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A001

Responding to the Challenge of New and Emerging Forms of Creutzfeldt-Jakob Disease and Bovine Spongiform Encephalopathy

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Creutzfeldt-Jakob Disease (CJD) is the major form of human transmissible spongiform encephalopathy. Underlying the transmissibility is a conversion of a normal cellular glycoprotein (the 'prion' protein PrPc) into an insoluble, relatively protease-resistant conformer (PrPCJD). Certain mutations in the PrP gene (PRNP) also cause disease. The apparent lack of any nucleic acid in this conversion process marks the disease as an unique genetic and infectious process. It also serves as an important paradigm for the other adult onset neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

Transmissibility between humans is often difficult to document because of long incubation periods and the lack of any immune response. Contamination of surgical instruments, tissue grafts from donors with CJD (eg. dura mater) and medicinal products contaminated with CJD infected tissue (eg. human pituitary derived hormones) have been implicated in about 200 cases of CJD worldwide. There is no epidemiologic evidence supporting the possibility of contamination by blood or blood products. However, experimental studies suggest that cellular fractions of blood may contain infectivity. With the emergence of a new variant form of CJD in the UK, which is linked to the occurrence of bovine spongiform encephalopathy, it is prudent to take extra precautions in ensuring that the risk of transmitting CJD by blood is minimised until such time as the nature and extent of the BSE/vCJD epidemic is fully appreciated.

A002

Living with vCJD – the UK Experience

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Background
The United Kingdom (UK) has been living with the unknown risk of variant Creutzfeldt-Jacob (vCJD) disease since 1986 when reports first began to emerge of Bovine Spongiform Encephalopathy (BSE). The origin of this disease may never be known with certainty but was spread by the recycling of ruminant protein to cattle. In 1996 vCJD, differing in a number of ways from sporadic CJD, was first described in humans. In the intervening years it became clear that the UK was facing a BSE epidemic and a number of stringent measures were introduced to halt this. It was in March 1996 that the Government announced that BSE was likely to have transmitted to people. Further stringent regulations were introduced, governing the preparation and sale of beef for human consumption.

Current situation
There is now compelling evidence that vCJD is caused by exposure of humans to the agent that causes BSE and up to 5 August 2002, 125 definite or probable cases of vCJD had been reported to the UK CJD Surveillance Unit. Follow up of previous blood donations from patients with vCJD and of blood donation recipients who later developed vCJD has not identified any matches. Donor deferral criteria to prevent the possible transmission of CJD have been in place for some years and since 1997, the UK Blood Services have imported plasma for fractionation from the US and have leucodepleted all blood components since 1999. There is still no scientific evidence that vCJD has ever been transmitted between humans via blood transfusion but there are reasons for considering that this could occur. A recent study showing BSE and natural scrapie transmission in blood transfusions between sheep confirms the risk of transmissible spongiform encepahalopathy via blood components suggesting that measures taken so far have been fully justified.

Preparing for the future
This background, with its strong emphasis on the precautionary principle has helped to shape the pro-active approach, which the NBS is taking with respect to blood safety issues. Another strong influence was the Burton judgement on Hepatitis C which, under consumer protection legislation, found that recipients had a legitimate right to expect that the product they received was free from any known infection. As a result, the NBS formed the Blood and Tissues Safety Assurance Group (BTSAG), chaired by the Chief Executive and comprising senior members of the NBS. Although initially focussing on vCJD issues, it was quickly realised that these could not be considered in isolation from other blood safety threats. The work of the BTSAG is supported by Department of Health analysts in order to reach a blood safety strategy which is able to prioritise

actions which could be taken against known and unknown risks whilst at the same time ensuring that an adequate blood supply is maintained.

A007

Evaluating the Evidence - Viral Screening

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Blood transfusion can never be totally free of the risk of transmitting infective agents. This is because we cannot routinely test for all agents potentially present, and because current tests for specific known agents cannot identify every infective blood unit with 100% reliability. However recent developments have extended our knowledge and continued to reduce the risk of clinically significant infection in recipients. For example, (i) three viruses identified since 1995 (human herpesvirus 8, GBV-C/hepatitis G virus, TT virus) have been carefully assessed for their possible relevance to blood transfusion (ii) the true natural history of hepatitis C virus infection has been defined with greater accuracy (iii) residual risks of transmission of major infections by screened blood have been critically assessed, and nucleic acid-based test strategies have been introduced to identify additional infective units (iv) further experience has been gained with procedures designed to inactivate or remove viruses.

Strategies to further reduce transmission risks come with exponentially increasing costs; it would appear desirable that, as with other expensive areas of modern medicine, mechanisms to quantitate the benefits should be applied to allow comparison of priorities between competing demands for health expenditure.

A009

Transfusion Associated Sepsis –UK SHOT Programme

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Background
SHOT is the UK's confidential, anonymised haemovigilance scheme for the reporting of serious adverse events of blood transfusion. It collects data on incorrect transfusions of blood and blood components (IBCT) and major immunological and infectious complications. Cumulative data are available about transfusion–transmitted infections (TTI) reported between October 1995 and September 2001. Approximately 3.5 million blood components are issued annually by the UK Blood Services of which approximately 250,000 are platelets. The UK Blood Services do not perform universal screening of platelets for bacterial contamination. Improved arm cleansing techniques and diversion of the first 20 ml of each donation are in the process of being introduced.

Methods
Suspected cases of TTI are reported to supplying blood centres to ensure rapid withdrawal of other implicated components and appropriate investigation and donor follow-up. Cases are then reported onwards to the National Blood Authority/Public Health Laboratory Service Communicable Disease Surveillance Centre (NBA/PHLS CDSC). In Scotland the Scottish National Microbiological Reference Unit collates reports. Details of all cases reported in the UK are presented in the annual SHOT reports.

Results
Of 21 cases of bacterial contamination over the 6 years from October 1995 – September 2001, 17 were due to platelets and 4 to red cells resulting in 6 deaths, 5 in recipients of platelets. In all except one, implicated platelets were at least 3 days old. In 8/21 cases the donor's arm was positively identified as the source. In some other cases investigation was incomplete or inconclusive but the nature of the organism was suggestive of a skin contaminant that was most likely to have been introduced to the pack at the time of collection. Additional data from the National Blood Service (NBS) derived mainly from time-expired platelets and more recently from limited prospective screening using BacTAlert™ has shown a bacterial contamination rate for platelets of 0.5%. Improved arm cleansing plus diversion of the first 20 ml of donation has been shown to reduce this by 70%.

Conclusion

Bacterial contamination, particularly of platelets, is a cause of transfusion-related mortality/morbidity in the UK. Two strategies to reduce the risk of contamination, improved arm cleansing and diversion are in the process of implementation. Further measures under active consideration are bacterial screening of platelets and pathogen inactivation.

A019

Elimination of Pathogens During Manufacturing of Plasma Derived Products

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The safety margin of plasma products achieved through the processing of screened human plasma has two main aspects. Firstly, the manufacturing process of a given plasma product will eliminate potentially present pathogens either through removal or inactivation. The second facet deals with cleaning to ensure that there is no cross-contamination between batches. Today, two types of pathogens are of concern, namely non-enveloped viruses, that inherently are much more stable than enveloped viruses, and the prions (causative agents of TSE (vCJD)). These pathogens are relatively resistant, to “classical” inactivation techniques such as solvent detergent treatment or pasteurisation when compared to the enveloped viruses.

We have investigated several steps in manufacturing processes for their potential to remove small non-enveloped viruses and/or the TSE agent. One technique in manufacturing dedicated to pathogen removal is that of nanofiltration. Nanofiltration has been shown to efficiently eliminate viruses larger than the nominal pore size of the nanofilter. We have also demonstrated efficient removal of small viruses (smaller than the nominal pore size of the filter) can be achieved when present as virus antibody-complexes. Nanofiltration also has the potential to eliminate prions (PrP^{res}) as has been shown using a Scrapie model suggesting that this manufacturing step reduces risk of transmission of vCJD should this agent be present in plasma.

Several inactivation methods have been investigated for both manufacturing processes and cleaning procedures. For manufacturing processes two irradiation techniques and pasteurisation in the presence of a chaotropic agent have been assessed. All methods showed excellent potential to inactivate non-enveloped viruses. However, these promising new techniques require detailed investigations on the protein of interest in order to minimize damage while maximising virus kill. Inactivation capacity of cleaning solutions and conditions were investigated utilizing minute virus of mice, a member of the family *parvoviridae*. This virus provides a good model for “worst case” scenarios as it shows high resistance to wet heat treatment and alkaline conditions. Studies examining the effect of NaOH on PrP^{res} showed that this agent is destroyed by 0.1M NaOH at room temperature as detected by a sensitive Western blot assay.

In conclusion, several techniques are now available to assess inactivation and removal of viruses and prions during manufacturing. These techniques help to ensure the safety of plasma products. Appropriate cleaning regimes also guarantee proper batch to batch segregation.

A020

Pathogen Inactivation of Red Cells: The INACTINE™ Process for the Preparation of Pathogen Reduced Red Cell Concentrates

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The development of a safe, practical and effective pathogen reduction technology for the preparation of red blood cell concentrates (RBCC) would represent an important advancement in transfusion medicine. The ideal approach for achieving the safest red blood cell concentrates should address pathogen, prion and immunological risk factors associated with transfusion. This objective can be best achieved by combining a broad spectrum microbial inactivation treatment with a red cell purification process. The presentation will discuss the INACTINE compound PEN110 which has been demonstrated to have an extremely broad range of anti-microbial activity including inactivation of a large panel of enveloped and non-enveloped viruses, bacteria, parasites and lymphocytes in the presence of red cells. INACTINE™ PEN110 belongs to a family of ethyleneimine oligomers which are all low molecular weight, non-aromatic, anti-microbial molecules with the ability to disrupt nucleic acid replication by modification of nucleic acid bases with preferential reactivity to guanosine. As a result of the base modification, polymerase enzymes cannot fully transcribe the genome resulting in inactivation of the pathogenic agent (virus, bacteria, parasite or leukocytes) potentially present in blood. INACTINE™ PEN110 is a highly water-soluble cation and capable of readily diffusing through cell membranes. The ability of the compound to uniquely inactivate a broad spectrum

of non-enveloped viruses is attributed to PEN110 being sufficiently stable in blood to allow time for penetration to occur and second, the compound has a sufficiently small molecular radius that facilitates penetrate into the viral capsid protein shell to access the viral nucleic acid target. Compounds which have a short half life in blood or that are bulky in size due to heterocylic structure have not been demonstrated to achieve a broad spectrum non-enveloped viral inactivation effect. In addition, the red cell purification process achieved using a closed, automated cell washing system will be discussed which in addition to removing PEN110 from the blood has been shown to have high efficiency removal of cytokines and immunoglobulins which can elicit adverse immunological transfusion reactions, and prion proteins which, in their pathogenic form, are associated with transmissible spongiform encephalopathy diseases. The advantage of a combined approach of microbial inactivation and red cell purification will be discussed as a process that provides a greater global safety to the red cell concentrate that can be achieved by either technology alone.

A028

Immunohematology in Taiwan

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Since 1982, extensive study on distribution of blood groups antigens and antibodies was performed at the Mackay Memorial Hospital (MMH). Marked differences have been observed between Taiwanese and Caucasians. Accordingly the standard Western pretransfusion testing procedures were simplified in Taiwan as follows:

The frequency of D- (RhD negative) individuals in the Taiwan population is only 0.33% and since one third of these also are D_{el} phenotype. The incidence of anti-D is extremely low (ie one in 295,000 routine blood donor), and no anti-D was detected in 95 D- pregnant women (94 had D+ babies) from 38,136 cases of antenatal screening performed at MMH (1984 – 1988). Finally only one in 87,040 babies born in MMH suffered from HDN due to anti-D (1982 – 1993). As a result, the Department of Health, Taiwan decided that RhD typing would be only optional in pretransfusion testing. MMH has now discontinued routine D typing for all Taiwanese patients since 1988 except for Caucasians.

It is known that for the detection of some examples of anti-K, the manual Polybrene method (MP) requires a supplementary antiglobulin phase. However, the frequency of K in Taiwanese was found to be 0%. We have never previously encountered any anti-K except a unique anti-Ku found in a patient with K_{null} phenotype. This particular account is the only alloantibody ever found against Kell blood group system during past 20 years in Taiwan. MP is known to be very sensitive for Rh antibodies, and in Taiwan anti-E, -c account for 48% of all clinically significant alloantibodies. In addition, anti-"Mi^a" is also easily detected by this method. Therefore MP without supplementary antiglobulin phase has become the routine method for antibody screening / cross-matching throughout Taiwan, resulting in an extremely rapid (about 3 minutes), cost effective (reagents prepared in house), sensitive and easy procedure.

Anti-"Mi^a" with the frequency of 0.8% among patients, is the most common clinically significant alloantibody as it causes intravascular hemolytic transfusion reaction and hemolytic disease of the newborn. The overall frequency of MiIII phenotype was found to be 7.3%. This phenotype is most common in three indigenous tribes in the east coast of Taiwan (Ami 88%, Yami 34%, and Puyuma 21%). It has been decided that for all antibody screening, a cell with the "MiIII" phenotype (these cells react with anti-"Mi^a") should be included in routine pretransfusion testing. A_2 and other subgroups of A were found to be rare in Taiwanese, for this reason routine testing of patients' serum with A_2 red cell and testing patients red cells with anti-A,B were stopped.

A029

Blood Group Phenotypes in Ethnic Groups: The Impact of Molecular Techniques on Routine Blood Group Serology

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In a number of ethnic groups in Asia and Oceania, some blood group phenotypes that are rare in Caucasians are relatively common. These blood group antigens can be the basis of incompatibility, resulting in transfusion reactions or haemolytic disease of the newborn. Antisera for typing of these polymorphisms are often difficult to obtain. The molecular basis of the polymorphism has been described for many of these blood groups, and nucleic acid amplification techniques can be used to genotype (and predict the phenotype) when antisera are not available. In East Asian peoples phenotypes initially collectively

known as Mi(a), resulting from gene conversion between GpA and GpB genes, are common. In this report, three examples of the application of molecular techniques to the investigation of Mi(a)-related problems are described. Use of ELISA as an alternative for detection of antibodies to red cell antigens provides the first example. Peptides representing the MNS7, MNS10, MNS26 and MNS20/34 antigens were captured on ELISA plates and 150 sera from Taiwanese people were screened. When undiluted, all 150 sera agglutinated Mi(a) positive cells. At a 1:20 dilution, 43% of the sera reacted with at least one of the peptides by ELISA. In a second example, polyclonal antibodies of a defined specificity were recovered using affinity chromatography. An agarose gel displaying a peptide representing the MNS26 (Hop) antigen was prepared. Antibodies to this antigen bound to, and were recovered from, the gel. This technique may be useful for the production of monospecific polyclonal typing sera. In the third example, three Individuals serologically defined as Mi(a) positive were typed by PCR with a GpA and a GpB specific primer followed by *Msp*I digestion. *Msp*I cleaves only PCR products amplified from a gene with a reactivated exon 3/4 splice site. For these three serologically Mi(a) positive individuals, a PCR product was amplified and cleaved by *Msp*I, a result consistent with a GpB-A-B gene conversion and the observed phenotype. While there are limitations in applicability of these techniques, in the absence of specific antisera or cells of defined phenotype, molecular techniques can be applied to resolve complications in immunohaematological investigations that may be caused by blood group antigens characteristically found in at higher frequency in specific ethnic groups.

A030

Blood Groups in Our Region – What is their Clinical Significance?

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There is a mixture of ethnic groups resident in Australia and New Zealand and the blood group genotype frequencies of each group can vary significantly. These variations can cause problems in antibody screening and identification testing, as well as in the supply of compatible blood. In order to fulfil their designated role, antibody screening panel cells should represent the blood group antigens found in the local population, preferably in homozygous state. If they fail to do so, then antibodies in the patient's serum may not be detected in pre-transfusion screening. Equally, the screening cells do not need to carry antigens which are absent, or very rare, within the relevant population.

In a number of Asian races, there is a higher incidence of some antigens that are only present at very low frequency in Caucasians. As a result, there is an increased likelihood of antibodies to such antigens being present in the patient population. In addition, these antibodies may be immune IgG forms, rather than the IgM forms that are generally found in Caucasians. Now that it is common practice to ABO homologous blood (based on a rapid saline crossmatch) to patients with a negative antibody screen, the lack of representation of these "Asiatic" antigens on the screen cells is capable of causing an incompatible transfusion and subsequent transfusion reaction. It may also result in an unrecognised haemolytic disease of the newborn.

Conversely, some of Australia's neighbouring countries have populations in which there is an increased frequency of some "double negative" or "null" phenotypes. If persons of such phenotypes are transfused, they can develop antibodies that will react with almost all Caucasian blood types. The rapid recognition and identification of such "null" phenotypes and antibodies to high frequency blood phenotypes can prevent delayed transfusion and save the patient much distress. In addition it relieves the blood bank scientist of a great deal of stress! Compatible blood for such patients can often be more easily obtained by selecting donors from the same ethnic group, but in these days of privacy laws, such information may not be available on the donor records.

There are well-documented cases of antibodies to both low and high frequency antigens in Asian, Melanesian and Polynesian races that have caused transfusion reactions and haemolytic disease of the newborn.

A037

Tracking Donor Eligibility in the UK

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The selection of blood donors against a set of medical-history and lifestyle criteria provides the first line of defence against infection entering the blood and product supply chain. It also limits supply by reducing the potential and actual donor pool.

Selection criteria have become progressively tighter in the past three decades; this has led to a safer blood supply and a declining base of active donors who supply an increased volume of blood.

In England and north Wales some 29 million people are eligible by age to give blood of whom 6% are (at any time) donors. However survey and population modelling suggest that 60% of people will, at some point in their life, be a donor. Those who never donate form two groups:

- Those who present but are unable to donate.
- Those who never present.

Eligibility amongst new recruits is approximately 95%. This has decreased from 96% over three years. Medical history represents the major cause of permanent ineligibility. However permanent ineligibility amongst new recruits is relatively rare reflecting the young and healthy target market, and effective pre-screening. Much more common, at around 30% incidence in the first 2 years, is temporary ineligibility caused by skin piercing, travel and low Hb level. Temporary suspension of first-time donors has a profoundly negative effect on their propensity to return resulting in a very low conversion rate of recruits who are rejected at first attendance. A more creative and sensitive approach to handling the screening of new recruits is required.

A particular challenge is posed by vCJD. Many blood services have excluded donors who have travelled to Britain and Europe, but this is not an option in the UK. Whilst further understanding about the transmissibility and epidemiology of vCJD awaits scientific development, preventative measures are under consideration. Key amongst these is the possible exclusion of all donors who have themselves received a transfusion in the past. There is evidence to show that this would reduce collections in the short-term by between 8% and 16%. The impact on supply to hospitals would be severe with lasting shortages across all product types.

In summary the balance between blood safety and sufficiency is finely balanced in the UK. While the pressure to tighten eligibility criteria in the light of better understanding of infection pathways is constant there is evidence that the impact on supply sufficiency is becoming severe.

A039

Strategies to Predict Platelet Demand and Reduce Wastage

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Platelet concentrates are frequently in short supply because of their 5-day expiry. To maximise the availability of platelet concentrates and minimise wastage, we developed a system to estimate the platelet requirements for the five haematology units around metropolitan Adelaide.

Hospital haematologists, registrars and transfusion scientists e-mailed or faxed an estimate of their platelet requirements for the following week to ARCBS-SA. A standard template was designed to record the number of platelet concentrates required by each haematology patient by day of the week. It also recorded their ABO blood group and any special requirements, such as CMV negative or irradiated product. These details were used for production planning for the following week.

This system has established a pathway of communication between ARCBS and the hospitals about platelet needs. This allows pro-active and timely changes in production. Predicting the ratio of group O to group A platelets needed and collecting group B platelets when required, can help reduce the transfusion of ABO non-identical platelets. The use of ABO identical platelets has a number of advantages that are particularly relevant for haematology patients.

Patients needing frequent and/or multiple doses of platelet concentrates can readily be identified and a formal assessment of refractoriness instituted. Planning for patients who require intensive platelet support for surgery or ongoing bleeding has been valuable. Platelets needed for haematology outpatients or surgery on Monday mornings can also be pre-empted. The other situation where it is useful to estimate platelet requirements is prior to long weekends.

A further initiative has been to provide a spreadsheet detailing the number, ABO group and CMV status of platelet concentrates available at each hospital and at the blood service at the end of each day. This is e-mailed to the ARCBS medical officer on call to facilitate the transfer of stock when platelet concentrates are in short supply.

Wastage of platelet concentrates has declined since the introduction of the system. Adelaide is of a size that makes these initiatives workable. Good communication between the hospitals and the blood service is central to their success.

A040

Immediate Spin Compatibility Testing – Is It As Reliable As We Think?

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Immediate spin (IS) compatibility testing is widely supported as a means of detecting ABO incompatibility prior to release of blood in abbreviated crossmatch protocols. Many laboratories have ceased to use washed red cells in this protocol. The possibility of soluble blood group antigen neutralising weak ABO antibody is a theoretical possibility under these circumstances and the present protocol was designed to test whether it is necessary to wash cells when this technique is applied.

Aim
The aim of the present study was twofold:
1. To establish the ability of immediate spin techniques to detect ABO mismatch
2. To clarify the necessity for a red cell wash step in the application of the procedure

Method
Random routine serum samples received by the transfusion laboratory that had been tested and subsequently frozen for 7 days were thawed for testing. Serum blood group was unknown at the time of testing.

3-5% red cell concentrations were made in buffered saline from non group “O” red cells that had been received from the Australian Red Cross Blood Service. The blood group of the unit was not identified at the time of testing.

A standardised immediate spin technique was applied. The ABO records for the patient sample and the donor unit were then retrieved and any discrepant results were repeated. If the discrepancy remained, the donor red cells were washed 3 times in buffered saline and the test repeated

Laboratory Findings
A total of 7708 immediate spin tests were performed on 543 random patient serums using 202 individual non group “O” donor units. 139 (1.8%) tests involving the serum of 23 patients gave discrepant results. 105 discrepant results were resolved by washing donor red cells leaving 34 (0.44%) unresolved tests. Bone marrow transplant accounted for 13 of these, leaving 21 (0.27%) tests that did not detect ABO incompatibility.

Conclusion
The results suggest that washing red cells will improve the opportunity to detect ABO incompatibility. Immediate spin tests are not totally reliable as a sole means of detecting ABO incompatibility.
1. 75.5% of the missed incompatibility was resolved by the washing of red cells prior to the test.
2. Washing red cells prior to immediate spin test reduced the non-detection rate of ABO incompatibility from 1.8% to 0.44%.
3. Exclusion of minor mismatch BMT where ABO incompatibility will not produce clinical sequale reduced the non-detection rate to 0.27%.

A041

Automated Interfaced Pretransfusion Testing and Electronic Release

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In recent years several major changes in transfusion laboratory practice have occurred. There has been a growing need for cost efficient laboratory procedures with considerable rationalisation and abbreviation of pretransfusion testing.

Automated interfaced pretransfusion testing and electronic release of red cells contributes to less labour intensive testing procedures, increased safety, cost savings and improved efficiency. Automation leads to increased standardisation, faster

specimen processing and reporting, elimination of manual specimen identification, uniform interpretation of serological reaction patterns and objective reading of haemagglutination endpoints.

Electronic release or computer crossmatching, when coupled with fully automated pretransfusion testing offers further potential for increased productivity and more efficient use of red cells. In more than 90% of cases of pretransfusion testing the antibody screen is negative and the need for a serological crossmatch has been questioned. Electronic verification of ABO incompatibility can be used as the crossmatching procedure for patients without clinically significant antibodies. The computer crossmatch or electronic verification provides several advantages over traditional crossmatch techniques. The speed at which computer crossmatched blood can be made available decreases the need to have crossmatched blood available when only a group and antibody screen may be more appropriate.

Fully automated interfaced pretransfusion testing when coupled with electronic release of red cells compliment each other's benefits. We have been able to enhance patient care by eliminating unnecessary delays in specimen processing and provision of compatible blood following introduction of such a system. 90-95% of our routine pretransfusion testing has now been fully automated and more than 95% of red cells issued are computer crossmatched. Operationally workflow has been streamlined by more efficient work practices along with improved accuracy and reliability of test results. Specimen processing and reporting times have fallen by 30 to 40% and laboratory management has benefited through increased productivity and decreased outdating of red cells.

A042

Lessons from the UK SHOT Programme

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Background and aims
SHOT is a confidential, anonymised scheme for the reporting of serious adverse events of blood transfusion. Launched in November 1996 SHOT data can be used to inform policy in the transfusion services, improve standards of transfusion practice, aid production of clinical guidelines for the use of blood components and educate users on transfusion hazards and their prevention. Approximately 3.5 million blood components are issued annually by the UK Blood Services.

Methods
SHOT invites reports of major adverse events complicating blood transfusion in the UK. It collects data on incorrect transfusions of blood and blood components (IBCT) and major immunological and infectious complications. Recently data collection has been widened to include "near miss" events.

Results
With 413 hospitals eligible by 2001, participation was running at 92%. Cumulative data for the period 1 Oct 1996- 31 Sept 2001, from 1148 fully analysed questionnaires, has shown that 699 (60.9%) were IBCT incidents, comprising the largest single category. 161 were ABO incompatible transfusions leading to 9 deaths and together with Rh and other red cell incompatibility, 58 cases of major morbidity. Other errors included failure to meet special requirements e.g. for irradiated components. Incidents resulted from multiple procedural failures in 49% of cases. 55% of errors arose at the point of collection (from hospital storage site)/administration, 28% in the hospital blood bank and 13% at the point of prescription, sampling or request. Approximately 36% of hazards were immune complications. The outcome in 70 fully analysed cases of TRALI included 18 deaths and 49 cases of major morbidity, making this the 2nd most common cause of transfusion-related morbidity/mortality after ABO incompatibility. Infectious complications comprised 2.8% of cases (32/1148) of which 21 were due to bacterial contamination resulting in 6 deaths, 5 in recipients of platelets. 52% of 812 "near miss" events over 3 years involved bedside sampling errors.

Conclusions
SHOT's 5 annual reports together offer a comprehensive picture of transfusion complications in the UK and powerful data from which to make firm recommendations for improvements in transfusion safety. Guidelines for the administration and management of transfusions followed the first annual report but there remains an urgent need to tackle the problem of failure of the bedside checking process using computerised methods such as hand-held scanners. Greater emphasis on training and education and the appointment of hospital transfusion practitioners will contribute to improved transfusion practice. Strategies for the reduction of bacterial contamination and TRALI must be considered. An overarching organisation/ framework is needed to assess and prioritise competing blood safety initiatives.

B087

Implementing Haemovigilance in a Major Teaching Hospital – Overcoming the Obstacles!

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There is increasing awareness of the importance of haemovigilance as an integral component of quality transfusion practice. We are creating a structure on which to implement and further develop this in our hospital, and report some of our early experiences.

We identified the following limitations:
- Knowledge: lack of understanding of clinical transfusion best practice among medical and nursing staff: e.g. decision-making/indications for transfusion (especially in relation to published guidelines) and management of transfusion reactions.
- Context: limited appreciation of the significance of 'near-miss' episodes (e.g. blood product request form and specimen labelling errors), and the importance of attention to clerical detail at all stages of the transfusion process, and how this relates to safety.
- Communication: often poor between clinical staff and blood bank.
- Information technology systems of hospital pathology and clinical services: especially difficulties with data extraction and reporting/collection.
- Human resources: very time-consuming to investigate/follow up clerical errors and clinical reactions and collect data.
- Framework and integration: prior absence of structured hospital-based model for haemovigilance and lack of integration of the reporting system into the hospital quality management system. Errors were previously managed on a case-by-case basis, without systemic corrective action or a vertical reporting structure.

Actions proposed/underway include:
- Strengthen the role of the hospital Blood Transfusion Committee.
- Improve framework for clinical liaison: further definition of role of transfusion specialist, nurse and fellow; develop improved clinical unit contacts e.g. trauma nurse.
- Improved education/dissemination of information e.g. development of a transfusion newsletter, hospital email and transfusion intranet site.
- Develop/introduce structured hospital-wide reporting to clinical divisions and hospital management, including clinical risk review committees.

Conclusion
These programs are designed to improve patient care and manage risk, and require time, authority and appropriate resources (personnel, organizational and IT support). Integration of haemovigilance into the hospital quality system and its endorsement by hospital senior management is essential.

B012

Immunomodulatory Effects of Factor IX Concentrates *In Vitro*

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Clinical and subclinical immunological abnormalities have been reported in HIV-seronegative haemophilia patients. The mechanisms by which these abnormalities arise remain unclear.

Cytokines are important biological response modifiers. As we have recently shown plasma-derived factor VIII concentrates to modulate leucocyte cytokine production, the effect of FIX concentrates (MonoFIX-VF, AlphaNine SD and Prothrombinex HT) on production of a range of cytokines by leucocytes was investigated.

Whole blood cultures were activated with various stimuli and cytokine production by T-cells and monocytes was investigated using multiparameter flow cytometry.

In cell cultures exposed to FIX, T-cells showed reduced production of TNF-α, IL-2 and IFN-γ but there was no alteration for IL-4; monocytes showed reduced production of TNF-α, IL-1α, IL-1β, IL-6, IL-8 and IL-12 but an increase in IL-10 synthesis. All changes in cytokine synthesis were shown to be directly proportional to changes in pdFIX concentration.

Upregulation of CD69 (Activator Inducer Molecule) and CD25 (IL-2Rα) was also reduced in a dose dependent manner in cultures exposed to FIX concentrate. MonoFIX was the least immunomodulatory FIX concentrate, followed by Alpha Nine, then Prothrombinex HT.

The decrease in production of the Th1 cytokines, together with the increase in production of Th2 cytokine IL-10, may be one basis of altered immunoregulation resulting in increase in rates of certain types of infections reported in these patients, which require Th1 cytokine production for an effective response.

B061

Cellular Fraction of Red Blood Cell Concentrates has Minimal Neutrophil Priming Activity

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Adverse reactions in recipients of fresh blood products are a recognised risk of blood transfusion. Using *in vitro* functional assays, we have previously shown that immunomodulatory factors capable of stimulating homologous neutrophils accumulate in the supernatant of red blood cell concentrates (RBC) during storage. These effects were virtually eliminated by pre-storage reduction of leucocytes. The aim of this study was to determine whether the cellular fraction was capable of activating homologous neutrophils. Three types of RBC were investigated: 1) routine "hard spun" RBC (Group 1); 2) buffy coat-depleted RBC (Group 2); and 3) leucocyte-filtered RBC (Group 3). RBC cellular fractions were assessed for the level of IL-8 and ability to induce increased expression of CD11b.

RBC were prepared, according to standard procedures, from whole blood (WB) collected into packs containing CPD anti-coagulant and Adsol solution (Baxter). Group 2 RBC were collected into Optipacs and processed by the Optipress (Baxter). Group 3 RBC were leucocyte filtered by a 4-log removal filter (BPF4, Pall). All RBC were stored at 2-8°C for 42 days. Samples, collected aseptically on day 1 and each week thereafter, were centrifuged, supernatant removed and cellular fraction stored at -70°C. IL-8 levels were determined by a sandwich ELISA (R&D Systems). Induction of CD11b expression on neutrophils was determined by incubating fresh WB with RBC cellular fractions, or fMLP as control, followed by staining with FITC-labelled anti-CD11b and flow cytometric analysis.

Level of IL-8 in the cellular fraction of leucocyte-depleted RBCs (Group 2 and 3; n = 6 and 5, respectively) was negligible throughout 42-day storage. These data are consistent with those obtained for supernatant from these RBC, which similarly showed no detectable IL-8. In contrast, moderate levels of IL-8 (mean 141 ± 53.4 pg/ml; n = 7) were detected in the cellular fraction of Group 1 RBC at early time-points and gradually declined during storage. Compared to IL-8 levels in supernatant of Group 1 RBC, which showed a progressive accumulation during storage, an inverse relationship exists for the IL-8 levels in the cellular fraction of these RBC. Cellular fractions from Group 2 and 3 RBC induced minimal (5%) increase in the expression of CD11b, whereas Group 1 RBC induced significantly higher (30%) expression of CD11b.

These results demonstrate that the cellular fraction of RBC is not itself capable of inducing significant activation of homologous neutrophils. However, leucocytes present in RBC products, such as the Group 1 RBC, appear to be the primary source of immunomodulatory factors capable of inducing neutrophil activation as evidenced by detectable levels of IL-8 in RBC cellular fractions and the ability to cause increased CD11b expression. These effects were virtually eliminated by pre-storage reduction of leucocytes and hence provide evidence for a benefit of leucocyte removal from RBC.

B026

The Effect on Iron Stores by Polymorphisms in *HFE, TfR* and *DMT1* in Blood Donors

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The incidence of iron deficiency rises with increasing donation frequency. This effect is most marked in pre-menopausal female donors. It is hypothesised that the presence of polymorphisms in genes involved in iron regulation may affect iron status in these donors. To address this, polymorphisms in iron transport genes, *HFE,* transferrin receptor (*TfR)* and divalent metal transporter 1(*DMT1*) were examined in relation to iron deficiency in a cohort of female blood donors.

206 female blood donors under 50 years of age were recruited to the study with 5 donors excluded after failing to meet all acceptance criteria. Donor records were accessed to obtain previous donation behaviour. *DMT1* polymorphisms G185R, C1303A, 1254T/C and IVS 7 +12 G/A and *TfR* S142G were directly sequenced. Serum ferritin levels were measured and *HFE* polymorphisms C282Y and H63D were genotyped using polymerase chain reaction-sequence specific primer (PCR-SSP).

Iron deficiency was detected in 32% of women (defined as ferritin <12ng/ml), with the incidence increasing as donation number increased. Improved iron status was not associated with carriage of a single *HFE* mutation. *DMT1* 1254C was associated with reduced incidence of iron deficiency when donation was limited to two per year (0.05 v 0.28, p=0.017). The G185R mutation was not observed. Two donors were heterozygous for the C1303A mutation. *DMT1* IVS7+12 G/A was also not significantly associated with iron status. The *DMT1* polymorphisms analysed in this study were not associated with mutations in the HFE gene. *TfR* S142G was not associated with iron deficiency nor polymorphisms in *HFE*.

These polymorphisms in blood donors occur with similar frequency to previously published normal controls, as shown below.

Gene	Allele	Frequency
HFE	282C	0.91
	282Y	0.09
	63H	0.86
	63D	0.14
DMT1	1254T	0.82
	1254C	0.18
	IVS7+12 G	0.84
	IVS7+12 A	0.16
TfR	142S	0.58
	142G	0.42

Of the three genes studied only *DMT1* 1254C showed any association with iron deficiency. Because the 1254 T/C polymorphism is a silent mutation that occurs in exon 13, it may indicate linkage to another site. A trend towards a lower incidence of iron deficiency in donors with a single *HFE* mutation was also evident, however not significant.

B016

Reforms to the Blood Sector – A National Approach

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Australian Governments are now working towards far-reaching reforms of the structure and funding of the blood and blood products sector. The aim is to introduce a truly national approach to the planning and management of the supply of blood and blood products.

There are a number of unique relationships in the blood sector. Many different organisations play a vital part. By working with stakeholders Governments want to ensure that Australia's blood supply is as safe as possible and is of a high quality. We also need to plan for future needs, always striving for an adequate and safe supply of blood and blood products.

Governments spend about $370 million a year to produce and supply blood and blood products for the Australian health system.

Why is there a need for change? The recent Review of the Australian Blood Banking and Plasma Product Sector recommended that Governments move to a national system, with specific recommendations on:

- governance;
- funding;
- key operational functions;
- quality and safety; and
- information, monitoring and research.

What will the future hold? It is expected that in approximately 12 months time from now a new National Blood Authority will be established and operating. Governments are still deciding on its exact form. The new Authority will have direct responsibility for supply and production, planning and management, contracting suppliers of blood and blood products and providing expert advice. It will operate on behalf of all Australian Governments.

This paper will provide information to conference delegates about future directions for the Blood Sector.

B007

Fetal DNA in Maternal Plasma

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The aim of this study has been to answer the following questions:

1) Is fetal DNA always detectable in the maternal plasma at a time during the first or second trimester when chorion villus sampling (CVS) or amniocentesis is normally carried out?
2) Can paternally-derived blood group antigens be used as markers to prove unequivocally that fetal DNA has been extracted from the maternal plasma?
3) Is the fetal DNA cleared from the maternal plasma following delivery, such that it becomes undetectable before or shortly after discharge from hospital?
4) Does the mode of delivery influence the time of disappearance of the fetal DNA from the maternal plasma?

Patients were recruited at King George V and St George Hospitals during their consultation immediately prior to fetal sampling for cytogenetic studies. A small quantity of fetal tissue and blood samples from both the mother and her partner were collected at that time. DNA was extracted from the fetal tissue and from the maternal plasma using QIAGEN spin columns. PCR was performed using TaqMan reagent kits and the appropriate sets of primers for the detection of the SRY (male chromosome) and β-globin (housekeeper) genes. In certain pregnancies, primers for the RHD gene (4 instances) or K1 (Kell) gene (2 instances) were used to identify fetuses at risk of haemolytic disease of the newborn owing to the presence of maternal antibodies. Cord blood samples have provided a source of infant DNA to confirm the results of fetal sampling as well as allowing the determination of paternally-derived blood group antigens. Primers for an extended range of these antigens will enable confirmation by PCR of the presence of female fetal DNA in early maternal plasma.

The full complement of samples from 35 family groups have been collected. Fetal DNA was extracted both from amniocytes or chorion villus tissue and from the maternal plasma collected in early pregnancy, and tested for the presence of the Y chromosome. There have been no discrepancies in the PCR results to date. Collections from a further 23 families will be complete following delivery of the baby. In addition, there are 22 families from whom an incomplete range of specimens has been obtained. Computer records may enable much of this data to be utilised. A second cohort of patients is currently being recruited specifically to address questions 3 and 4 and establish that fetal DNA in the maternal circulation is cleared rapidly and completely after delivery. Once verification of the PCR method has been completed, it is anticipated that this will be a valuable and non-invasive means of performing fetal studies in early pregnancy.

B059

Use of 2-D Gel Electrophoresis to Identify Proteins Accumulating in Supernatants of Red Blood Cell Products During Storage

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A variety of proteins, peptides and lipids that may potentially contribute to adverse transfusion reactions accumulate in the supernatant of red blood cell concentrates (RCCs) during storage. These substances are largely derived from degrading leucocytes and platelets, disintegrating red blood cells and from residual plasma carried over during processing. The aim of this study was to obtain a clearer picture of the proteins that accumulate in the supernatant of RCCs during storage using the technique of two dimensional polyacrylamide gel electrophoresis (2-D PAGE). Using this technique, proteins may be separated in a first dimension according to their isoelectric point and in a second dimension according to their molecular weight allowing for the separation of individual proteins from complex mixtures of thousands of proteins.

Whole blood was collected from six donors into blood collection packs and prepared into either hard-spun RCCs or leucocyte filtered RCCs according to standard blood bank procedures. Red cells were stored in Adsol storage solution at 4^0C with supernatant samples collected aseptically on Day 1, 14, 28 and 42 post donation. Supernatant samples were precipitated to remove non-protein impurities and were completely solubilised and then stored at -80^0C. 2-D PAGE was initially performed using immobilised pH gradients with a wide pH interval (pH 3-10), however as most proteins were found in the pH range of 4-7, this narrower interval was used for subsequent experiments. This narrower range also provided higher resolution enabling the detection of proteins that were not detectable with the initial wide pH gradient. Large 8-16% gradient or 12.5% homogenous gels were used for the second dimension separation of proteins. Gels were then silver stained to visualise proteins. In supernatant collected from hard-spun RCCs, there were two major changes during the storage period (i) new proteins were detected at later collections (ii) the expression of more than 50% of proteins were found to increase. These changes were not as pronounced in supernatants collected from leucocyte filtered RCCs. We are currently analysing the 2-D gels in order to identify specific proteins of interest. Peptide mass fingerprinting by mass spectrometry will also be used to determine the amino acid composition of elusive proteins.

In conclusion, 2-D PAGE provides a powerful tool to identify new proteins and changes in expression of proteins in blood products during storage. These insights may help to identify specific proteins that contribute to adverse transfusion reactions.

B161

Recombinant Activated Factor VIIa for Massive Haemorrhage in Non-Haemophilia Patients: The Australian Experience

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Background
Recombinant activated factor VII (rVIIa) is approved for the treatment of bleeding in patients with haemophilia who develop inhibitors to blood coagulation factors VIII or IX. We describe the Australian experience with rVIIa for the treatment of massive bleeding in non-haemophilia adult patients without a coagulation factor inhibitor.

Methods and Results
Ten massively bleeding, multi-transfused, coagulopathic (median PT 18 sec [range 15-40 sec], median APTT 45 sec [31-94 sec], median fibrinogen 2.1 g/L [1.2-4.3 g/L]) patients (1 pelvic trauma, 1 haemorrhagic pancreatitis, 1 fatty liver of pregnancy, 5 post surgery, 1 chronic liver disease with coagulopathy, 1 aneurysm rupture) were treated. Patient age, the dose and timing of rVIIa administration in relation to onset of bleeding, and transfusion requirements prior to and after rVIIa administration are summarised in the Table. Transfusion requirements were dramatically reduced or eliminated in the majority of patients following rVIIa administration and 6 of the 10 patients survived the episode of massive haemorrhage (alive at 30 days or discharged from hospital).

	Age	Prior to rVIIa			rVIIa timing* (hrs)	rVIIa dose	First 24 hours after rVIIa		
		P Cells	FFP	Platelets			P Cells	FFP	Platelets
Median	41	23	24	18	14	100	2	0	2
Range	22-79	3-35	2-51	6-64	4.5-168	32-180	0-14	0-11	0-19

*Hours after onset of bleeding. Two patients received a second dose of rVIIa.
P Cells denotes packed red cells; FFP, fresh frozen plasma.

Conclusions
These uncontrolled data suggest a role for rVIIa as an adjunctive haemostatic measure in patients with massive haemorrhage for whom conventional measures to achieve haemostasis have failed. Randomised trials currently in progress should provide further information regarding the efficacy and safety, optimal patient selection, timing and dosing of rVIIa for the treatment of uncontrolled massive haemorrhage.

B104

Mini Dose Rh D Immunoglobulin Prophylaxis: Should this be the Standard Dose Routinely Administered to At Risk Rh D Negative Antenatal and Post-natal Women in the First Instance?

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Aim
To assess the level of foetal maternal haemorrhage (FMH) in pregnant women undergoing routine testing at this institution, and to determine the optimal dose of Rh D immunoglobulin (Anti-D) prophylaxis required for these women.

Methods
The results of all Rh D negative women undergoing routine FMH testing by flow cytometry between January 2000 and May 2002 were analysed retrospectively and divided into antenatal and post-natal groups. The majority of women tested were antenatal patients beyond the first trimester of pregnancy, and post-natal women whose infant was known to be Rh D antigen positive. Flow cytometry was performed using a Coulter EPICS XL-MCL flow cytometer. An internal control was assessed with each analysis, and comprised Rh D negative red cells (rr) spiked with Rh D positive cells (R1r) to a final concentration of 0. 5%. In addition when available, red cells obtained from the baby were used as a positive control.

Results

	Antenatal	Post-natal
Number (n)	134	159
Median FMH	0.01%	0.01%
Mean FMH	0.008%	0.014%
Range	0-0.03%	0-0.22%
Mini dose (50ug) Anti-D administration sufficient: % women	100% (134/134)	99.4% (158/159)

Conclusion
The 50ug (mini) dose of Rh D immunoglobulin is currently limited by the NHMRC for administration as prophylaxis to women who are in their first trimester of pregnancy. This dose is sufficient for a FMH of 2.5ml (0.1%) red cells or 5ml of whole blood.

The results obtained indicate that all antenatal women tested only required the administration of this dose. In addition, the vast majority of post-natal women would also have been completely protected had this dose been given immediately post-partum in the first instance.

The standard dose is sufficient for a FMH of 6ml (0.25%) red cells or 12ml whole blood. The routine administration of one standard dose (125ug) of Anti-D, as is currently recommended following each sensitising event in women beyond the first trimester of pregnancy and immediately post-partum, represents over usage of this precious resource in this study.

When extrapolated nationally, more rational usage of the lower (mini) dose in all at risk women, has major cost and resource implications for Anti-D usage in Australia. Further studies in other centres should be performed in order to determine if a change in policy can be confidently recommended.

B153

Transfusion Transmitted Viral Infections – Does the "Precautionary Principle" Apply to Agents Not Known to be Significant Pathogens?

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The emergence of new infectious hazards of transfusion has motivated a search for other agents that may be transfusion-transmitted. In this study two viruses, TT virus (TTV) and Borna virus, which may be transmitted by transfusion were studied. TTV, found in plasma and leukocytes, is a small virus with a circular 3.8 kb single-stranded DNA genome. In this study, three nested PCR tests, using different primer pairs, were used to investigate the prevalence of TTV infection. In PCR positive specimens the number of copies of the TTV genome were quantified using real-time PCR on a Light Cycler (Roche Diagnostics). The estimated frequency of infection in unselected Australians was up to 20% depending on the primer pairs used. In PCR positive specimens, the TTV concentration ranged from 10^4 to 10^8 genome equivalents per ml. There is evidence that TTV has been transmitted by transfusion, but the relationship between virus and host may be one apathogenic endosymbiosis. The second agent of recent interest is Borna virus (BV). BV is a cause of disease in horses, but evidence of infection and consequences of BV infection in humans remains controversial. A novel assay for BV circulating immune complexes (CICs), developed by our collaborators in Berlin, was applied to sera from healthy volunteers and multi-transfused patients in parallel in Berlin and Sydney. CICs were detected in multitransfused patients and healthy volunteers. Without isolation of the virus or amplification of the viral genome, the serological evidence presented is not conclusive proof of the presence of BV. In a hospital transfusion service, our most important obligation is to have sufficient blood available for transfusion to minimise morbidity in bleeding patients. If the "precautionary principle" were to be applied to agents of unknown significance the blood supply may be eroded further, with a risk of immediate harm to bleeding patients for whom blood may not be available.

A047

Blood Group Studies in Taiwan

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Several blood groups with special findings in Taiwanese have been studied.

The Lewis phenotypes distribution among Taiwanese (the major population) are Le(a+b-) 0%, Le(a+b+) 25%, Le(a-b+) 67%, Le(a-b-) 8%. In indigenous tribes (1.5% of the population) there is an additional Le(a+b-) phenotype. Six *Secretor* genes (*Se* or *FUT2*), including weak *Se* (Se^{w} or Se^{385}) and nonsecretor alleles (se^{571}, se^{685} and se^{849}) have been detected on 667 DNA samples by a PCR-RFLP in various population of Taiwan. A good correlation was observed between the *Se* genotype and the Lewis phenotype. The Se^{w} allele and the three *se* alleles are responsible for the Le(a+b) and Le(a+b-) phenotypes, respectively.

Taiwanese has high frequency of blood group B with B_3 phenotype being the most common ABO subgroup. Molecular genetic analysis of 14 unrelated B_3 individuals has revealed 2 different molecular changes, one individual with a 247G→T mutation and the other 13 with a G→A mutation at the +5 nucleotide of intron 3. The latter leads to a complete miss of exon 3 during

messenger RNA processing of B_3. The B_3 transcript without exon 3 corresponds a B-transferase product that lack 19 amino acids in the N-terminal segment.

The secretory H-deficient paraBombay phenotype (O_{Hm}), is the results of compound heterozygosity of 6 different *h* genes (*H* gene mutations). In Taiwan, O_{Hm} is not uncommon, and estimated to be 1 in 8000 among Taiwanese. It is estimated to be 2500 individuals with this phenotype in Taiwan. When indirect antiglobulin test with prewarmed crossmatching is performed, the individuals with this phenotype could be safely transfused with usual blood group (eg, group O or B red cells to O^B_{Hm} person).

Molecular study of Taiwanese RhD negative individuals revealed that about two third of them were real RhD negative with total deletion of *RHD* gene. Other one third of D_{el} phenotype revealed a deletion of a segment of 1,013bp between introns 8 and 9 including exon 9 of *RHD* gene.

In Asians most cases of adult i phenotype are associated with congenital cataract. Molecular analysis of 3 adult i pedigrees (5 cases) revealed a 1043G→A, and a 1148G→A mutations in *IGnT* gene in 2 families and a deletion of the *IGnT* gene in a third family, suggesting that the *IGnT* gene is the candidate for the blood group *I* gene. Confirmation of blood group *I* gene will further assist in the investigations of molecular basis for the association of the adult i phenotype with congenital cataract.

A048

Genetic, Chemical, Immunochemical and Serological Basis of the Weak Subgroups of ABO

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The ABO histo-blood group polymorphism, despite appreciation of its importance in transfusion and transplantation, is still amongst the least well understood of blood group systems. Following the discovery of ABO, subgroups of ABO were soon recognised. These subtypes were all essentially classified by their serological characteristics with polyclonal ABO reagents and salivary inhibition and elution studies, although today they are becoming less recognised due to the use of potent monoclonal reagents. Although we know some of the structures expressed on the common ABO types, the structural basis of the subgroups of ABO has still not been resolved. Recent evidence suggests that there is both a quantitative and qualitative basis. The genetic basis of ABO subgroups can be characterised as two basic types. That is, those with mutations which result in inefficient glycosyltransferases and those with apparently unmutated *A* or *B* genes and for whom the molecular basis of their phenotypes lies elsewhere, perhaps in earlier exons or more likely in the regulatory elements of the promoter region(s) of the ABO gene. In both instances it is predicted that the mechanism of causing A or B subgroup status is a consequence of inefficient formation of A or B antigens, either by ineffective or insufficient enzyme activity. The consequences are both less ABH antigen is formed and other glycosyltransferases are now able to better compete for the precursors previously used by (or regulated by) the A and B glycosyltransferases. Furthermore, this changed equilibrium will allow the biosynthesis of novel precursors, some of which may be utilised by the subgroup enzymes, thus resulting in a qualitative variation(s).
In summary, we know that the subgroups of ABO exist at the phenotypic level and they may have either a simple (*ABO* mutation) or complex genetic basis. We also know that the subgroups probably have both a quantitative and qualitative basis. What we don't yet know is what is the structural basis of the qualitative differences and can they be serologically recognised or targeted?

A049

Preparing the Patient for Surgery Without Blood Transfusion

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Avoidance of allogeneic blood whenever possible is the overall goal of blood management in preparing any patient for surgery. Several essentials must be determined to achieve this goal. These include the operation planned, any pre-existing conditions, the patient's cardiopulmonary status, the preoperative hemoglobin/hematocrit and the patient's wishes regarding blood or alternatives. Is the operation one that you do most of the time without the need for transfusion, e.g., laparoscopic cholecystectomy? Or, do you normally use blood for this type of operation, e.g., aortic aneurysm repair? To answer these questions, you will need your own personal data, not something from the literature. You can create your own database using a simple Excel spreadsheet. Using this data, you can follow your own patients to determine your transfusion parameters. By

applying appropriate strategies, such as modifying your transfusion trigger, you can gradually reduce your use of allogeneic blood. This will also help you discover if you are transfusing patients just because you've always done it that way!

A complete history and physical exam are essential. The impact of pre-existing conditions on the need for transfusion support is still unknown. Some studies have suggested that the patient's cardiopulmonary status is a significant factor in deciding when or if to transfuse. Remember that your primary concern is if patient can mount an appropriate response to acute anemia. Be prepared to stop medications such as platelet inhibitors to help reduce operative blood loss. Check the patient's hemoglobin/hematocrit at the time of the surgery planning visit. This can be done simply in the office with a fingerstick and a portable, Hemocue device. If the patient is anemic, you must decide if the anticipated blood loss from the planned operation is great enough that the existing anemia could lead to transfusion. If so, you can delay the operation until you have been able to correct the patient's anemia using iron and recombinant human erythropoietin.

Remember to ask the patient about the use of blood or alternatives. If the patient refuses blood for any reason, ask what alternatives he/she will accept. Remember to obtain informed consent. Create a blood management plan and put in the records for all to read and follow. Consider alternatives such as preoperative donation, ANH and the cell saver based on the planned operation.

Preoperative planning, involvement of the patient in the transfusion decision and tracking, analysis and modification of your results will help you attain the goal of appropriate blood management for all patients.

A051

Haematologist's Role

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The Retrieval team, Emergency Department staff, surgeon, and operating theatre staff are all asking for more blood now, hospital blood bankers are racing to keep up with the demand for blood and blood products to be issued, ARCBS staff are advising shortages – who should be conducting this cacophony? – the haematologist??

And the haematologist's role is to exhibit in the heat of all the action:

- interest and knowledge of transfusion science and medicine
- management and communication skills
- commitment, "availability" and leadership
- encouragement and support of all front-line workers

What is required of us so that our transfusion service can reliably sponsor 'best' outcomes?

- fostering of excellent working relationships between the hospital blood bank and hospital departments
- use and familiarity with protocols for patient management in massive transfusion, which are constantly reviewed by all 'stakeholders' including funders
- regular audit activities
  positive feedback to staff

B082

Using Pretransfusion Samples Collected more than 72 hours before Transfusion. Is it Safe?

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A pre-transfusion specimen should reflect the current antibody status of the patient so that compatible blood may be provided at the time of transfusion. Ideally, for patients who have recently been transfused or are pregnant, the presence of alloantibodies will be detected in the pre-transfusion sample should they be emerging. The time of collection of a pre-transfusion sample should be as close as possible to the proposed transfusion to ensure the detection of newly developed alloantibodies. The

maximum age of a pre-transfusion specimen has traditionally been set at 72hours and is currently recommended by the ASBT. The 3-day limit has been selected as an arbitrary interval expected to be both practical and safe.

Monash Medical Centre has a protocol of holding specimens for up to 7 days for their thalassaemia and antenatal patients.

Thalassaemia patients provide an example of a multiply transfused population; those patients who are most susceptible to the development of alloantibodies. Their lifestyle is intrinsically dependent on the regular transfusion regime. The offer of a less restrictive schedule of pre-transfusion testing is of advantage to this group.

This study will present Monash Medical Centre data of transfusion outcomes for over 170 multiply transfused thalassaemia patients including pregnant thalassaemia patients. 55,000 crossmatch samples were tested between 1994 and 2002. The data will include DAT and transfusion reaction investigations and demonstrate that the extended age of a pre-transfusion sample is safe as well as desirable for these patients.

B086

Blood Product Request Form and Specimen Labelling Errors – The Tip of the Haemovigilance Iceberg?

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Quality transfusion practice requires monitoring of all stages of the process.

Staff of The Royal Melbourne Hospital (RMH) Blood Bank (BB) have been collecting transfusion incident data and we report our initial experience.

Transfusion related incidents as reported to, or discovered by, BB staff were recorded on our Transfusion-Related Incident Form (TRIF). The ward clinical teams reported clinical transfusion reactions using a separate form.

From December 2001 to the end of May 2002 there were 665 transfusion incidents captured, out of a total of 7938 transfusion requests. Of these, 549 (83%) were blood product request form and specimen labelling errors, defined as any non-compliances with RMH requirements and the ASBT 1999 Guidelines for Pretransfusion Testing.

The labelling errors were grouped according to the potential for serious adverse outcome; 19% were classified as serious, where the patient's name, date of birth or hospital unit number was incorrect or absent. There were no adverse clinical outcomes during the observational period. However, such errors may be considered as 'near-miss' events. Corrective actions were taken in all such cases.

There were 116 other incidents (17% of total incidents) and these consisted of: other specimen and form errors (lost/unsuitable/wrong specimen or form) - 66; clinical reactions - 16; ward transfusion-related incidents (excluding clinical reactions) e.g. blood product lost / damaged / expired / delay / wrong patient - 9; laboratory incidents - 3; database (discrepancies between pathology and hospital computer systems) -15; other / multiple errors - 7.

The large number of clerical errors (7%) indicates there is non-compliance with existing guidelines concerning specimen labelling and request forms, and this has serious implications for patient care and risk management. In our experience, there appears to be very limited appreciation of the significance of transfusion 'near-miss' episodes. This vital component of haemovigilance needs to be better understood by the wider hospital community. The integration of these haemovigilance activities into hospital quality systems is essential. Authority, and appropriate resources are additional critical elements to implementation.

B038

Monoclonal Anti-PrP, a Candidate for Detection or Treatment of vCJD?

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Creutzfeldt-Jakob Disease (CJD) is the human form of transmissible spongiform encephalopathy (TSE), a neurodegenerative disorder. CJD has become a major public health problem, as it appears that human have developed a new variant form of CJD (vCJD) through consumption of meat from cattle infected with bovine spongiform encephalopathy (BSE, also known as Mad Cow Disease). The infectivity of vCJD is linked to Prion protein in an abnormal conformation (PrP^{sc}). The theoretical risk of transmission of vCJD via blood transfusions has led to many countries pacing a ban on blood donations from people who have lived in the UK and/or other European countries for certain periods during the height of the Mad Cow Disease outbreak. Further deferral policy has been implemented in USA and more restrictions on blood donations will be introduced. The screening of infectious agent PrP^{sc} is therefore of the highest priority. Most detection systems, such as immunohistochemistry and Western blotting, have been optimised for brain or lympho-reticular tissue, while there is no diagnostic test available to detect PrP^{sc} in the blood. Scientists and pharmaceutical companies are now racing to develop a blood test for vCJD. Is a Mab to PrP^{sc} the answer? In our laboratory, a total of ten anti-PrP monoclonal antibody-producing cell lines were generated and some Mabs were strongly positive with CJD-positive but not normal brain tissue while others only reacted with normal brain tissue. To apply these Mabs to blood or plasma, a commercial partner is needed for developing a specific and practical assay for large-scale screening. More recently, researches have found that antibodies against PrP^{c} may be able to prevent prion infection *in vitro* and *in vivo*, so a Mab to infectious Prion could have therapeutic potential.

B169

Biological False Reactive Results: Do They Persist Indefinitely?

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The Australian Red Cross Blood Service tests all blood for a number of different viruses including HIV, Hepatitis B and Hepatitis C. Testing of blood by screening assays can sometimes lead to biological false reactive results (BFR). In current methods used this is estimated to be about 0.3% of donors. BFR's are a recognised problem for blood services throughout the world. Although the testing has showed that the donor does not have the disease in question, the products cannot be used once this result is obtained. All donations which are repeat reactive by ABBOTT PRISM are subjected to the ARCBS Viral Testing Algorithms which specify a specific sequence of assays to use in order to determine a donor status for each virus. Once a donor status is established, the appropriate donor notification, counselling and re-entry follows in accordance with the ARCBS donor protocols.

The current practice in accordance with this protocol is to notify donors of a BFR status if this result is obtained on more than one occasion within a 12-month period for the same virus. It is also common practice to advise the donor not to attend for donation for one to two years These donors are generally listed as 'off service' and the expectation is that they will be accepted to donate only if they re-present in person. No formal invitation is made to recommence donation.. This results in the loss of a significant number of donors. Are we losing a pool of donors unnecessarily? Should we be making more effort to invite these donors back for retesting after a year or two? Are some virus's different than others in their persistent BFR status?

Anecdotal evidence suggests that once a donor has a BFR from PRISM testing , then that shall remain forever. With advances in technology and new algorithms this may not necessarily be true In an effort to answer these questions.

A review of donor data will be presented.

A057

The Use of Fresh, Unrefrigerated Whole Blood to Control Massive Bleeding – From Two Centres in Western Australia

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The term "fresh blood" has been defined in a number of ways. For the purpose of the talk, fresh blood refers to blood that has not been refrigerated, nor separated into components, and is less than twenty-four hours old.

At Royal Perth Hospital, the Transfusion Medicine Unit has facilitated the provision of fresh blood in situations of uncontrollable life threatening haemorrhage for the past ten years, (three years at Fremantle Hospital). The practice is only considered following transfusions with multiple units of stored blood products.

The use of fresh blood to control massive bleeding has been shown to be effective, however the decision to use this protocol should not be taken lightly. The use of untested blood increases transfusion risk and must be weighed against the risk of death from exsanguination. Fresh blood can be obtained from hospital staff, the patient's relatives, or the Red Cross Blood Service, however there are specific problems associated with obtaining blood from each of these sources.

The effectiveness of fresh blood in controlling haemorrhage may be due to the presence of large potent platelets that are not as effective as those in standard platelet concentrates. The resulting improved haemostasis indicates that fresh whole blood has a role to play in the treatment of patients with uncontrollable haemorrhage.

A review of thirty-one cases at the hospitals has demonstrated the effectiveness of fresh blood in the majority of cases.

A062

Policy, Regulation and Risk

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Risk management in the biologics area is highly dependent on the public's expectation of safety which, in blood and tissue-derived therapeutics, exceeds that of most everyday activities. Regulatory interventions have done much to enhance the risk profile of all biologics, but there is a growing realisation that efforts have been excessively focussed on product quality and safety features and insufficient attention has been paid to patient and outcomes-based issues. Also, the limits of regulation, in the overall policy development context of a health systems bureaucracy, are sometimes insufficiently appreciated. This presentation will discuss some examples of current decision making in the blood and tissues sector which illustrate some of these principles. The competing tensions provided by the current focus on evidence based health care interventions and the precautionary principle will be described. Some tentative principles on which to make risk based decisions will be proposed.

A065

Risk Management: Where To Next?

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Internationally three main themes dominate the world of transfusion. These are safety, sufficiency and concern over increasing costs. It is increasingly recognised that the goal of ‘zero risk’ is not achievable and indeed may not necessarily be desirable. New technologies and strategies to improve the safety of the blood supply however continue to emerge. Current ‘opportunities’ include extension of Nat testing to include detection of HBV DNA, pathogen inactivation systems for blood components and the extension of precautionary measures to reduce the risk, as yet theoretical, that vCJD might be transmitted by transfusion.

Regulatory authorities in a number of countries continue to promote a pharmaceutical model for Transfusion Services with an emphasis on the implementation of quality systems based on Good Manufacturing Practice (GMP) principles. This undoubtedly contributes to increased safety and effectiveness of blood components.

New technologies and changing population demographics combine to constrain the available health dollar. Clinicians will often view the Blood Service as a competitor for development monies and increasingly challenge the increasing costs of providing ‘safer’ blood components. The recent introduction of Universal Leucodepletion and NAT testing within New Zealand amply demonstrates this concern.

Numerous studies have demonstrated that potential recipients of blood components expect absolute safety. The process of informed consent should alert potential recipients to the risks associated with transfusion. Ongoing litigation both in Australia and New Zealand with respect to Hepatitis C transmission suggests however that informed consent alone will not necessarily protect against future legal risks. The recent legal opinion in the United Kingdom that identifes blood should be considered a product within the context of the Consumer Protection legislation is also of note in this regard.

In this environment the question inevitably arises as to how, and by whom, decisions on new safety strategies should be made. Within New Zealand, NZBS is undoubtedly responsible for ensuring that potential safety initiatives are identified and reviewed. In this context NZBS acts as a 'champion for blood'. Clearly decision making must however take into account other demands on the available health budget. Such decisions will need to be based on the available evidence. Other factors will however also contribute to the final decision which will often be political in nature. Systems should be developed to ensure that all stakeholders have an opportunity to contribute to the process. In this setting decision-making should be open, transparent and accountability must be clear.

A063

Is Zero Risk Possible?

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Patients admitted to hospital suffer an alarmingly high incidence of iatrogenic injury. In 1964, Schimmel[1] reported that 20% of patients admitted to a university hospital had an iatrogenic injury, of which 20% were serious or fatal. In 1981, Steel et al[2] reported that 36% of patients admitted had an adverse iatrogenic incident, of which 25% were serous or life threatening. In 1991, Brennan et al[3] reported a population based study of hospitalized patients in New York State in 1984; 4% of patients had an injury that prolonged their hospital stay or resulted in measurable disability and nearly 14% of these injuries were fatal. In Australia and in Utah-Colorado, a comparison of the Quality of Australian Health Care Study and the US study[4] showed that about 2% admitted patients had serious adverse events and a mortality rate of 0.3% admissions. For comparison, in 1981 Cossart et al[5] reported a 1.7% incidence of non-A, non-B hepatitis and 0.3% incidence of hepatitis B in Australian patients who had undergone cardiac bypass grafting. These findings have not produced a storm of litigation or changed practices to the same degree as for blood transfusion.

In blood service centers, a transmission of 1 HIV in 1.2 M donations in 1999 in Australia[6] was associated with a vote for funds to introduce NAT for HIV. For hepatitis C, recent UK figures[7] suggest that only 3 additional reactive samples were found by NAT in 7 M donations. This means that current HIV Ab and HCV Ab screens have reduced the risk to the range of six sigma quality espoused by leaders in industry[8]. NAT will reduce the risk further by 1 or 2 logs. In contrast, the voluntary SHOT system in UK hospitals in 99/00 reported an error rate for transfusion of an incorrect blood component of 1 in 10,000[9]. About 50% of reported errors were at the bedside and 27% in the laboratory. While these results are 2000 times better than for general hospital admissions outlined above, they remain preventable and do not meet blood center risk levels. There were 39 ABO incompatible transfusions in 2.7 million, resulting in 2 deaths.

The systemic process improvement to reduce risk in blood centers involved electronic failsafe checking systems, improved viral screening tests, reliable equipment and materiel and trained staff following documented procedures. In contrast, ward systems rely on variably trained humans undertaking complex tasks in cluttered environments, relying on manual transcription and visual checking for control[10]. Laboratories fall between these extremes.

It is concluded that hospitals are dangerous places and that zero risk of transfusion is not possible with the resources available in hospital wards. As always, allocation of resources to address the issue requires political processes to allocate a high priority and a willingness to provide the resources needed.

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A066

Achieving a Balance Between Risk, Cost Effectiveness and Patient Safety. Where To Spend?

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The emergence of HIV/AIDS, Hepatitis C, and more recently vCJD, has resulted in a high level of community concern over safety in the blood sector, in particular, the risk of disease transmission. This has resulted in increasingly more rigorous testing of blood and the adoption of donor referral policies, with arguably, only marginal gains in safety at high cost.

The Review of the Australian Blood Banking and Plasma Product Sector (March 2001), recognised the importance of ensuring appropriate policy responses to safety issues and The Review Committee was given the task of considering and recommending ways to improve system-wide decision making processes, including the provision of timely, expert advice on safety, quality and supply issues.

Numerous sound policy decisions can, however, be attributed to the AHMAC Blood & Blood Products Committee since its inception in 1995. These include but are not limited to advising on access to recombinant products, establishing national indemnity arrangements, reviewing alternatives to homologous blood donation and recommending the adoption of limited pre-storage leucodepletion. A high level of scientific rigour has been applied through evaluation of evidence and the use of a range of economic assessment tools.

This scientific approach to resource allocation parallels the evidence based decision making expected of clinicians but a range of other, potentially conflicting and competing factors inform policy, largely as a result of the complex funding arrangements in place in the sector.

The need to maintain public confidence in the blood supply is a high priority. This provides a sensitivity to coverage by the media which can ignite new debate or direct community expectations. The precautionary principle has been applied in recent blood sector risk management decisions and is expected to create further challenges. Variances between local policy and interstate or international policy create additional concern.

Most importantly, the competition for funding from within acute health is intense and directing funding to blood sector safety and quality initiatives such as the SA Bloodsafe project, is best achieved through dedicated funding.

It is proposed that the allocation of resources to improve safety and quality in the blood sector will become increasingly more problematic. It is critical that new governance arrangements enable governments, clinicians and the community to work together to encourage informed decisions to minimise risk.

A080

Why the Need for Transfusion Avoidance?

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In recent years homologous blood transfusion is no longer being regarded as essential for a wide range of medical and surgical conditions. Most major surgery can now be conducted without homologous blood component therapy. Stemming from the recognition that HIV could be transmitted by homologous blood transfusion there has been a resurgence of interest in methods for minimising exposure to homologous blood products. Blood transfusion in the past was generally not regarded in the same light as other risk/benefit decisions in clinical medicine. Appropriate attention to accurate diagnosis of the problem, considerations of alternative therapeutic options are essential to appropriate blood component therapy. Blood component therapy and its immediate endpoints are part of a process and not an outcome. Although appropriate endpoints may be achieved in terms of measurable parameters or clinical response, we need evidence that these traditional “outcomes” are relevant in relation to the final clinical outcome. There are multiple appropriate endpoints within any medical process that are central to a successful final outcome, but there are others where dogma rather than evidence has dominated clinical practice. Indeed many patients contracted HIV from homologous transfusion when, in retrospect, the causative transfusion was inappropriate. In the current era of evidence-based medical practice clinicians should ensure they have a good understanding of the potential benefits, indications and potential hazards of homologous blood component therapy.

Potential mechanisms for transfusion reactions include:

Immunologically mediated reactions

Homologous transfusion is a cellular or humoral transplant and immunological differences between the donor and recipient may result in varying degrees of component incompatibility. In general the recipient needs to have been previously immunised to a cellular or plasma antigen of the donor, usually by pregnancy or previous blood transfusion. Occasionally, high titre antibodies in donor plasma may be responsible for immune reactions, eg transfusion related lung injury (TRALI). Rarely grafting of transfused lymphocytes may cause devastating transfusion associated graft versus host disease (TAGVD). Immunomodulation induced by homologous transfusion may indirectly result in increased likelihood of infection and/or cancer recurrence rates.

Alterations in blood components secondary to preservation and storage

Changes due to storage may result in quantitative and/or qualitative deficiencies in the blood components reducing efficacy of transfusion or responsible for adverse clinical effects from degenerate material, released vasoactive agents, cytokines, procoagulant material and bilirubin load from non-surviving red cells.

Infection

Transmission of infectious disease or bacterial contamination.

A081

Evidence Based Challenges to Traditional Practice in Blood Management

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The practice of medicine is an amalgam of science and art that, when applied in appropriate doses and in the correct sequence, produce therapeutic benefit. However, should one end of the spectrum – whether science or art – overbalance the other, problems may arise. This has been amply demonstrated in transfusion medicine since its introduction into clinical practice. The demand for evidence-based medicine emerged in the early 1990's as multiple disciplines joined forces to promote the concept that "clinical practice should reflect clinical science". Conflicting evidence exists about both blood safety and transfusion practice. Transfusion-related errors and reactions have been with us since blood types were first identified. Yet, our way of dealing with this serious problem remains rooted in the dark ages. Many of our blood banks still rely on pen and ink to track blood resources and transfusion data. The challenge of dealing with transfusion reactions must address modernizing our facilities, computerizing our tracking systems and eliminating the human factor when is has more potential for harm than for good. Although the relationship between transfusion and immunomodulation was proven over 20 years ago, lack of education, tradition, and/or stubbornness keep many from accepting its existence. The challenge of TRIM today should not be to dispute its existence, but to clearly establish its clinical consequences, if any, and then develop strategies to protect our patients. We hear often that the blood supply is safer than it has ever been. This concept of relative blood safety is based primarily on the "evidence" that in 2002 the risk of getting a viral disease such as AIDS is approximately 1 in 1 million compared to a risk of 1 in 10,000 in 1982. We put a lot of faith in these risk estimates, but what is the clinical evidence that supports them? The knowledge that mandatory testing will detect and eliminate 99+% of positive donors, suppositions regarding transmission of disease based on both these donor screening tests and faith in the integrity of the system that supplies blood are the main sources. The actual number of <u>real</u> patients with disease <u>caused by transfusion-transmitted diseases</u> has never been calculated for all of the known transmissible agents. Similarly, we have scientific information about windows of infectivity but do we have the same evidence for periods of dormancy or latency before a disease becomes clinically manifest? Without this kind of evidence, can we truly say what the prevalence of any TTD is?

We are faced with the challenge of dealing with a Catch-22 today in dealing with blood safety. We must assure the safety of the blood supply by any and every means, <u>but</u> we must not do this at the expense of eliminating the blood we require. We mandate testing for some pathogens but not all. We develop more sophisticated ways to "sterilize" blood while refusing to admit that the cost for this will rapidly turn blood into an unaffordable commodity for many. And finally, we persist in looking to testing and sequestering blood for answers while casting a blind eye on the only truly evidence-based solution – transfusion avoidance.

Physicians have been transfusing blood for many years with the absolute certainty that comes only from blind faith that they were saving lives. However, if one looks for the sound, scientific evidence of this "truth", there is none. Our desire to believe in this benefit also flies in the face of evidence from several recent trials showing that blood is not life saving and, in fact, may be life-threatening for some patients. Transfusion decisions are most often made based on tradition, which leads to variability in transfusion rates from one physician to another for identical patients. Much of our transfusion "evidence" fails to recognize the truth that only total blood avoidance can eliminate the dangers caused by exposure and that the majority of patients can be treated with today's medical therapies without the use of blood.

Evidence-based medicine is useless if the "evidence" is not taught, is ignored or worse, is rejected because of personal bias or philosophy. Our challenges, then, are to recognize existing evidence, no matter how unpopular or un-traditional it may be, to teach what we know, to cooperate in both basic laboratory and clinical research to build our evidence base, and to use our progress to change not only the way we practice individually but also the collective field of health care policy. Once this is done, clinical practice can truly reflect clinical science in blood management.

A082

Joining Forces for the Future – How Conservation helps the ARCBS

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The blood supply of any country has always been a valuable and scare resource. Increasing exclusion of donors exemplified by the deferral of over 5% of our donors for vCJD puts increasing pressure on supply.

UK evidence suggests that their donor pool is becoming exhausted and the US supply is also diminishing. Managing this resource well enables ongoing supplies to be maximised. Careful management of production losses, inventory, expiry and utilisation will help everyone win, making the efforts of Australian volunteer donors effective and worthwhile while enabling the demands for safety expected of all of us achievable.

A091

Blood Conservation - Anaesthetist's Pivotal Role

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Traditionally the operating theatre and Intensive Care have been very significant users of blood. Although change is occurring, the anaesthetist remains at the forefront of a large number of strategies to restrict allogeneic blood usage. This is due to the numerous factors involving his exposure to the patient at a time when blood loss is relevant and his training which may present a circumspect outlook on the problems facing the patient. These strategies can be used to optimise the patient preoperatively, minimise blood loss intraoperatively and then maximise postoperative erythopoiesis. Some of these will be examined and comment will be made with reference to the speaker's considerable experience in this subject.

Blood conservation and alternatives are burgeoning, as are the areas in which these modalities are required. The scope of knowledge required to cover all of these transcends any individual specialty. Who will be the ultimate guardian of this knowledge or will this mean the evolution of a new subspecialty of medicine?

A102

Emerging Strategies in Blood Conservation

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An increasing number of biotechnological and pharmaceutical products are now available as alternatives and adjuvants to blood transfusion, some of which are listed in Table 1. Stimulants of red blood cell production now include recombinant human erythropoietin (epoietin alfa, epoietin beta), as well as an altered erythropoietin molecule (darbopoietin alfa) which has

a more prolonged half-disappearance time. Recombinant activated factor VII is now FDA approved for patients with hemophilia who have inhibitors, and there are ongoing clinical trials of this hemostatic agent in patients without pre-existing coagulopathies in peri-surgical hemorrhage, in patients with quantitative thrombocytopenia after peripheral stem cell transplantation, in patients undergoing liver transplantation, and in trauma patients.

Artificial Oxygen Carriers
There are many potential advantages for cell-free hemoglobin solutions and perflurocarbon emulsions (as synthetic oxygen carriers). These are listed below:

Stroma-free	Size ~ 1 micron
Not antigenic	Possibility of unlimited availability (bovine)
O_2 unloading devoid of 2,3,DPG effect	Extended shelf life - > 2 years
P_{50} ~ 20 mmHg -- normal Hb. P_{50} 27 mmHg	High oncotic properties
Pathogen inactivation	Altered oxygen affinity and unloading characteristics

Possible disadvantages of such products include interference with interpretation of some laboratory tests, and their relatively short time in circulation (24-48 h). One potential problem associated with hemoglobin solutions is vasoconstriction, a consequence of their ability to bind nitric oxide (NO).

Perfluorocarbon emulsions (PFC) are capable of dissolving large amounts of any gas, including oxygen and carbon dioxide. These have been shown to be effective for oxygen delivery during hemodilution in patients undergoing orthopaedic surgery at 0.9 and at 1.8 g/kg PFC dosage. In a multinational prospective, randomized study of a perflurocarbon solution to augment ANH during orthopedic surgery, perflurocarbon combined with 100% oxygen was more effective than autologous blood in reversing physiological transfusion triggers. With their high affinity to dissolve gasses, prevention of and therapy for micro-embolic bubbles from cardiopulmonary bypass or preservation of solid organs for transplantation are other possible and desirable applications for which PFC's appear to be ideally suited.

The two principal applications for the AOC's currently under clinical investigation are in patients with trauma and in patients who are undergoing surgery, with or without acute normovolemic hemodilution. The rationale for the use of AOC with hemodilution is three-fold: (i) the cellular hemoglobin collected during hemodilution would be used to replace the hemoglobin solution or other synthetic oxygen carrier as it is eliminated; and (ii) the use of AOC would permit more aggressive hemodilution with lower targeted cellular hemoglobin levels than would otherwise be tolerated and (iii) an AOC could serve as a replacement fluid during blood loss. At the present time, AOC products are in various stages of clinical development.

Table 1: Examples of biotechnology products in transfusion medicine

Erythropoiesis stimulants erythropoietin novel erythropoietin stimulating factor	Hemostasis recombinant factor VIIa recombinant factor VIII recombinant factor IX
Artificial oxygen carriers hemoglobin solutions perfluorocarbons	Anticoagulants antithrombin III activated protein C

A103

Blood Conservation - Perspectives from a Regulator

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The Therapeutic Goods Administration (TGA) is responsible for assuring the safety, quality and efficacy of blood products in Australia. The TGA's role is limited to the manufacturing of these drugs and does not include issues of medical practice. Nevertheless, proper implementation of regulatory requirements can contribute actively to the optimal usage of blood, limitation of exposure to homologous blood and other important safety measures. This presentation will focus on recent developments in the regulatory environment, which stimulate the development of blood conservation measures. Examples include:

- The development of recombinant alternatives to plasma protein concentrates has allowed the potential for replacing blood derived products for the treatment of life long bleeding disorders. However, a regulatory framework was required to demonstrate adequate safety and efficacy. This has been provided over the past years through the development of international guidelines and the TGA has adopted most of the guidelines of the European Medicines Evaluation Agency in this area. The requirement to demonstrate efficacy through clinical trials requires the recruitment of patient numbers which are small due to the rarity of some of these disorders. The international regulatory environment has proven to be flexible in guideline development in allowing smaller numbers than is conventionally required for these diseases. Recently, some of these agents have been trialed for indications other than those stimulating the initial pharmaceutical development. In one particular case, the use of a recombinant coagulation factor as a general haemostatic drug, allowing significant blood conservation in situations of massive blood loss, has been proposed. Appropriate and flexible use of the relevant efficacy guidelines should make it possible for this indication to achieve regulatory approval over the coming years.

- The use of epidemiological data to assess the viral status of a donor population is an established component of regulatory oversight of the blood system. Use of such data allows estimates of relative and residual risk from blood transfusion. Such estimates may then be used to draw up regulatory and policy guidelines to limit exposure. The examples of pooled versus single donor platelet transfusions and the use of apheresis for the manufacture of clinical fresh frozen plasma will be discussed in this regard. Development of other guidelines for appropriate product presentation may also contribute to this area.

- The continuing concerns regarding established and emerging pathogens has stimulated a precautionary approach to risk management in blood safety. The regulatory measures taken over the potential risk of variant Creuzfeld Jacob Disease (vCJD) exemplify this approach. It is noteworthy that the relevant regulatory drivers internationally have incorporated recommendations regarding the use of blood substitutes as a way of enhancing patient safety. The current measures applicable in Europe and North America will be described and their influence on blood usage assessed.

Nevertheless, it is recognised that regulatory measures cannot act as the main stimulus in developing blood conservation policy. Product regulation cannot impose undue conditions on the practice of medicine and historically, regulatory authorities have been limited in the extent to which they can influence inappropriate use of medicines. It is possible that the continuing focus on safety will result in regulators extending their traditional role. Ensuring this is done on the basis of sound science will contribute to improving the safety of the blood system.

A104

Cord Blood Expansion

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One issue with the utility of Cord Blood for adult transplantation is the total number of $CD34^+$ contained in the graft. We have been evaluating different methodologies for expansion of Cord Blood cells. Pelex et al. (British Journal Hematology 104: 643,1999) reported that the copper chelator, tetraethylenepentamine (TEPA) improved by long-term expansion of $CD34^+$ cells. We have recently begun pre-clinical validation studies using the reagent. $AC133^+$ cells are isolated from a fraction of a Cord Blood using the CliniMACS System (Miltenyi Biotec). The cells are incubated with 50 mg/ml of Flt 3, SCF, TPO, IL-6 and 3 mg/ml TEPA for 3 weeks. At the end of 21 days, the cells are harvested. $CD34^+$, $CD34^+$ 38^-, CFU-GM analysis is performed at days 0, 7,14 and 21 of cultures. Results of these experiments will be discussed during the presentation.

A111

Standards and GMP for Haemopoietic Stem Cell Manufacture - Approaching a Choice

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The TGA has implemented regulation through classification based on the degree of risk. This policy requires that the manufacture of human tissue products that are classifiable as moderately or highly manipulated must comply with the code of

GMP. Appropriate regulation of products manufactured from human tissue is critical, to ensure that such highly manipulated cell products are consistently produced to a standard that ensures that they are fit for their intended use, and pose no risk to the recipient due to inadequate safety quality or efficacy. However in striving to achieve compliance with regulatory requirements laboratories are confronted by the twin hurdles of budgetary constraint and the need to maintain the drive for innovation.

Most stem cell laboratories had their genesis in a research laboratory. Over the years the focus of these laboratories has shifted from research to the provision of a clinical function. However there remains a need for such laboratories to adjust quickly to the needs of its clinical users and implement evolving technologies. The dilemma that stem cell laboratories must address is how to maintain a commitment to research, innovation and flexibility in the face of ever more stringent regulatory control.

In implementing regulatory control laboratories must address the dilemma of budgetary constraint. There is a considerable fixed cost as well as ongoing operating costs that must be borne in meeting regulatory requirements. Risk management by the TGA is highly dependent on the public's perception of safety but it must be realised that these expectations can represent an unrealistic target for stem cell laboratories.

The implementation of regulatory control over stem cell processing requires cooperative interaction between the TGA and stem cell laboratories in a setting where there is mutual acceptance of the needs of both parties.

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A112

Two Years of Regulation of the Fresh Blood Sector – A Clinical Pathologist's Perspective

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The provision of blood and blood products in Australia is a complex system, involving the ARCBS, CSL, hospitals, pathology services, cord blood banks and the ABMDR. Regulation of this sector is even more complex involving all levels of government and NATA. One of the government bodies is the TGA.

Regulation of fresh blood products commenced in August 2000 with gazetting of Therapeutic Goods Order No. 66 (TGO66) and publication of the "Australian Code of Manufacturing Practice – Human Blood and Tissues". TGO66 mandated the Council of Europe document titled "Guide to the preparation, use and quality assurance of blood components" (COE guidelines) as the minimum standard.

Clinical haematologists require a safe and adequate blood supply. How does the average clinician find out about changes which impact on this safety and adequacy? Rarely do government agencies communicate directly with clinicians. Rather, it is via the speciality Colleges and other professional bodies. The Royal College of Pathologists of Australasia (RCPA) received a copy of TGO66 but there was no accompanying documentation in "plain English" explaining the order. This led to protracted discussion regarding which blood products were covered by the order.

Similarly, the potential implications of the COE guidelines were not clear until it was realised that it included not just regulations for the production of blood products but also regulations regarding donor selection. The selection criterion of most concern was the changing of the minimum haemoglobin levels for donors, which was estimated to result in the deferral of 19% of current blood donors. This occurred at the time of imminent deferral of donors considered at risk of variant Creutzfeldt-Jakob disease. As a result the RCPA became concerned about the consultation process that had occurred regarding legislation that could have such an impact on the adequacy of the blood supply.

Although regulation of the fresh blood sector initially most obviously applied to the ARCBS, it has now included the cord blood banks and individual hospitals that collect matched unrelated stem cells. Concerns persist about the level of consultation and information regarding the regulation of the blood products, as well as the accountability of the TGA with respect to its decision making processes. It is to be hoped that this situation will improve with the establishment of the National Blood Authority.

A113

Carbohydrate Blood Group Terminology - Are Serological Concepts Enough?

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Traditionally immunohaematologists have viewed the ABH blood group antigens in their simplest terms – that is, are they present or absent? From a blood transfusion perspective, determining the simple positive or negative status of these antigens is critical yet adequate for safe blood transfusion. But is this enough? Today we use potent monoclonal reagents selected to show the broadest specificity, so broad that we may have in fact lost (serologically at least) many of the subtleties of ABO. Part of the key to unlocking the complexities of the ABO system lies within understanding the actual structures of ABO that are expressed on red cells and tissues.

At the moment a positive result means the presence of an antigen, but what is the glycotope(s) your monoclonal antibody has detected? Furthermore, are there different structures or ratios of structures expressed on the red cells/tissues of individuals with the same basic A, B or O phenotype that may be important to transfusion and/or transplantation? Are some more important than others?

Carbohydrate blood group antigens e.g. ABH, have the potential to be extremely complex. The glycotopes and the chains which carry them (and influence the antibodies which recognise them) are all affected by variability in characteristics such as sugar type, linkage, branch positioning, shape, charge, ring size and epimeric and anomeric configuration. For example, if a six sugar molecule is considered then it would have theoretically 1,000 billion possible structural combinations, which is 21 million times more combinations than are possible with six amino acids! Some ABH blood group antigens can consist of as many as 60-80 sugars. Herein arises a problem, that is, how to describe each and every variation of these molecules so that information from different sources can be cross-referenced. We have therefore had to develop a relatively simple yet logical nomenclature capable of describing carbohydrate histo-blood group antigens. It is hoped that this nomenclature will be another tool towards understanding the complexities of ABO.

The final and perhaps most critical issue is do serologists need to expand their horizons and understand the complexities of the most important blood group system in man? We think so, because only by critically and knowledgeably examining the complexities of the ABH system will be we able to make sense of the wealth of information embedded in this evolutionary gem – ABH.

A114

Terminology of Red Cell Surface Antigens

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Inherited variations of human red cell membrane proteins, glycoproteins and glycolipids, from the ABO blood group were discovered more than a century ago, since then several hundreds of new red cell antigens have been identified. As a consequence many different terminologies were introduced. In 1980 ISBT established a Working Party (WP) on Terminology for Red Cell Surface Antigens to develop a genetically based numerical terminology for blood groups. In 1990 a 6-digit numerical terminology was proposed by the WP for 242 red cell antigens. This was followed with continuous updatings afterwards. Up until Wien meeting in 2000, 272 red cell antigens were classified into 26 blood group systems, 11 antigens of 5 collections, 21 low incidence antigens (700 series), and 11 high incidence antigens (901 series). This numerical terminology was used primarily for easy computer storage and retrieval of information on blood group antigens and to provide a framework for a genetical classification of blood group antigens.

The 6-digit, genetically based terminology for red cell surface antigens has been in operation since then and is generally well accepted as a method of classifying and cataloguing blood group specificities. However the complexity of this system will be greatly increased in the future by additional upcoming issues, such as numbering epitopes of the RH1 (D) antigen defined by monoclonal antibodies and new terminology describing carbohydrate histo-blood group antigens / glycotopes.

For most serologists the current ISBT terminology for red cell antigens will be enough to work with, and the proposed complex nomenclature of biochemical structure probably at first will be of importance only to researchers.

A115

The UK Approach to Understanding Donor Behaviour

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Mass unremunerated homologous blood donation is a remarkable and unlikely phenomenon. In the UK there are signs that the potential of the population to meet its own demand for blood is reaching the upper limit. In this context it is vital to understand donor behaviour as a means to safeguarding the blood supply by gaining the greatest benefit from the donor pool.

There are two elements to understanding donor behaviour:

1. Describing the activity patterns of blood donors.
2. Explaining donor behaviour in terms of motivation and cause.

Historically in the UK there has been a remarkable imbalance between these two elements, with much work aiming to understand motivation (particularly of first-time donors). In contrast very little work was done to describe the behaviour.

The 1990s brought a revolution in information technology enabling large databases to be built and quickly manipulated. This has led to the present situation in which a single database holds details of over 5 million past and present NBS donors – and several billion transactional records which can be manipulated to extract information.

The National Blood Service has adopted a hybrid approach to understanding donors combining data analysis with traditional and innovative market research techniques. Key to describing donor behaviour has been a series of cohort studies tracking the development of new recruits from initial expression of interest to permanent withdrawal or retirement. This has provided critical information on recruit conversion and retention and led to improvements in practice.

Analysis of medium to long term trends in donorbase parameters has uncovered troubling structural aspects of the donor pool and led to a reassessment of recruitment and retention policy. In particular an unsustainable rate of attrition and replacement has been discovered, leading to a newly launched programme to generate donor loyalty.

Qualitative and quantitative market research has provided insights into the donor perspective. Innovative research techniques have been adopted to extract sub-textual and subliminal meaning from group and interview research. Donors have been shown to be a heterogeneous group, and to exhibit a distinct progression in terms of behaviour and expectation.

An important outcome of the research has been a realignment within the NBS to place customer service and the generation of donor loyalty on an equal footing with technical standards, to ensure a blood supply that is both safe and sufficient.

A116

Systems to Develop Predictive Production Models

Dr S Thomas
The Australian blood system relies on non-remunerated voluntary blood donors, with around 3% of the population donating for the country's needs.

With the introduction of vCJD deferral in December 2000, the Australian Red Cross Blood Service (ARCBS) carried out planning for the replacement of the anticipated donor loss. Early estimates predicted a loss of on average 5.3% of donors, with some states such as WA particularly hard hit with an estimated 7.9% donor loss.

Despite recruitment planning for increased attendances from regular donors and the attraction of new donors, the impact of vCJD deferral, at least in WA, exceeded initial expectations.

The loss of 8% of the whole blood and 12% of the apheresis donor base flowed through to significant product loss despite recruitment efforts. In order to develop better systems to model the impact of multifactorial strategic drivers on product

availability, ARCBS explored the use of 'iThink' software. This software allows the input of multiple parameters relating to population characteristics, regular and new donor deferral and return rates, and the flow on impact on product availability.

With the extension of the vCJD ban in the United States, and the anticipated introduction of new, more stringent haemoglobin guidelines for donors, ARCBS sought to utilise the scenario modelling software to determine the combined impact of these new restrictions on its donor base.

Although the software is capable of modelling the end-to-end functions of the organisation, from donor attendance through collection, processing, testing and dispatch, for this session just the donor section will be presented.

A117

Factor VIII Inhibitors in Children

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High titre factor VIII inhibitors occur in 10-15% of children with haemophilia A, posing a significant risk of increased morbidity and mortality. Inhibitor pathogenesis is strongly influenced by genetic factors: There is a high concordance rate in siblings of inhibitor patients but not in their extended family members. Furthermore patients with large deletions, stop mutations and intrachromosomal recombinations are more likely to develop inhibitors than patients with small deletions and missense mutations. Inhibitor pathogenesis is also influenced by patient immunological factors: Inhibitors are generally IgG antibodies, often IgG_4 subclass, indicating immunoglobulin isotype switching has occurred and the requirement for T-cell participation.

The main clinical risk to a child with high titre inhibitors is the lack of efficacy of human factor VIII for the treatment of bleeding episodes and for prophylaxis. Alternatives treatments such as porcine factor VIII, activated prothrombin concentrates and recombinant factor VIIa have all been used to arrest bleeding episodes and provide prophylaxis with variable success. The other avenue of therapy is the eradication of the inhibitor by an immune tolerance programme. These programmes consist of high dose factor VIII (although optimal dose is under investigation presently) as a single agent or in combination with immunosuppressive or immunomodulating therapy. This therapy may abolish inhibitors in approximately 70% of patients, which leads to normalisation of the factor VIII half-life, near normalisation of the patient's quality of life, and a marked reduction in the cost of treatment. Prognostic indicators of immune tolerance success are the inhibitor titre (<10BU) at the start of immune tolerisation, possibly the time from diagnosis to initiation of immune tolerisation, and young age.

A118

Transfusion in Neonatal Medicine

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Although transfusion practices in neonatal medicine are determined by the same principles and processes that apply at other times of life, they are complicated by issues related to the small absolute circulating blood volume in newborns (80-90 mls/Kg; eg: 70-80 ml for a 900 gm preterm infant), the need for frequent blood sampling for monitoring of blood gases/biochemistry/haematology, and the fact that neonatal haematologic problems often reflect a continuum of pathologies which have their origins in fetal life. As at other times in life, it is important also to minimize transfusion risks. The following examples serve to illustrate these issues, and the treatment strategies used to minimise transfusions in this population.

1. Rhesus Isoimmunisation (and other haemolytic disorders)

In severe disease, fetal surveillance (via umbilical venous sampling) and serial intrauterine transfusions of O (-) ve packed RBCs are needed. Delivery is often preterm, and although haemolytic jaundice is often not a significant clinical problem, these babies often develop a severe hyporegenerative hypoEPO anaemia, which may require transfusion and/or EPO therapy. In less severe disease, neonatal problems typically involve haemolytic jaundice, which is managed with intensive phototherapy and possibly exchange transfusion; other therapeutic options include high dose IgG therapy, haem oxygenase inhibition, and the gastrointestinal sequestration of bilirubin.

2. Anaemia of Prematurity

This problem is akin to the physiologic anaemia experienced by all babies, which co-incides with their transition from HbF → HbA production. In the premature infant, a relatively sluggish EPO response, and ongoing blood sampling exaggerate the problem, which may cause cardiovascular instability, and poor growth. Treatment options include serial transfusions (using quad packs to minimize donor exposure), and rhEPO therapy (200 IU/Kg alt daily). Although rhEPO therapy has been shown to reduce the number of transfusions, it produces only a modest reduction in donor exposure. A directed donor program may help in the management of this problem.

3. Marrow Suppression (secondary to intrauterine growth failure) - thrombocytopenia and neutropenia

This is a common problem, particularly in settings of placental insufficiency. The resulting chronic fetal hypoxemia results in an (appropriate) intense erythropoiesis that can be so dominant that other marrow activities are suppressed. Typically, there is a high peripheral nucleated red cell count, with marked neutropenia and mild thrombocytopenia. The neutropenia usually recovers by 5-7 days, though is associated with an increased risk of infection, while the thrombocytopenia typically worsens and then recovers by day 7-10; platelet transfusions are sometimes required.

A119

Intrauterine Transfusion

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A brief history of the management of haemolytic disease of the newborn is given from its identification in the early 1600s to the formal recognition as a disease process in the 1930s. A historical profile of the methods of management including the introduction of timed premature delivery, exchange transfusions, amniocentesis for the assessment of foetal risk, anti D prophylaxis to reduce maternal sensitisation and, finally, the use of intrauterine transfusion is presented. The technical aspects of intrauterine transfusion, the indications for us of this procedure and the observed outcomes will be discussed.

A129

Transfusion Serology Quality Assurance Program 2002

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Transfusion laboratory Services have changed considerably over the last ten years with introduction of gel/column technology and computerisation of data entry and reports. Quality Assurance Programs have traditionally assessed usual laboratory practice. The large variation of approaches, in particular computerisation, has produced unique challenges for a Quality Assurance Program in Transfusion.

Recently a program to assess feto-maternal haemorrhage estimation has been introduced as a graded exercise. Wide variation of results from Kleihauer testing has been observed and exercises and being designed to determine variables associated with the test and proposals for improvement.

Recognition of poor performance laboratory work is required by accrediting and government bodies. Assessing poor performance in Transfusion and providing a graded score is a challenge. Any system of grading needs support from participants for the grading system success and validity.

B180 **P51**

Autoimmune Haemolytic Anaemia Post Allogeneic Bone Marrow Transplant Associated with an Antibody Displaying Apparent A_1, C_w , C and D Specificity

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Haemolysis is a well recognized complication of stem cell transplantation. Post transplant immune-mediated haemolysis can be due to autoimmunity, passenger lymphocyte syndrome, passive transfusion of antibody and major blood group incompatibility. Autoimmune haemolytic anaemia is recognized as a complication of allogeneic bone marrow transplants, which is difficult to

treat and the prognosis is poor. This is a case report of autoimmune haemolysis presenting in a 15 year old male thirteen months post unrelated bone marrow transplant for relapsed ALL. The patient had successfully converted from group O Rh_o (D) positive to group A_2 Rh_o (D) positive but complications such as CMV retinitis and ventriculitis and graft failure were evident. A weak anti A_1 had been detected after group conversion, but the appearance of the antibody appeared to coincide with intravenous immunoglobulin (Intragam®P) administration. A haemolytic anaemia was evident and at this time, an antibody was detected which reacted preferentially with R_1, R_{1w}, R_2, and r^1 cells. The Direct antiglobulin test (DAT) was positive for IgG and C_3d and an anti A_1 was eluted from the patient's red cells. As the disease progressed, the patient's group showed autoagglutination. It was necessary to transfuse the patient with group A_2 Rh_o (D) Negative red cells as these were the most compatible. Despite transfusion of more than 100 units of red cells over a period of 3 months, splenectomy and plasmapheresis treatment, the patient died from multiorgan failure due to CMV reactivation.

B145 **P52**

Anti-Jk^a Autoimmune Hemolytic Anemia in a Singaporean Infant

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Jk^a is a high incident antigen of the Kidd system in most populations. Autoantibodies with Jk^a specificity are rather rare in association with autoimmune hemolytic anemia. Recently in Singapore, we report a case with a complement binding IgG auto anti-Jk^a in a 1-year-old boy.

A 1-year-old boy who was febrile with viral infection was hospitalised on 28 May 2002. Blood test was ordered on the child, and the hemoglobin was 4.2g/dl with a hematocrit of 12.5%. Therefore, one unit of red blood cells was ordered to overcome the anemia crisis. The initial antibody screen was positive with 1+ and the direct antiglobulin test (DAT) was 3+. An EDTA sample was requested for further antibody investigation. The boy had no history of blood transfusion.

Materials and Methods

Serologic testing and reaction grading were done according to the standards of blood bank practice. Antibody screening was performed with indirect anti-human globulin tests. DATs were performed with polyspecific anti-IgG and C3d antiglobulin reagents and read after the immediate spin. All DATs were performed on EDTA samples. An acid eluate was performed with a commercial kit. Chloroquine diphosphate was used to dissociate IgG antibody from red cells.

Result

Red cells from the patient before blood transfusion showed a positive DAT (3+ with polyspecific, 4+ with C3d and 4+ with anti-IgG). An antibody screening test by indirect antiglobulin test with polyspecific AGS was positive with 1+. Our panel results with polyspecific AGS indicated an antibody of Jk^a specificity in both serum and eluate. After a 2-hour incubation with chloroquine diphosphate, the Jk^a typing on the patient cells was positive with 3+ and Jk^b tying was negative. In conclusion, auto anti-Jk^a was identified in the serum of young child.

B001 **P53**

Reactivity and Incidence of Cefotetan Induced Haemolytic Anaemia Antibodies

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Cephalosporins have been previously implicated as a common cause of drug induced haemolytic anaemia, predominantly second and third generation cephalosporins and rarely first generation cephalosporins. Cefotetan is a second-generation cephalosporin which is now commonly given as a prophylactic antibiotic for gynaecological, obstetric and other abdominal surgery. We recently investigated 4 cases of Cefotetan related haemolytic anaemia with the unusual serological feature of cross reactivity with the first generation cephalosporin, Cephalothin.

The four cases were all females who had undergone recent surgery (vaginal hysterectomy, caesarian section x2, cholecystectomy) and received a single dose of Cefotetan prophylactically. All presented 10-14 days postoperatively with severe anaemia (40-62 g/L) without evidence of bleeding, but with evidence of immune mediated haemolysis (reticulocytosis

and raised bilirubin, Lactate Dehydrogenase and a positive Direct Coomb's Test with IgG and Complement). Antibody which caused red cell agglutination only with Cefotetan coated red cells and in the presence of Cefotetan was detected in the serum of all four patients. Tests for reactivity with other cephalosporins were negative with Ceftriaxone, Cefotaxime, Cephalexin, Cefuroxime, Ceftazidime, but did show some reactivity in all 4 cases with Cephalothin.

Subsequent retrospective testing of 75 female patients who had received prophylactic Cefotetan under similar circumstances within the previous year without subsequent clinical problems did not show any evidence of cefotetan dependant antibodies. Low titre reactions were seen in 5 patients, but these were considered to be non-specific.

B027 **P54**

Searching the Extended Family for a Bone Marrow Donor

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The aim of an extended family search is to explore the option that relatives other than siblings may be suitable as bone marrow or stem cell donors.

Relatives such as aunts, uncles and cousins, may share one of the patient’s haplotypes and there is the possibility that the other haplotype is introduced into the family via spouses. With this in mind, there is the possibility that an extended family member of the patient may be HLA identical (6/6) or mismatched by only one antigen (5/6) with the patient.

Tissue types are very much related to ethnic backgrounds. In the Northern and Western European Caucasoids for which haplotype data is available, the probability that this may occur is dependant on the frequency of the non-shared haplotype in the general population. In ethnic groups other than Caucasoid where the haplotype frequencies are not known or hard to establish, extended family searches are still worthwhile for finding a potential bone marrow or stem cell donor.

At the Australian Bone Marrow Donor Registry in NSW (ABMDR-NSW), out of a total of 45 extended family searches performed there have been 6 in which a relative has been shown to be HLA identical or mismatched by only one antigen with the patient and therefore a suitable candidate as bone marrow or stem cell donor.

Valuable time and resources can be saved by careful evaluation of the order in which family members are to be tested. It is important that family trees are obtained from the family before beginning an extended family search to ensure that a genuine 6/6 or 5/6 genetic family antigen match is obtained.

B146 **P55**

Prevalence of HTLV in Singapore Blood Donors

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Human T-cell lymphotropic virus type I and II (HTLV I/II) infection is known to associate with adult T-cell lymphoma-leukaemia (ALT) and HTLV-associated myelopathy (HAM). These viruses are transmissible by cellular blood products like whole blood, red cells and platelets, hence posing a risk in acquiring HTLV infection through transfusion. However this risk is greatly reduced as HTLV viruses can be eliminated by refrigerated storage for more than 10 days. Several countries e.g. US, Australia, Japan and Hong Kong have included HTLV antibody screening in their mandatory testing of their blood donations. The seroprevalence for these countries range from 0.07 % to 25%. In 1991, the presence of HTLV-I antibodies was looked at in Singapore donor population and none of the less than 1,000 samples tested were confirmed positive, hence Singapore does not screen donors for HTLV till date. With advancement of testing technology, a study on the prevalence of HTLV was performed at Centre for Transfusion Medicine in early 2002 on a larger population of 3,105 donors. Using a highly sensitive assay kit from Abbott Laboratories, 3 out of the 3,105 donors were screened repeatedly reactive for HTLV I/II antibodies, giving a seroprevalence of 0.10%.

B129 **P56**

Monitoring Effectiveness of Skin Disinfection Procedures Across Australian Red Cross Blood Service

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Background
Normal skin flora can usually be removed by disinfection procedures prior to blood donation. Effective, convenient and reliable disinfection procedures are essential to reduce the bacterial load at the venepuncture site sufficiently, as bacterial contamination of blood components can cause potentially serious transfusion reactions. This study aimed to establish a standardised method for monitoring the effectiveness of skin disinfection techniques across the Australian Red Cross Blood Service (ARCBS).

Methods
An evaluation of routine skin disinfection procedures was performed at sites throughout ARCBS. Donors used were random blood donors from each collection site. Samples were collected using a sterile swab, placed immediately into a transport tube containing Aimes medium without charcoal in a gel format. Swabs were taken pre and post-disinfection by the staff member collecting the blood donation and were sent, at 2-8C, to a central microbiology testing laboratory. Swabs were vortexed in saline and an inoculum of the saline solution was spread over a horse blood agar plate. Plates were incubated aerobically for 48h at 35C.

Results
Samples (n=1337) were received from 45 collection sites in 7 states. Skin disinfection agents were similar nationwide (essentially all 70% isopropyl alcohol), but there were some variations in application technique, including the number of alcohol swabs used (1-3 applications), the area and pattern of skin wiping, and the time the skin was left to dry before commencing venesection.

Overall, skin disinfection was achieved as expected:

- The national ARCBS target of <5 cfu/plate in 75% samples was met by 44/45 sites (state range 91-99%), with the more stringent target of <10 cfu/plate in 95% samples met by 37/45 sites (state range 93-100%).
- An arbitrary international target of >90% reduction in bacteria post-disinfection (McDonald et al, Vox Sang 2001) was met in 5/7 states (state range 86-99.5%).
- Mean post-disinfection counts ranged from 0.09 - 6.31 cfu/plate (SD 0.5-30.8) across various states.

Conclusion
Although the disinfection agents were essentially the same nationwide, variations in effectiveness of skin disinfection were apparent. This may be due to application methods, local donor or collection centre factors (e.g. temperature, other environmental effects), but the clinical implication of these variations is unknown and further evaluation is warranted. A standardised method of monitoring pre- and post-disinfection levels of bacteria allowed results to be directly compared across Australia, including from regional collection sites. The monitoring system was practical and efficient, and the results will serve as a baseline for future comparisons.

B095 **P57**

The First Case of HCV Seroconversion in a New South Wales Blood Donor after the Introduction of HCV NAT Screening

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The Australian Red Cross Blood Service introduced routine HCV and HIV-1 NAT screening of all blood donations in June 2000, to reduce the diagnostic preseroconversion window of infectiousness for these viruses in the general blood supply. We report the first case of HCV seroconversion in a New South Wales blood donor after the introduction of NAT.

A 31-year-old white male NSW blood donor made a donation in early October 2001 that tested NAT HCV positive by TMA (Chiron). Testing by anti-HCV chemiluminescent immunoassay (Abbott PRISM), anti-HCV EIA (Murex VK 48 version 4.0)

and anti-HCV immunoblot assay (Genelabs) was negative. A follow-up sample, collected 7 days post index donation, produced the same pattern of test results. In addition, the ALT was normal and testing for HCV RNA by RT-PCR assay (Roche Amplicor) was positive. Genotyping of PCR product (Inno LiPA HCV II, Innogenetics) revealed type 3a infection. On the occasion of initial follow-up, the donor reported a recent parenteral risk factor for HCV infection and symptoms of anorexia, nausea and fatigue. The donor had made 2 previous donations in May 2001 and July 2001 that had screened anti-HCV and HCV NAT negative. A provisional diagnosis of acute HCV infection therefore was made.

Serial blood samples were collected from the donor at 7-day intervals for a further 5 weeks and tested in the same manufacturers' assays. The donor initially developed an abnormal ALT associated with persisting negative HCV serology at 2 weeks post index donation. The first significant serological change was an indeterminate anti-HCV immunoblot assay result at 4 weeks post index donation. The donor subsequently developed reactivity in the anti-HCV chemiluminescent immunoassay and anti-HCV EIA at 5 weeks post index donation. Finally, at 6 weeks post index donation, the anti-HCV immunoblot assay also became reactive. The donor remained viraemic and symptomatic during this period.

A look back was performed for the red blood cell component of the donor's donation collected in July 2001, which was transfused in early August 2001. A blood sample obtained from the recipient in mid October 2001 tested anti-HCV and HCV RNA negative.

Although the risk of transfusion-transmitted HCV infection is extremely low with current anti-HCV testing, this case demonstrates the improved safety of the Australian blood supply with the addition of HCV NAT.

B041 **P58**

Filtered Washed Red Cells – A New Approach

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Introduction
To meet the needs of end users the ARCBS-SA introduced Filtered Washed Red Cells (FWRC) as a new product. Products with reduced contaminating leucocytes reportedly reduce the risk of febrile, urticarial and anaphylactic reactions post transfusion. Previous data gathered by ARCBS-BASS demonstrated that FWRC prepared using Baxter products could be stored for 28 days.

Methodology
Variations in suppliers, filter types, extraction equipment and processing temperature within the ARCBS resulted in the need for ARCBS-SA to perform additional validation prior to implementation of FWRC in SA. Quality parameters were examined up to 28 days post collection and processing of the units.

Fresh units of Buffy Coat Poor Red Cells with additive, prepared using the Terumo Automated Component Extractor (T-ACE), were filtered using the Terumo Imugard III Red Cell Filter. These units were subsequently washed three times using the Baxter 0.9% Sodium Chloride Triple Transfer Pack. The washing process entailed mixing 200mL of saline with the cells, centrifuging using a hard spin and extracting the supernatant with the T-ACE. After completing this process three times the red cells were suspended in Optisol, Terumo's red cell preservative solution.

The ARCBS-SA Quality Control Laboratory tested all validation units on Day 0, Day 14 and Day 28. Testing parameters included white cell counts, haemoglobin, % haemolysis, pH, potassium and volume. Development and validation of an assay for total protein testing of the final product supernatant was achieved with the cooperation of QC Laboratories in the ARCBS-SA and the ARCBS-BASS.

Results
The results of all testing performed on the 30 units processed during the validation trial met current ARCBS National Specifications. Council of Europe Guidelines (6th Edition) does not contain parameters for a filtered washed red cell product in additive. However the trial units did meet Council of Europe parameter specifications for "Red cells in additive solution, buffy coat removed", "Red cells, leucocyte depleted" and "Red cells, washed".

Conclusion

Filtered Washed Red Cell units prepared pre-storage (20-24°) using Terumo Imugard III RC filters and Baxter 0.9% Sodium Chloride Triple Transfer Packs meet all required specifications at all stages of testing, and may be stored for up to 28 days at 2-6°C.

By providing a validated, controlled and quality assured product the ARCBS-SA is now able to improve upon current hospital practices to the benefit of the patient.

B028 **P59**

Comparison of Pall BPF4, Terumo Immugard III and MacoPharma Red Cell Filter Units at ARCBS-SA

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ARCBS-SA performed an evaluation of three red cell filter units due to requests from our end user hospitals to provide pre storage filtered red cells for a specific patient group. Red cell filter units from Pall, Terumo and MacoPharma were compared for their ability to produce leucodepleted red cells which were then assessed for specific quality parameters in accordance with the Council of Europe (6th Ed). This evaluation was in line with ARCBS National strategies of leucodepletion to provide improved customer service and to satisfy the existing clinical demand by ARCBS-SA end users. This process is an improvement on current hospital practices by providing a validated quality assured and controlled filtration process. Leucodepletion provides benefits to the patient by reducing the potential for non haemolytic febrile transfusion reactions, decreasing cytokine levels, lowering the risk of infection and reducing immune suppressive effects.

Thirty Terumo buffy coat poor red cell units were filtered in the Processing, Inventory and Distribution Department of ARCBS-SA by each of the Pall BF4, Terumo Immugard III and MacoPharma red cell filter units. The red cells were assessed pre-filtration, post-filtration and at expiry (42 days post collection) for volume, white cell count, red cell count, haematocrit, haemoglobin and haemolysis (at expiry).

All red cell units tested met the Council of Europe (6th Ed) requirements for white cell count post filtration regardless of the filter type used. The efficiency of filtration was not significantly different between the different filter types with all filters removing >99.9% of white cells. Haemoglobin loss per unit was similar for all filter types but was most marked with MacoPharma filters. The largest discrepancy in red cell recoveries was observed with MacoPharma filters (71-102%) with Terumo filters providing the most consistent red cell recoveries (86-93%). The filtration process resulted in a loss of unit volume that was most marked with MacoPharma filters (average volume loss of 42 ml).

All filters provided excellent white cell removal from the Terumo buffy coat poor red cells evaluated. Reduction of contaminating white cells was accompanied by a volume loss, which largely accounted for the reduced haemoglobin and red cell loss per unit. Despite these factors there was no significant difference between the three filter types evaluated. In consultation with ARCBS-SA end users the Terumo Immugard filter units were selected for filtering buffy coat poor red cells at ARCBS-SA.

B060 **P60**

Introduction of Automated Component Extraction in ARCBS-SA: the laboratory perspective

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Aims

To maximise plasma yield and improve component consistency, automated component extraction was identified as an appropriate strategy for ARCBS-SA. The Terumo Automated Component Extractor (T-ACE) was selected, as it was compatible with collection bags in current use. The aim of the study was to develop protocols for the implementation of T-ACE in PID for the production of therapeutic components.

Method

To develop protocols for the manufacture of therapeutic components various parameters were investigated. T-ACE parameters that affect component quality were; volumes removed, speed of extraction, and optical sensor settings. Centrifugation

parameters of interest were speed and duration. The storage conditions of whole blood pre- separation were also considered as it was reported to affect component quality. Quality parameters of blood components prepared under different conditions were assessed by the Quality Control Laboratory to determine compliance with specifications. All whole blood was centrifuged in a Beckman J6MI and components extracted by T-ACE.

Results
Whole blood collected in triple bags were processed on the day of collection or following storage overnight at 20-24°C. A primary centrifugation of 3800rpm for 5 minutes followed by extraction enabled production of "red blood cells buffy coat removed". The triple bag configuration required a second centrifugation of 4200rpm for 10minutes to separate the plasma from the buffy coat. The plasma, if separated within 18 hours, was suitable for clinical use. The buffy coats prepared were not suitable for platelet production. The quadruple pack configuration was required to produce buffy coats suitable for this purpose.

Whole blood intended for platelet production was collected into quadruple bags and held initially at room temperature for four hours. Components subsequently prepared did not meet all quality parameters, particularly with respect to contaminating leucocytes in the red cell product. A minimum hold of 18 hours at 20-24°C and centrifugation at 3200 rpm for 10.5 minutes produced acceptable components; "red blood cells buffy coat removed", plasma for fractionation, and buffy coats suitable for platelet pooling. Four buffy coats were then pooled using T-Sol, centrifuged at 1300 rpm for 5 minutes and extracted on a T-ACE to give rise to platelet pool suspended in T-Sol. This product met quality parameters for "buffy coat pooled platelets".

For the preparation of fractionation plasma, double bags were centrifuged at 4000rpm for 8 minutes and extracted automatically using T-ACE. This product met quality specifications.

Conclusion
Protocols were developed for automated component extraction that delivered consistent components meeting ARCBS Blood Component Quality Control Specifications version 1.5, enabling the implementation of T-ACE in ARCBS-SA.

B010 **P61**

Immunomodulatory Effects of Post-Storage Leuco-Depleted Plasma on Lymphocyte Proliferation *In Vitro*

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Prior blood transfusions have been documented to improve the success of renal allografts and have been associated with increased postoperative infections and possible cancer reoccurrence. Pre-storage leuco-depletion has been shown to reduce the tumor growth promoting effect of stored blood, while post-storage leuco-depletion did not. These data suggest that leucocyte/platelet products are released during storage, and following transfusion may modulate the recipient's immune system.

To investigate this hypothesis, blood was collected from 5 consenting adults and stored for 30 days at 4^0C. Plasma was then removed and mixed with fresh packed cells from the same donors. Both fresh plasma + PBMC and post-storage leuco-depleted (plasma) + PBMC were cultured with phytohaemagglutinin at 37^0C in 5% CO_2. At 72 hours, cultures were pulsed with ^{3}Hthy for 18 hours and lymphocyte proliferation determined using a cell harvester and liquid scintillation counter.

Upregulation of the activation inducer molecule CD69 (involved in lymphocyte signal transduction) and the interleukin-2α receptor (IL-2αR) was also determined using multiparameter flow cytometry.

T-cell proliferation was inhibited in a dose-dependent manner in cell cultures exposed to post-storage leuco-depleted plasma.

CD69 and IL-2αR upregulation by T-cells was inhibited in a dose-dependent manner in cell cultures exposed to post-storage leuco-depleted plasma.

The decrease in T-cell proliferation together with the inhibition of CD69 and IL-2αR upregulation in the presence of stored plasma, may explain the clinical findings in post-storage leuco-depleted transfused patients.

B048 **P62**

Managing the Introduction of Automated Component Extraction at ARCBS-SA

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Introduction
Process change within the production environment of the Blood Service must be managed to ensure continued product quality. Where this change involves the introduction of new equipment, rigorous quality parameters must be met. The introduction of automated component extraction was a planned process in ARCBS-SA, however, only during the evaluation process were major issues identified which ultimately required creative solutions.

Method
An impact analysis was performed prior to the introduction of Terumo Automated Component Extractors (T-ACE's) into Processing, Inventory and Distribution (PID) in ARCBS-SA. A change control plan was developed to address these impacts in PID. Evaluation commenced and, because of the identification of major organizational issues, an Organizational Consultative Change Committee (OCCC) was assembled to manage these impacts.

The evaluation stage of the T-ACE implementation process suggested that a holding period of eighteen (18) hours prior to processing would optimise quality of blood components intended for the preparation of platelets. The decision to introduce this process required revision of working hours to optimise the collection, processing and testing of blood components

Results
Within PID the changes needed were as follows:

- Refit of the Processing area to adequately accommodate the introduction of six (6) T-ACE's. A plan for the area was designed in consultation with the Facilities Department and approved by TGA.
- Development of new Standard Operating Procedures (SOPs).
- Training and assessment of staff in the new SOPs.
- A communication plan was developed for our End Users for notification of new components, Buffy Coat Poor Red Blood Cells and 4 unit Platelet Pools suspended in T-Sol.
- Departments impacted upon by this process change were identified and a team formed to communicate proposed changes to procedures.

The OCCC comprised both management representatives and staff representatives from the areas most affected by the change. Following consultation staff were rostered over six (6) days between the hours of 8.00am Monday to 4.00 pm Saturday. While other staff were required to work their standard daily hours rostered for duty between 7.00 am and 11.00 pm Monday to Friday.

Conclusion
The introduction of new technology involves careful consideration of all stakeholders' needs. It is necessary to develop a detailed project plan with constant review as the evaluation progresses to identify further changes throughout the process that may have significant organizational impact.

B068 **P63**

Effect of Preparation and Storage Conditions on Activation Potential of Platelets

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Platelets collected by apheresis typically display a different basal activation status to those prepared from pooled buffy coats. This may be due to the collection process, method of production, or storage medium. This study aimed to determine whether the basal activation status affected the potential for activation and aggregation under the influence of different agonists in vitro.

To determine whether the basal activation status of apheresis and buffy coat platelets was in fact different, eight samples of each were assessed for expression of CD62P, an activation dependent platelet marker, by flow cytometry. The expression of CD62P was found to be significantly higher in buffy coat platelet concentrates than in platelets prepared by apheresis. To assess whether the difference in the basal activation status led to a difference in the ability of platelets to respond to agonists,

buffy coat and apheresis platelets were activated with ADP and assessed by flow cytometry for CD62P expression. Apheresis platelets displayed a four-fold increase in the percentage of platelets expressing CD62P following ADP activation compared to the basal level. Buffy coat platelets displayed only a two-fold increase in the percentage of CD62P expression.

To assess whether the decreased responsiveness of buffy coat platelets to agonists was due to the storage medium, buffy coat platelets were resuspended in plasma and incubated at 37°C. The basal and ADP stimulated activation status was then measured. Following resuspension in plasma, buffy coat platelets displayed a decreased expression of CD62P under basal conditions. The level of expression of CD62P following stimulation with ADP was decreased compared to the response of buffy coat platelets in TSol. This demonstrated that despite a lower basal activation level being observed there was no increase in the potential for buffy coat platelets to express CD62p in response to ADP in vitro.

Apheresis and buffy coat platelets were also tested functionally by aggregometry to measure the ability of platelets to change shape in the presence of ADP. Apheresis platelets displayed a significantly higher response than buffy coat platelets on day one of storage. The response decreased over the storage period with both buffy coat and apheresis platelets displaying an equal response on day five of storage. When the buffy coat platelets were restored to plasma the aggregation response was equivalent to that of apheresis platelets suggesting that platelet function is restored on return to physiological conditions.

B149 **P64**

Getting to the Point- Problem Solving through Effective Relationships

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The ARCBS Adelaide detected a problem with the retraction of needles into a needle-guard safety device used in conjunction with Terumo blood collection bags for the collection of blood units. This problem placed the safety of staff at risk as it reduced the effectiveness of the device. The problem was reported to the Team Leader, Donor Service Manager, Quality Assurance Manager, the OHS Coordinator, Therapeutic Goods Administration Incident Reporting and Investigation Scheme, the supplier (Baxter Healthcare) and the manufacturer (ITL Corporation).

An investigation was launched by ARCBS Adelaide to identify the cause of the problem. Thorough examination of the device revealed minor differences in the batch in use from previous lots. No significant defect was identified.

The frequency of the failure was difficult to assess as most reports were anecdotal and there was no hard data to support the reports. The work area involved has a high throughput so a simple system was implemented to record incidents and examples of device failures were retained.

The device supplier was contacted and they, along with the manufacturer flew to Adelaide to meet with the ARCBS staff and to try to understand the problem.. Examples of the failed devices were sent to Sydney for examination and investigation by both the Supplier and the Manufacturer. A new lot of the devices was supplied and staff retraining was performed to eliminate any issues related to device handling and assist in identification of the cause.

The manufacturer's investigation revealed that in certain circumstances the minor variations in tolerances of the device did not enable sufficient space for full needle retraction when the Terumo Blood Collection bag needle hub is in a diagonal position. After reviewing their manufacturing tools they were able to provide, as an interim measure, devices from an existing tool that gave reduced failure events.

The manufacturer, as part of their policy of continuous improvement, also undertook to design new tools to eliminate the retraction issues and to make the product more "user-friendly". Two thousand samples of a re-designed device were supplied to ARCBS Adelaide to trial. These were placed into routine use, a simple sheet was designed to collect data on the new device, and feedback was provided to the supplier and manufacturer.

This incident shows that simple monitoring systems can be used to collect data valuable to assist in investigation of incidents and that with good communication and relationships between user, supplier and manufacturer resolutions can be reached that satisfy all stakeholders.

B130 **P65**

What and How Much Should We Teach Medical Students on Transfusion Medicine?

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The medical student of today is bombarded with an ever-increasing volume of medical knowledge that requires teachers to carefully judge what should be presented at the undergraduate level. Too much on specialized subjects can be counter-productive, while too little may result in an adequate basic knowledge of the fundamentals of clinical medicine.

Understanding of transfusion medicine has expanded greatly in the past few decades.

The extent to which this subject is taught to medical students throughout the world varies greatly, but as it is so important for the care of patients, careful attention is required to ensure that young doctors are well trained.

Experience suggests that graduates do not always have a good understanding of key areas of transfusion medicine. In view of the recent development of clinical guidelines for transfusion by the ASBT/NHMRC Working Party, perhaps it may be time to redefine the key areas that medical students must be competent in, prior to graduation.

Some details of a local programme for medical school training in transfusion medicine will be outlined as a basis for comparison to the activities of other medical schools in Australasia. Experience suggests that better training in transfusion medicine will result in improved use of blood, increased safety for patients and a lessening of the demands on the blood donor service.

B174 **P66**

Blood Utilisation in Hysterectomy

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Introduction
Hysterectomy is one of the most commonly performed major surgical procedures in Australia today with a rate of 3.3 per 1000 women. There are different procedures to perform hysterectomy, mainly abdominal hysterectomy (AH), vaginal hysterectomy (VH) and laparoscopically assisted vaginal hysterectomy (LAVH). Blood transfusion, as a major adverse event associated with hysterectomy, was reviewed over time at a single tertiary institution and compared with Victorian State data.

Methods
Data from the Royal Women's Hospital (RWH) and Victorian admissions data set (VAED) were analysed to identify blood transfusion associated with hysterectomy. For RWH patients, individual patient medical records and transfusion data from the Pathology Service database (medipath) were used to independently verify transfusion data. All hysterectomies associated with a code for malignancy were excluded.

Results
Specific coding regulations for ICD9 regarding blood transfusion took effect on 1st July 2000. Prior to this, patients were coded for blood transfusion except if they had blood transfusion in the course of a procedure. The amended codes after 1st July state that all transfusion is to be coded.

At RWH, rate of blood transfusion can be considered reliable from 1997 due to the availability of the Pathology Database. The alterations in rates of transfusion before and after the introduction of reliable data show that using coding alone, transfusion is underestimated by as much as 50%. Verified RWH transfusion rates for AH, VH and LAVH were 7.9%, 4.8% and 8.4% respectively. When logistic regression is performed from 1997 onwards, LAVH is the only predictor for blood transfusion $p<0.01$, odds ratio 1.76, while other procedures were not predictors. Victorian transfusion rates for AH, VH and LAVH were 5.8%, 2.2%, and 3.5% respectively for the year 2000/2001. Autologous blood transfusion is utilised more in the private sector as compared to the public sector. In the public sector, autologous transfusion as a ratio to all blood transfused is 2.4% for AH, 7% for VH and 6% for LAVH. In the private sector, rates are 30% for AH, 20% for VH and 40% for LAVH.

Conclusion
Difficulties with coding for blood transfusion prior to 1st July 2000 make all coding based reviews of blood utilisation potentially unreliable due to underestimation of transfusion. Information on likelihood of transfusion for various types of hysterectomy should be provided to women to enable them to make informed choices regarding surgical options and could be used to target autologous blood collection to procedures with high likelihood of transfusion.

B121 **P67**

Use of Cryoprecipitate for the Treatment of Factor XIII Deficiency

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Patients with Factor XIII (FXIII) deficiency often present shortly after birth with umbilical bleeding. Most major centres do not have supplies of FXIII concentrate readily available to treat these patients. To achieve haemostasis, Fresh Frozen Plasma (FFP) is usually used. There is some controversy in the literature as to whether cryoprecipitate contains adequate FXIII to treat these babies. Bleeding in FXIII deficiency usually only occurs when FXIII is <1%, and the level necessary to stop further bleeding is approximately 10%.

Recently a bleeding neonate was admitted to our hospital with a suspected FXIII deficiency. This raised the question with which blood product to treat her. We used FFP because of the doubt about the FXIII content of cryoprecipitate.

Subsequently we assessed 50 units of cryoprecipitate for their FXIII content and their volume to determine their total FXIII content. Content was assessed in both Blood group A and O cryoprecipitate. The assessment was carried out using the Dade Behring Berichrom FXIII kit, which employs a photometric determination of the coagulation factor. The test was performed on the BCS instrument
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The cryoprecipitate was manufactured at ARCBS-NSW by the standard method. FFP was frozen to -25^0C then thawed at 4^0C. The resultant cryoprecipitate and cryosupernate were separated by centrifugation then refrozen to -25^0C prior to use.

The results show differences in FXIII content and volume between blood group O and A cryoprecipitate. There may also be an effect of shelf life on reducing content. We make recommendations for treatment of FXIII deficiency in neonates with regard to blood group and shelf life of the cryoprecipitate.

Obviously in a larger patient calculation of the replacement units required should be performed using weight in a standard formula as for FVIII. In this case the problem of any one unit of cryoprecipitate being of lower content is diluted by the greater number of units being given.

B090 **P68**

A Review of Intravenous Immunoglobulin (Intragam P) usage in NSW

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Introduction
Intragam P is currently supplied to patients in NSW by Australian Red Cross Blood Service NSW (ARCBS-NSW) according to Australian Health Ministers Advisory Council (AHMAC) Guidelines that were published in June 2000. These guidelines provide for IVIG to be supplied for a variety of conditions either as first line therapy or as an alternative therapy after other specific treatments have failed. In the guidelines, some clinical prerequisites, dosages and review intervals are specified.

Aim
To determine the usage pattern of Intragam P in NSW following the introduction of the AHMAC guidelines.

Method
Intragam P is issued on a named patient basis. Records of indication and dosage are kept by ARCBS as required by the AHMAC guidelines. Data collected for the period July 2000 to June 2002 was analysed for indication and product usage.

Results
Since July 2000,the issue rate of Intragam P in NSW increased by 30%, from an average of 20000 g per month to 27000 g per month.

AHMAC Category 1 indications are those for which there is now convincing evidence of benefit. These indications account for 98% of the Intragam P issued in NSW. The indications most commonly supplied in this category are Primary Hypogammaglobulinaemia, CIDP and ITP in adults.

AHMAC Category 2 indications are defined as indications for which there is inconclusive evidence of benefit. These indications account for 2% of the Intragam P issued in NSW. Examples of these include autoimmune haemolytic anaemia, thyrotoxicosis and haemophagocytic syndrome. Requests for Intragam P for patients with a diagnosis listed in Category 2 are assessed on a case by case basis.

Anecdotal reports suggest that increasing numbers of autoimmune conditions may respond to intravenous immunoglobulin, so requests for Intragam P for conditions listed as Category 2 are expected to increase.

Conclusion
From July 2000 to June 2002 in NSW, the majority of Intragam P has been supplied for patients with Category 1 indications. The number of requests for Intragam P have increased, stressing the available supply and impacting on the ability to supply Intragam P especially for patients with Category 2 diagnoses. It is expected that demand for Intragam P will continue to increase as the list of indications increases.

B002 **P69**

Blood Transfusion in Obstetric Practice

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Introduction
Blood transfusion is an uncommon but life-saving procedure for some obstetric patients. There is little contemporary Australian data related to obstetric transfusion. Blood utilisation in obstetric patients at the Royal Women's Hospital (RWH) was reviewed retrospectively for the period July 2000-June 2001. Transfused patients were identified and their characteristics analysed to determine blood utilisation practice at RWH. The prevalence of red cell alloantibodies in the obstetric population was determined.

Methods
Data was obtained from the RWH obstetric database (ROBIN), computerised pathology records (Medipath) and medical records for all deliveries performed during the review period. ROBIN was evaluated to ensure its accuracy. The medical records of all transfused patients were reviewed and information regarding the pregnancy extracted. The data for transfused and non-transfused women was compared for several variables.

Results
The transfusion rate at RWH was 1.8%. Various risk factors for transfusion were identified. The rate of transfusion for placenta praevia was 37.2%; 34.7% for retained placenta; 25.0% for abruption; 4.5% for uterine atony; 3.4% for caesarean section; and 1.6% for trauma to the lower genital tract. With respect to modes of delivery, caesarean section had a higher transfusion rate (3.4%) compared with normal (0.8%) or instrumental delivery (3.2%). Patients having non-elective caesarean section had a higher transfusion rate (3.7%) compared to those having elective caesarean section (3.0%). Transfusion risk also varied according to timing of delivery. Pre-term delivery had a higher transfusion rate (3.9%) compared to post-term delivery (2.3%), with the transfusion rate being lowest during term delivery (1.5%).

No antenatal transfusion was observed. RWH performed only responsive transfusion but not elective transfusion. Patients were generally transfused with more units of blood compared with other studies.

The rate of red cell alloimmunisation was 1.3%, which is identical to the rate reported 20 years ago. 0.4% of the population had antibodies commonly associated with some degree of HDN, and 0.3% had high levels of antibodies requiring intervention.

Conclusions
This study provides useful information for clinical quality appraisal and helps to identify conditions in which intensive management and immediate transfusion facilities are required. It will also enable clinicians to provide obstetric patients with improved and up-to-date risk assessment of likelihood of transfusion in a given clinical scenario.

Validation of ROBIN revealed many discrepancies between ROBIN and the medical records. This study recommends that improvements to the practice of data recording at RWH are needed.