidemiology, pathophysiology, diagnosis and prevention of TRALI is substantially incomplete and/or controversial. Acute lung injury (ALI) unrelated to transfusion has long been recognized in intensive care (ICU) patients and is believed to have a 2-event pathogenesis involving adhesion of primed neutrophils (PMNs) to pulmonary endothelium with subsequent PMN activation by some inducing agent. Although TRALI has traditionally been thought of as having a one-event pathogenesis (passive donor anti-leukocyte antibody interacting with cognate recipient leukocyte antigen with resultant leukocyte activation), evidence has been accumulating that presence of cognate anti-leukocyte antibody/antigen pairs is neither necessary, nor often even sufficient in isolation, to provoke TRALI. Rather TRALI appears to be a multifactorial syndrome, and is likely a true 2-event subtype of ALI, with both recipient predisposition and biological response modifiers (BRMs) generated during storage of blood products also often playing major pathogenetic roles. This session will highlight recent advances in our knowledge of the pathophysiology of TRALI, both antibody-mediated and non-antibody mediated, and the public health implications regarding prevention implied by the two models (which are non exclusive). The recent international consensus definition of TRALI will be discussed and information provided to guide the attendee as to the recognition, investigation and clinical management of TRALI.

ANZSBT Symposium: Practising Smarter

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Lessons Learnt from Scotland

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In 2000, we evaluated a programme of clinical effectiveness in transfusion practice utilizing the role of the transfusion nurse specialist (TNS). The study demonstrated that where the TNS was deployed there was a general trend towards improvement in practice, and also showed the importance of adopting a national co-ordinated quality improvement approach.

Following this study, in 2003, NHS Scotland (NHSS) launched a 3-year 'Better Blood Transfusion programme' (BBTP). Central to the BBTP programme, each hospital has access to a transfusion practitioner (TP) as part of a hospital based transfusion team. A standardized educational package has been introduced across NHSS (www.learnbloodtransfusion.org.uk) for all staff involved in the transfusion process, supported by a learner management system (*ORASGOLD*^{TM}). Over an 18-month period, approximately 30% of staff have successfully completed the Level 1: Safe Transfusion Practice module. Another key component of BBTP is to provide clinicians with transfusion data so they can review their clinical practice. The development of blood usage data has resulted in a range of reports, which will shortly be disseminated to users in NHSS. The BBTP teams have also instigated over 100 local blood saving initiatives and audits resulting in changes to regional blood ordering and cross-match policies. It has

been agreed however, to explore a more nationally co-ordinated approach to blood saving initiatives. The BBTP team also works with the transfusion service to ensure that in times of blood shortages the available stock is managed efficiently.

We have demonstrated that a co-ordinated clinical effectiveness programme with the appropriate support and relevant data, can improve transfusion practice. This collaborative approach between BBTP, Scottish hospitals and the Transfusion Service, is seen as the cornerstone of the NHSS response to recent European Union legislation (2002/98/EC / 2004/33/EC) and will allow us to benchmark with the wider UK ensuring safe, efficient and effective transfusion practice.

ANZSBT Symposium: Practising Smarter

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The Hong Kong Experience

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Transfusion medicine has evolved from a mostly laboratory-centred service with a focus on the serological aspects of blood, into a clinically oriented discipline that emphasis patient care. With the rising cost of quality and safety, increasing number of blood borne pathogens, demand for more and enhanced plasma products, increasing litigations and drive for public sector efficiencies, blood centres are in general facing a serious issue of insufficient resources. For blood centres, the term 'resources' should not be limited to the traditional quantifiable definitions of financial support, infrastructure and investment record, but should also include the considerations of expertise, local social and cultural setting, community understanding and acceptance, and, most of all, a pool of willing and suitable blood donors that is sufficient to provide for the community needs. To maintain blood sufficiency and to ensure blood safety, blood centre has to be creative and be able to practise smarter.

The Hong Kong Red Cross Blood Transfusion Service (BTS) is the only organization responsible for donor recruitment, blood collection and supply of processed blood products to hospitals. Blood products are supplied free of charge to hospitals as the BTS is fully funded by the Government. Funding is allocated on an annual basis and based on previous year's allocation with adjustment. Since the regional economic downturn in 1997, there has been continual cut in the BTS budget. We have to be creative in resources management to maintain sustainability. Notable examples included the contracting out of delivery of blood products to hospitals, contracting out of NAT tests, implementation of automated component processors, introducing phlebotomists to perform venepuncture, etc.

In pursuing efficiency and cost-effectiveness, we have not sacrificed service quality service nor blood safety. The BTS is both ISO9001-2000 and TGA cGMP accredited. Recently, we have also achieved ISO14001 accreditation.

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Ross Wilson: What could Australia's model system look like?

ANZSBT Free Communications 1: Transfusion Practice Improvement

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Best Practice in Transfusion Specimen Labelling – Declaration of Positive Patient Identification

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Inadequate patient identification during the specimen labelling process instigated involving consumers to check their details on the tube and form.
Evidence reveals that incorrectly labelled Transfusion Specimen can be fatal. Miscollection of blood is commonly referred to as 'Wrong Blood in Tube' (WBIT). A WBIT occurs when identical patient details are found on the tube and form however it is not that patient's blood in the tube. WBIT are detected when comparing current blood group results with an historical blood group and they differ. Majorities of WBIT go undetected as a high proportion of patients do not have an historical record.
As a result of a fatal incident due to mis-labelling of blood, a recent Australian Corner recommended that all organisations undertaking Pre-Transfusion testing follow the Australian New Zealand Society of Blood Transfusion

(ANZSBT) Specimen Labelling Guidelines. These guidelines state the collector must sign a declaration verifying positive patient identification at the time the blood is taken. It is also recommended that a second witness sign verifying the patient's identity. The coroner stated that where possible, especially with the young and aged that a relative or carer be involved in identifying the patient.

Reviewing the organisations pre-transfusion specimen labelling standards revealed that they did not meet the ANZSBT requirements regarding a declaration of positive patient identification by the collector or a second witness. Wrong blood in tube incidents and a recent root cause analysis in the hospital exposed that a problem did existed.
A small project team, involving a consumer was formed and Clinical Practice Improvement (CPI) methodology was utilised to resolve the declaration of positive patient identification when collecting blood samples in an emergency service. A mission statement aiming to eliminating miscollection and mislabelling was created. The specimen labelling process was studied and a detail flow chart developed highlighting areas of concern. Team voting revealed the inadequate transfusion request form was a priority.
Interventions such as a new sticker with a collectors and second witness declaration of patient identification was developed and placed on the original form. Staff education occurred and posters were developed explaining the new process. Each new intervention followed the CPI, Plan, Do, Study, Act cycle and interventions where adapted and changed according to the results.

Results were tallied weekly and Patient Identification Declaration by the collector was completed 100%, except for twice when it dropped to 97%. No WBIT have been detected during the 28 week trial and a second witness signature has been provided 74% to 100%.
Staff have embraced the practice change with consumers stated they are comfortable being included in the labelling process.

ANZSBT Free Communications 1: Transfusion Practice Improvement

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Transfusion Practice Improvement Strategies, Raising Awareness and Knowledge of Transfusion Issues in Victoria and Tasmania

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Aim: The Better Safer Transfusion (BeST) Program of the Department of Human Services Victoria, aims to improve transfusion safety and practice in hospitals.
The goal is to achieve practical and sustainable improvements in four main areas:
(1) improve awareness and knowledge of transfusion practice within hospitals;
(2) implement appropriate and best practice for clinical decision making and blood administration;
(3) develop and implement a state-wide haemovigilance system; and
(4) engage and support the private and rural sectors.
Method: The program is overseen by a multidisciplinary departmental advisory committee, supported by four expert working parties and a secretariat.
Improvement of knowledge and safety of transfusion within health services is through:
- support of existing transfusion nurse roles
- support and promotion of the transfusion practice course; an on-line course accredited as equivalent to a graduate certificate.
- audits encouraging review of practice against evidence-based guidelines have been sent to health services
- development of a haemovigilance system
- information on BeST program initiatives has been discussed in regional forums
- improvement practice tools and information for transfusion have been made available through the BeST website at: http://www.health.vic.gov.au/best

Results:
- on-line transfusion practice course continues with national & international participants in 2005, resulting in knowledgeable transfusion practitioners (medical, scientific, nursing) who can lead transfusion improvement
- 19 Transfusion Nurses working in Victorian & Tasmanian public hospitals
- education of health professionals by Transfusion Nurses within health services
- development of consumer education material
- response to transfusion audits (initial audit looking at storage & handling of blood products had >80% response rate).

Conclusion: Enthusiastic participation in transfusion improvement initiatives in Victoria and Tasmania continues to improve transfusion practice and patient outcomes. Building on the initial success of the Blood Matters program, rural and private hospitals are now also involved in this program.

ANZSBT Free Communications 1: Transfusion Practice Improvement

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Adapt, Adopt or Augment: Strategies for Change in the Blood Service?

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Safety and supply are two issues that universally concern Blood Services throughout the world, whatever their configuration. Measures to safeguard against the risks of HIV, Hep C and vCJD have required radical changes in many Blood Service organisations in recent years. In New Zealand, risk management was a major driver leading to the inauguration of a 'vein-to-vein' National Blood Service (NZBS) in 1998. In Australia the change imperatives were different, but the challenges nonetheless pressing. Prior to the establishment of the Australian Red Cross Blood Service (ARCBS) in 1996, each Australian State and Territory had an autonomous Red Cross Blood Service. Our purpose in presenting this paper is to discuss ways in which strategies for making radical changes were identified, appropriate to the specific contexts of change within the two countries. We discuss the merits and benefits of the strategies adopted. We believe the organisational development lessons learnt Down Under have wide application in Blood Services undergoing change around the world. There are, we believe, very real merits in adapting, adopting or augmenting other Services' experiences. Successful choice of change strategies, however, for the design, development and implementation of service and organisational change, depends on appropriate analysis of the tasks and challenges being confronted, and the careful marrying of theoretical principles with effective best practice in both clinical and organisational development areas.

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A Retrospective Audit of Irradiated Component Use in New Zealand

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Background: Transfusion Associated Graft vs Host Disease (TA-GvHD) is a fatal complication of blood transfusion. Practically, there is no treatment for TA-GvHD. The disease is prevented by providing irradiated components to at-risk patients. These patients are treated by a diverse group of health professionals. The challenge is to ensure that such patients always receive irradiated blood components.
Aim: To ascertain if patients with an absolute indication for irradiated components (as per ANZSBT Guideline) received only irradiated components. To assess whether patients who have an irradiated components protocol in place have appropriate diagnoses.
Method: Transfusion Nurse Specialists at six main centres across New Zealand retrospectively collated lists of patients with absolute indications for irradiated components for 2004. The clinical data included the diagnosis and treatment dates. Sources included case mix analysts, paediatricians, haematologists, pharmacists and blood banks. The units transfused to these patients were sourced from Progesa.
Interim Results: 498 patients were identified as having attended hospital in 2004 with an indication for irradiated components. 294 (59%) received transfusions. 4580 units in total were transfused of which 341 (7%) were not irradiated. 71 (24%) transfused patients received a mean of 4.8 non-irradiated units (range: 1-34). The diagnosis most strongly associated with patients receiving non-irradiated components was Hodgkin's Disease followed by Aplastic Anaemia and purine analogue therapy (23 and 20 patients respectively). Of the 338 patients with irradiated protocols in place, 68% were absolute indications and 25% were probable indications as per ANZSBT guidelines. Some neonatal units had standing policies to provide irradiated components to all patients. No cases of TA-GVHD were reported in 2004.
Conclusion: A significant proportion of patients with absolute indications for irradiated components received unirradiated components. Where protocols are in place, indications were mainly appropriate.

ANZSBT Symposium: **The Iron Maiden**

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Iron Metabolism and Assessment of Iron Stores

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Type 1 hereditary hemochromatosis is a common disorder of iron overload occurring in individuals homozygous for the C282Y *HFE* gene mutation. It can be a progressive and fatal condition. Early detection and phlebotomy prior to the onset of cirrhosis can reduce morbidity and normalize life expectancy. It is readily identified through biochemical testing for iron overload using serum transferrin saturation and genetic testing for C282Y homozygosity. Recent advances in non-invasive magnetic resonance imaging have substantially improved the diagnosis and risk stratification of patients with iron overload. General population screening has been waived in preference to targeting high-risk groups such as first degree relatives of affected individuals and those with clinical features suggestive of iron loading. This screening strategy is likely to continue until uncertainties regarding the natural history of the disease, age-related penetrance, and management of asymptomatic individuals are clarified.

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Iron Nutrition in Pregnancy and Early Life

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Aim: Iron deficiency is a relatively common problem in pregnancy in both developed and developing countries. In industrialised countries there is some debate about usefulness of routine iron supplementation in pregnancy. The aim of the Adelaide Mothers' and Babies' Iron Trial was to investigate whether routine low dose iron supplementation in pregnancy has beneficial effects for the mother and child.
Methods: Randomised controlled trial of a daily iron tablet (20mg) vs placebo from 20 weeks gestation until birth. Primary outcomes included maternal iron status at the end of the pregnancy and at 6 months post partum, as well as childhood IQ at 4 years of age. Other outcomes included pregnancy outcome, maternal health and well-being and childhood behaviour at 4 years of age.
Results: 431 women (215 in the control group and 216 in the iron group) were recruited from the Women's and Children's Hospital, Adelaide. The prevalence of iron deficiency anaemia (IDA) in the iron group (3%) was lower than the control group (11%). By 6 months post-partum, the frequency of IDA did not differ between the two groups but women in the iron group had less iron deficiency compared with control. There were no differences between the groups in pregnancy outcome, or any indices of maternal mood and well-being. Similarly the mean IQ and mean behavioural scores of children born to mothers in the iron and control groups did not differ. However, the percentage of children with abnormal total behavioural scores was higher in the iron group compared with the control group.
Conclusions: Although routine iron supplementation in pregnancy was associated with improvements in maternal iron status and a lower risk of iron deficiency, there were no improvements in indices of maternal well-being or childhood development in this well-nourished population.

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Tony Keller: Iron and the Blood Donor: What Have We Learnt?

Plenary: Ruth Sanger Oration

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Every Evening at The Bun Shop

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ARCBS, Sydney, NSW

Characters:
Sir Ronald Fisher- mathematician and geneticist
Dr Robert Race- red cell serologist

Scene I: **The Bun Shop – Cambridge, 1943**
Corner, Downing & Corn Exchange Streets

Ronald Fisher and Robert Race are seen, as they are every evening, discussing the latest interpretations of Race's serological findings.

They are drinking beer. Fisher is writing on a sheet of paper.

Scene II: **Caius College – Cambridge University**

Fisher has returned to his rooms in College. He works for two hours, studying the sheet of paper. He then retires to bed.

Scene III: **The Bun Shop**

At Bun Shop time the next evening, Fisher shows Race the sheet of paper. On it is revealed a quantum leap towards safer blood transfusion and a defining step in the prevention of a devastating disease.

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Novel Approaches for Blood Group Genotyping

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Blood Centre, Lund, Sweden

Studies show that approximately 1-2% of transfused patients will produce one or more alloantibodies to antigens absent on their RBCs but present on the transfused RBCs. The incidence is greatly increased in those patients who are regularly and/or multiply transfused and it is estimated that 35-50% of all transfused patients with sickle cell disease become alloimmunized. Additionally, alloimmunization may occur during pregnancy with potentially fatal consequences for the unborn foetus. Aside from any morbidity caused by the antibody, the provision of antigen-negative blood often requires the screening of hundreds of RBC units to find compatible blood. Current practice in many blood centres and transfusion services is to maintain a limited inventory of RBCs that have been typed for common antigens in the Rh, Kell, Duffy, MNS and Kidd systems. This testing is costly and the availability of test reagents is dwindling.
The molecular bases of 28 of the 29 blood group systems have been elucidated. Techniques to detect genetic polymorphisms that encode blood group antigens have been developed and refined. These are used primarily for the resolution of weak or unusual antigens, as an adjunct to serological investigations of samples from previously alloimmunized patients and for genotyping foetal DNA to determine at-risk foetuses. These mostly manual techniques are not practical for mass screening such as donor testing.
Most blood group antigens are encoded by single nucleotide polymorphisms (SNPs) and have the potential to be analyzed by more high-throughput technology e.g. microarray. Several groups have been exploiting the use of SNP microarrays for the rapid detection of blood group polymorphisms. Although in their infancy, initial results look promising and microarray platforms look likely to be the way of the future for genotyping donor blood.

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Toward *In Vitro* Production of Blood Products

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Transfusion of blood products is a common, life saving medical procedure used in trauma and surgery as well as in treating blood clotting disorders, cancer, sickle cell anaemia, and during organ transplantation. The Australian Red Cross Blood Service is responsible for the collection, processing and testing of almost one million blood donations a year from healthy volunteer donors. The challenges and costs of sustaining this very successful system are ever increasing. The potential contamination of blood products by adventitious agents is driving up costs for performing detailed donor interviews and testing of products, while reducing the number of eligible and willing donors.

Production of blood products in bioreactors mimicking haematopoiesis is an emerging alternative strategy. It is already technically possible to produce fully functional red blood cells (RBC) and neutrophils from haematopoietic stem cells and technologies are emerging to replace haematopoietic stem cells with embryonic stem cells, thus providing an abundant, well-characterized starting material. Irrespective of starting material, however, a substantial hurdle to in vitro production is the cost and logistic of producing meaningful numbers of cells. A single unit of red blood cells contains 2

trillion cells and with current technologies it would require 20 days operation of a 2,000 L bioreactor to produce a single unit of RBC. In scale, this corresponds to the production of 1 kg of monoclonal and an expected price of $1 million per unit.

This talk will highlight some of the engineering challenges as well as some solutions illustrated with examples from our own work on both RBC and neutrophil production.

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The Introduction of a National Haemovigilance Programme

Simon Benson
New Zealand Blood Service, Auckland, New Zealand

On 1 May 2005 following a four-month pilot at three North Island hospitals, New Zealand Blood Service (NZBS) introduced its national haemovigilance programme.
The programme, which embraces Council of Europe requirements, is modelled on similar schemes in the UK and Eire and aims to capture data on the prevalence of all types of transfusion-related adverse events not only so-called transfusion reactions.
The reporting process centres on hospital 'Transfusion Safety Officers' (TSO), nominally a hospital's blood bank charge scientist, who is responsible for ensuring all events are reported.
Events are initially reported using a dedicated haemovigilance form. An accompanying 'user guide' provides information for completing the form, definitions of types of events and anticipates some frequently asked questions. Completed forms are then submitted to NZBS National Office where they are entered into a Microsoft Access™ database for subsequent analysis. If further investigation is indicated additional event-specific forms are sent to the hospital concerned.
From the start of the pilot until 30 June 2005, 112 reports of events have been received from 22 hospitals. Whilst the majority of events have been non-haemolytic febrile transfusion reactions or allergic reactions (58% and 30% respectively) other events include 'transfusion associated circulatory overload' (TACO; 4%), 'incorrect blood component transfused' (IBCT; 4%) and possible 'transfusion associated acute lung injury' (TRALI; 3%).
The Haemovigilance Programme appears to have gained acceptance within the New Zealand transfusion sector with 16 of the country's 21 District Health Boards represented in the reports received. However there is still much effort required in raising awareness of the programme particularly among nursing, medical and quality personnel. Regular communication with these groups, hospital transfusion committees, newsletters, the NZBS website and ultimately release of an annual report are all tools that are being, or will be used, to raise the profile of haemovigilance in New Zealand.

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The NZBS 'DHB Clinical Oversight Programme'

Simon Benson
New Zealand Blood Service, Auckland, New Zealand

On 1 January 2005 New Zealand Blood Service (NZBS) introduced its District Health Board (DHB) 'Clinical Oversight Programme'.
NZBS has statutory responsibility for collection and distribution of blood being appointed by Parliament to ensure DHBs maintain efficient blood banking systems.
In discharging this responsibility NZBS has traditionally supported DHB transfusion activities with clinical audits, site visits and regional blood bank meetings. However delivery was inconsistent from region to region and not nationally coordinated.
To gauge what support was expected consultation with blood banks was undertaken, responses shaping the eventual proposal for a formalised 'clinical oversight programme'. Subsequent feedback showed support for the proposal providing a clear mandate for its introduction. The proposal was also endorsed by International Accreditation New Zealand (IANZ; the accrediting agency for medical testing laboratories) in line with requirements of standard NZS/ISO15189.
The programme is now well established. DHBs participate by formal agreement with NZBS and so far only one DHB has chosen not to participate.
12 site visits have been performed with 7 sites receiving corrective action requests. Copies of site visit reports are forwarded to IANZ and issues raised in them have been used by IANZ during their assessment visits to other blood banks.

Regular regional meetings are held by the four main NZBS centres. Attendance particularly by the smaller, geographically isolated blood banks is inconsistent, as staff cannot be spared to attend. However IANZ expects blood banks to attend at least 2 (out of 3) meetings per year which they will monitor. Finally 20 sites are scheduled to receive their (biannual) clinical audits.
It is hoped the NZBS 'DHB Clinical Oversight Programme' offers DHBs the support required to provide transfusion activities which are consistently practiced across the country, which meet the expectations of NZBS and IANZ and which conform to best international practice.

ANZSBT Free Communications 2: Adverse Events and Governance

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First Yield for HIV-1 NAT in the Australian Blood Donor Population – A Repeat Donor with Acute HIV-1 Infection

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Background: In June 2000, the Australian Red Cross Blood Service (ARCBS) implemented nucleic acid amplification testing (NAT) of all blood donations for HIV-1 and HCV to reduce the residual risk of transfusion-transmitted viral infections.
Case report: On 7 May 2004, a 55-year-old donor made a blood donation that was negative on routine screening for anti-HIV-1/2. However, the donation was within a minipool of 24 that tested positive for HIV-1/HCV RNA (Chiron, Procleix TMA HIV-1/HCV Multiplex Assay). Testing of individual donations resolved the pool to this donor. The donation was subsequently confirmed HIV-1 RNA positive and HCV RNA negative (Chiron, Procleix TMA HIV-1 and HCV Discriminatory Assays). A follow-up sample collected 7 days post index donation remained anti-HIV-1/2 negative and HIV-1 RNA positive. Seroconversion was documented on the testing of a sample collected 12 days post index donation.
At the time of donation, the donor reported being in good health and had responded negatively to all ARCBS risk factor screening questions. Two days post index donation, the donor became mildly unwell, with lethargy, cough and nasal congestion. These symptoms persisted for one week and resolved spontaneously. The donor subsequently remained well and was not commenced on anti-retroviral therapy.
Sequencing of the HIV-1 reverse transcriptase and protease genes from a follow-up sample collected 124 days post index donation demonstrated no drug resistance mutations and identified the HIV-1 subtype as CRF01_AE.
The donor had visited Thailand during the period of March 2004 and April 2004 for the purpose of elective surgical treatment and had returned to Australia 11 days prior to the index donation. The donor specifically denied risk behaviour in Thailand, including sexual activity or injecting drug use, suggesting the possibility of iatrogenic transmission.
Conclusion: This case represents the ARCBS' first NAT yield for HIV-1 and demonstrates the improved safety of the blood supply following the introduction of NAT.

ANZSBT Free Communications 2: Adverse Events and Governance

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Reducing the Risk of HBV Transmission

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Aim: Hepatitis B infection is endemic within New Zealand. Blood donations are tested for HBsAg (Abbott Prism CLIA). Approximately 160000 donations are transfused each year with 1-2 reports of probable transfusion-transmitted Hepatitis B received annually. The feasibility and likely benefits of additional Hepatitis B testing were investigated using either HBV DNA (Chiron Ultrio assay) or Hepatitis B Core antibody (Abbott Prism CLIA).
Method: 10000 donations were tested for HBV DNA (single donation testing). Reactive specimens were investigated with anti-HBc, anti-HBs (IMx Abbott) and real-time HBV DNA PCR (ESR, NZ). 10000 separate donations were tested for anti-HBc with repeat reactive donations tested with anti-HBs and an independent anti-HBc assay (Murex). HBV DNA testing was undertaken on anti-HBc positive donations with anti-HBs less than 100IU/L.
Results: 6.8% of donations were anti-HBc reactive. 64% of anti-HBc reactive donations had anti-HBs greater than 100IU/L. 6.3% had no detectable anti-HBs. 1 anti-HBc reactive donation showed low level Ultrio reactivity. Anti-HBs was 8.6IU/L with negative Ultrio HBV discriminatory and repeat Ultrio testing. To date, 7820 donations have been

tested by Ultrio. 13 donations were reactive but only 1 was reactive in both discriminatory and confirmatory assays. This donation was anti-HBc strongly reactive and anti-HBs was less than 10IU/L. Only 1 stored aliquot from 12 previous donations tested was Ultrio reactive but HBV discriminatory assay was negative. Lookback on the recipients of components from this donor's donations found 7 of 15 recipients alive. None of 4 tested had markers of past Hepatitis B infection.
Conclusion: The high level of anti-HBc reactivity suggests this would not be an appropriate screening test to reduce the risk of transfusion-transmitted HBV in New Zealand. Further study is needed to improve understanding of the significance of occult HBV infection and the utility of the Ultrio assay to reduce the risk of HBV transmission.

ANZSBT Free Communications 2: Adverse Events and Governance

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Transfusion Risk Perceptions of the Australian Public

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Aim: Transfusion risk perceptions of the general public have been investigated in Canada and the USA. However, to the best knowledge of the authors, there have been no studies investigating how the Australian population perceives the safety of transfusion.
Methods: A questionnaire was adapted for the study based on that developed by R.D. Davenport and D. Henrard from the University of Michigan, USA (unpublished, used with permission). The questionnaire included questions to explore both elective and emergency surgery transfusion preferences, risk perceptions, personal experience with blood transfusion and adverse transfusion outcomes, blood donor status, and demographic information. The questionnaire was distributed by mail to a proportionally stratified random sample of 3,014 adults drawn from the Australian electoral roll during October-December of 2003. Data was analysed with chi-square tests using the Stata8 software package.
Results: When asked to rate their level of concern with the safety of blood transfusion today, 69% of respondents stated a low level of concern. The majority of respondents (60%) were able to correctly estimate the risk of contracting a serious viral infection as very low (1 in 100,000 or 1 in 1,000,000). When asked about transfusion preference during elective surgery, similar proportions preferred autologous blood (43%) and homologous blood (42%), 13% preferred a directed donation, and less than 3% said they would not accept a blood transfusion at all. For a transfusion in a medical emergency, 60% of respondents indicated they would prefer blood from the "blood bank", with 40% seeking alternatives. These results can be directly compared to those of the University of Michigan study.
Conclusion: Relative to the general public of the USA, the Australian public has lower levels of concern about blood safety. This is reflected in the higher proportion willing to accept a homologous transfusion in elective and emergency surgery.

Free Communications 3 – Clinical Immunohaematology

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Neonatal Alloimmune Thrombocytopenia – A 5 year Victorian Experience

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Aim: To review cases referred for investigation of neonatal alloimmune thrombocytopenia (NAIT) in Victoria from 2000-2004. NAIT is a major cause of morbidity and mortality in the thrombocytopenic foetus and neonate. It results most commonly from parental human platelet antigen (HPA) incompatibility.
Methods: Retrospective review of database results (HPA, HLA, crossmatch investigations etc) and clinical records.
Results: Over the last 5 years we have seen increased numbers of referrals for investigation of NAIT: n=14 in 2000, 23 in 2001, 26 in 2002, 45 in 2003 and 40 in 2004. Confirmed cases averaged 7/year, corresponding to an incidence in Victoria of 11/100,000 live births. Other cases were unable to be confirmed or excluded because of inadequate specimens, confounding HLA antibodies, or complex clinical scenarios including other potential diagnoses. Intravenous immunoglobulin (IVIG), infused to the mother antenatally, is used to augment the foetal platelet count prior to delivery and reduce the risk of intracranial haemorrhage. From March 1999 to June 2005, requests for 161 doses of IVIG were made to treat NAIT (0.4% all requests for IVIG), corresponding to 0.75% grams IVIG issued, with the average antenatal dose being 58 grams on each occasion. Many of these pregnancies also required HPA-matched intrauterine platelet transfusions and neonatal platelet and IVIG support.
Conclusions: Our referral centre provides coordinated testing, specialised platelet therapy and clinical advice for the management of these complex cases. Referrals for labour intensive and time-consuming NAIT investigation and support have increased in recent years. Optimal therapy remains to be defined. Development of a national registry

would further the knowledge about pathogenesis and management of NAIT and may provide new insights into the course and prognosis of the disease.

Free Communications 3 - Clinical Immunohaematology

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The Mystery of the Missing Miltenberger – What is the Basis of Non-B-A-B Mi(a)-Pos Phenotypes in Asia?

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Aim: The antigens of the MNS system are located on erythrocyte glycophorins. GpMur (Mi III) results from gene conversion. A GPA sequence is inserted into GPB, leading to reactivation of a splice site and expression of a hybrid of GpA and a GpB pseudoexon. Other variant MNS glycophorins such as GpHop, GpBun, or GpHF (Mi types IV, VI or X) have been thought to be low frequency with GpMur the basis of Mi(a)-pos types in Asia. A-B crossovers, such as GpHil (MiV) have not been thought to contribute to Asian Mi(a)-pos phenotypes. The aim of this study was to study the frequency of B-A-B gene conversions and A-B crossovers in specimens serologically defined as Mi(a)-pos.
Method: A PCR for detection of B-A-B conversions and a PCR designed by Shih et al for detection of A-B crossovers were optimised.
Result: A 145 bp product consistent with B-A-B gene conversion was detected in 80% (16/20) of serologically Mi(a)-pos samples from Vietnamese subjects. B-A-B gene conversion was detected in 3% of Australian Chinese tested. A-B crossovers were detected only in two individuals of GpJL (Mi XI) phenotype.
Conclusion: Nadarajan et al found 60% of Mi(a)-pos Malaysian Chinese were B-A-B pos but that less than 50% of Mi(a)-pos Malays were B-A-B pos and while up to 2% of Indians were Mi(a)-pos none were B-A-B PCR-pos. Lin has reported that 60% of serologically Mi(a) pos Taiwanese are of types associated with B-A-B conversion. For Australian Chinese the proportion that was B-A-B positive was similar to those reported by Nadarajan and Lin. For Mi(a)-pos samples from Vietnamese the 80% B-A-B pos frequency was a higher than for other Asian ethnic groups. Whether some of these Mi(a)-pos individuals react with anti-Vw found in some batches of intravenous immunoglobulin is a question currently under investigation.

Free Communications 3 - Clinical Immunohaematology

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First Example of the Para-Bombay Phenotype in an Australian Proband is Encoded by a Novel *FUT1* Allele

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Aim: The rare H-deficient Bombay and para-Bombay RBC phenotypes are characterised by sporadic mutations in the *FUT1* gene in different populations. Samples were investigated from a pregnant Australian female with a suspected para-Bombay phenotype. Our aim was to characterise the RBCs and to identify *FUT1,* and possibly *FUT2,* mutations responsible for the phenotype. **Methods**: Standard serological methods were used, including adsorption/elution of human anti-H. Genomic DNA was isolated from the proband and a cord sample drawn at delivery. Routine *ABO* genotyping assays and sequencing analyses of *FUT1* and *FUT2* were performed. **Results**: The proband's RBCs typed as O, Le(a–b+), and were H– by direct testing with human, monoclonal and lectin anti-H. However, an eluate from her RBCs following adsorption contained anti-H that was reactive with papain-treated RBCs only. Analysis of *FUT1* in the proband revealed homozygosity for a mutation 661C>T, predicted to change Arg221Cys. No mutations were identified in her *FUT2* sequence. *FUT1* from the cord sample demonstrated heterozygosity: 661C/T, and this sample was also heterozygous for a common silent mutation in *FUT2*, 357C>T. The *ABO* genotype of both proband and cord sample was O^1O^1. **Conclusions**: We report the identification of a para-Bombay phenotype in an Australian woman that is due to a novel mutation in *FUT1* (661C>T). Weak H antigen was detected on her RBCs. This might be due to low activity levels of a fucosyltransferase encoded by the mutated *FUT1*, but it is more likely due to adsorbed soluble H antigen produced by the normal fucosyltransferase encoded by *FUT2*.

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Alloimmunisation in the Setting of Apheresis Single Donor Platelets. A Review of Haematology Patients Undergoing Chemotherapy or Bone Marrow Transplantation

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Introduction: Foreign antigen exposure may result in alloimmunisation leading to immune platelet transfusion refractoriness with requirements for specialised platelet products.
Aims: Study aims were to assess the alloimmunisation rate in haematology bone marrow transplant (BMT) and chemotherapy patients, the impact of alloimmunisation on transfusion requirements and address the value of continued routine platelet antibody (PAI) screening in the setting of leucodepleted apheresis-collected single donor platelets (PSD).
Method: Routine PAI screens performed over 33-months on BMT and chemotherapy patients for pre-emptive detection of alloimmunisation were reviewed. Patients were classified using clinical and laboratory information.
Results: The audit identified 283 patients; 148 BMT patients (autologous 71, allogeneic 77) and 135 chemotherapy patients. 43 patients had positive PAI tests at any stage; 21 BMT and 22 chemotherapy patients. Alloantibodies were detected in 40 patients (alloimmunised 22 patients, transient 18 patients) and autoantibodies in 3. Alloantibody specificity was anti-HLA (20 patients) with anti-HPA/platelet glycoprotein detected in 2. Three times as many female patients were alloimmunised. Alloimmunisation was detected earlier in females, usually on first PAI screen and before transfusion. The overall alloimmunisation rates were 8.8% in BMT patients (allogeneic 13.0%, autologous 4.2%) and 6.7% in chemotherapy patients. Risk factors for secondary alloimmunisation were present in 86.4%; 13 of 17 alloimmunised women had children, 2 of 4 nulliparous women and 4 of 5 male patients had extensive transfusion histories. Alloimmunised patients required specialised platelet products including crossmatched, HLA, directed donations or frozen-thawed PSD. Four BMT patients lost evidence alloimmunisation post-engraftment allowing return to standard PSD.
Conclusion: Alloimmunisation continues to occur despite the use of leucodepleted transfusion products. Evidence for secondary alloimmunisation was present in the great majority of patients. Early detection of alloimmunisation assists transfusion decision-making.

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Incidence of Red Cell Alloantibodies in Two Multi-transfused Populations

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Alloimmunisation to red cell antigens is a recognised risk of transfusion and may complicate provision of blood products to multi-transfused patients. We hypothesised that patients with haematological malignancies, either as a result of chemotherapy and/or underlying disease process, may have impaired immune responses and therefore a lower rate of alloimmunisation. To address this hypothesis we performed a retrospective analysis comparing red cell alloantibody formation in two cohorts of multi-transfused patients. The first cohort consisted of 424 patients with haematological malignancies (HM) admitted between July 2003 and June 2005; the second included 116 patients with major burns (MB) admitted between April 2002 and April 2005. Both cohorts had similar demographics: 44% female, median age 55 (17-93) for HM; 36% female, median age 42 (16-85) for MB. Alloantibodies developed in a lower proportion of HM patients compared with MB patients; 16/424 (3.7%) versus 9/114 (7.9%), however this did not reach statistical significance ($p \leq 0.1$). Overall, the median number of red cell units received was greater for HM than MB; 16 (1-185) versus 5 (1-109). Preliminary results suggest the rate of alloimmunisation per red cell unit transfused to be less for HM than MB; 0.19% versus 0.46%. Rhesus antigens (E, D, C, Cw c, e) were the commonest specificity in both groups with Kell being the second commonest specificity in the MB group. Kell alloantibodies rarely developed in HM patients due to widespread use of Kell negative units in this cohort. Universal usage of Rh D and E compatible blood may have reduced the rate of alloimmunisation by 40% in patients with HM. This policy with the addition of Kell negative blood in MB patients would have reduced alloimmunisation to a similar degree. These data support the practise of Rh phenotyping of all patients where recurrent transfusion is anticipated.

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Laboratory Investigation of Drug Induced Thrombocytopenia

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Introduction: Drug induced thrombocytopenia (DITP) is commonly caused by antibody specific destruction of platelets. Evidence of causation is provided by detection of drug-specific platelet reactive antibodies.
Aims: Drug specific platelet antibody detection (DRG), (excluding heparin) at RPH was reviewed to examine the range of drugs tested, methods used and detection rate. The adequacy of current investigation protocols was also examined.
Method: DRG investigations performed over 5 years were reviewed (hospital & referred tests). Testing involved a solid phase red cell adherence method (SPRCA) in presence and absence of drug plus flow cytometric assessment of platelet surface immunoglobulin. For patients with indeterminate results suggesting presence of circulating drug, testing was repeated once the drug had cleared the circulation.
Results: For 67 suspected DITP episodes, 77 drugs were tested on 66 patients (36 quinine, 11 glycoprotein IIb/IIIa inhibitors, 20 antibiotics, 10 others). Initial DRG test results were 33 negative, 9 indeterminate and 25 positive. Eight patients with indeterminate results had strong evidence of DITP on repeat testing (6 SPRCA, 1 MAIPA, 1 clinical). Positive DRG tests included: quinine(15), quinidine(1), abciximab(10), tirofiban(1), beta-lactams(3), phenytoin(2), thiazide(1). Only 5 patients with negative DRG results had repeat testing (0/5 were positive). 5 patients with negative DRG results had strong clinical evidence of DITP. Drugs tested in these cases were quinine, rifampicin, ceftazidime, cephazolin, urokinase, timentin, cephazolin.
Conclusion: Positive DRG testing provides strong laboratory evidence of DITP. DRG testing should be performed when DITP is first suspected, as timing of testing is critical. Patients with negative or indeterminate DRG results should be reassessed within 24-48 hours. The laboratory investigation protocol has been amended to improve drug detection in suspected DITP by adapting current methodology to better suit the proposed drug mechanism of action.

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The Challenges of Providing Safe Blood in Africa

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Africa is a large and varied continent with some 46 different countries at varying levels of development. As of 2002, only 30% of the countries had drawn up their transfusion policies and, of these, not all were in place. Only a minority of systems has a fully voluntary donor system and fully 60% of blood is collected from family or replacement donors. In most parts of the continent, there is a critical shortage of blood, amounting to 70% of estimated need, exacerbated by shortages of qualified staff. The majority of blood is used to treat anemia of parasitism and maternal bleeding. Blood is most often collected in the hospital environment. In many countries, the cold-chain is fragile or non-existent. Financial issues create additional difficulties, particularly as equipment, supplies and test kits have to be imported. Testing may be incomplete, despite the very high prevalence rates for transfusion transmissible infections. In sub-Saharan countries, the prevalence of HIV infection may reach 20% and there may also be very high frequencies of infection with HBV. HCV prevalence rates vary, but are generally lower. Consequently, testing loss is very high, further affecting the blood supply. Additionally, much of the continent is highly endemic for malaria. In some locations, creative approaches have been developed for donor management (such as the "Club 25" concept) and these have been effective in reducing the prevalence of HIV among the donor population. Additionally, significant efforts are being made to introduce and maintain quality systems throughout the continent. There is an increasing trend towards provision of financial and technical support from developed nations and the US PEPFAR program will be outlined. The major difficulty with such external support is establishing sustainable outcomes.

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Bloody Obstetrics: A Developing Country Perspective

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Maternal mortality: ~ 600,000 women die worldwide each year as a direct result of a pregnancy or childbirth complication. Most are preventable using cheap and simple measures. For every woman who dies, there are another 100 who are permanently disabled from the complications they survive.
Where does Obstetric Haemorrhage fit in with respect to Maternal Morbidity and Mortality?
Aetiologies

- Miscarriage and ectopic
- Unsafe abortion
- Antepartum haemorrhage
- Intrapartum haemorrhage
- Postpartum haemorrhage

Aided and abetted by high levels of intercurrent diseases and high prevalence of anaemias, eg, malaria, nutritional anaemias, chronic diseases eg TB and HIV

Aided and abetted by:

- Corruption: funding and supply lines
- Social Determinants of Health eg Low education levels in the community, Low status of women, transport issues, cultural taboos
- Lack of resources: appropriately trained health staff, equipment, communication infrastructure, IT, appropriately banked and tested blood, medications, infrastructure, Standard Treatment Guidelines, agreed an measurable benchmarks, audit, monitoring and evaluation

Some examples:

The way forward, at least for obstetric haemorrhage…….

- Addressing the Social Determinants
- Health Promotion
- Blood banking and distribution
- Service planning, funding, audit and M&E
- Partnerships

Poverty Reduction and The Millennium Development Goals

- Poverty reduction strategies
- The debt crisis
- Global trade policies
- Science for Development
- Effective international development assistance

Myths and magic bullets

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China – The New Blood Service

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Before 1978, most clinical blood in China were sourced from paid donations. As an effort to eliminate paid blood donations, from 1978 to 1998, the provincial governments coordinated annual blood donation plan and assigned blood donation quota to all enterprises and organizations for them to fulfill. It was the prototype of non-remunerated blood donation in China. However, it was not voluntary as individuals had to take turns to be selected for blood donation. In deed, there were many instances of enterprise paying money or offering incentives such as promotion or vacation to selected staff to donate blood, or one person paying another person a good sum of money to make a donation instead of oneself.

On 1 October 1998, China enacted a blood donation law which laid down the system of voluntary non-remunerated blood donation and stipulated the duty of healthy citizens to donate blood. Since then, voluntary non-remunerated blood donation has been rapidly increasing across the country. According to the statistics released by the Ministry of Health, China, non-remunerated donations had jumped from for 22% in 1998 to 91.3% of clinical blood in 2004, and voluntary non-remunerated from 5% to 71.5%.

% of clinical blood	1998	1999	2000	2001	2002	2003	2004
Voluntary non-remunerated	5	13.6	21.1	39.4	58.6	61	71.5
Directed non-remunerated	17	32.1	37.5	33.1	29.9	24	19.8
Paid	78	54.3	41.4	27.5	11.5	15	8.7

The development of blood programme has also stimulated the establishment of blood centres. In 1978, there were only 40 blood centres in China. In 2000, there were 441 blood centres. Except Tibet, every province in China has established a provincial blood centre in the capital city. The roles of provincial blood centres are to provide technical support and training to prefecture blood centre as well as co-ordinating local blood supply.

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Voluntary Non-Remunerated Blood Donation – Establishing Cultural Change in Developing Countries

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Aim: Voluntary, non-remunerated blood donation is the cornerstone of a safe blood supply. As part of the World Health Organisation's (WHO) Global Blood Safety Programme, a collaborative partnership has been established with the International Federation of Red Cross and Red Crescent Societies (the Federation) to effect cultural change in those developing countries where blood services are not currently based on voluntary blood donor programmes.
Method: WHO and the International Federation have jointly prepared a set of materials for training in the basic principles of education, motivation, recruitment and retention of voluntary blood donors. The *Developing a Blood Donor Programme* curriculum has been produced to provide the basis on which workshops can be conducted. These workshops are designed to be interactive and focus on practical issues and the challenges associated with the phasing out paid or family replacement donor systems.
Result: Since November 2004, regional and local workshops have been conducted in Singapore, the PR China, Nigeria, Vietnam and Egypt. Throughout the workshops, participants learn about the key elements of a successful blood donor programme, including identification of safe donor populations, audience-specific strategies for donor education, motivation and recruitment as well as pre-donation counselling, donor care and retention. They also undertake a Gap Analysis of their blood donor programmes. The ultimate outcome is the development of a Plan of Action for their local, regional or national Blood Service.
Progress on with the implementation of their plans is monitored regularly and ongoing support is provided by a regional contact, usually one of the workshop facilitators. Follow-up workshops after 12 months are planned to measure the results to date, in particular, improvements in the number of voluntary, non-remunerated blood donors.
Conclusion: *The Developing a Blood Donor Programme* is an important initiative in developing countries, ultimately contributing to the delivery of safe and adequate blood supplies, a situation which is largely taken for granted in the developed world.