An Improved Method for the Recovery of Total Nucleated Cells in Cord Blood

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A significant proportion of the cost of cord blood banking lies in the resources required for the distribution of information to donors, obtaining informed consent, interview, collection and processing of cord blood donations which do not translate into bankable units. A collaborative cord blood pilot program undertaken by the Australian Red Cross Blood Service and King Edward Memorial Hospital identified a 19% loss of bankable units at the processing stage. The aim of this study was to improve the total nucleated cell recovery in buffy coat preparations thereby increasing the potential for banking success and cost efficiency.

Cord blood was collected from delivered placentae into CPD-A anticoagulant and processed within 24 hours. Cord blood was transferred into a Stemcare (Fresenius HemoCare) cord blood processing set. The set was aligned with a full saline bag prop and secured with a centrifugation support (BagRap). The assembly was centrifuged at 1000g for 20 minutes with slow acceleration and no break. Buffy coat cells were separated using a Baxter Optipress II (back plate: standard, force: 10, buffy coat volume: 40 mls and buffy coat level: 6.8). Whole cord blood, buffy coat, plasma, and red cell fractions were tested for the total number of nucleated cells, CFU-GM and CD34+ cells.

11 cord blood donations were processed. In all but one haemolysed donation, the adopted processing assembly and centrifugation method facilitated a clean undisturbed interface. In this exception, 42% of total nucleated cells (TNC) were detected in the red cell fraction as a result of the inability of the Optipress II to recognise the interface. The TNC recovery in the buffy coat was 49.6%. In one case, a genuine flow restriction during the Optipress II program resulted in a TNC recovery of 7.4% with 88% of cells detected in the red cell fraction. In the remaining 9 procedures the mean TNC recovery was 87.9% (range 76.2 - 95.7%). When compared to TNC yields obtained during the pilot program (mean recovery: 70.8%, range: 61.1 - 80.6%), these results represent a 17.1% increase in TNC recovery.

This processing technique consistently produces a high yield of nucleated cells in buffy coat derived from cord blood. Separation is achieved in a closed system without additives, thereby preserving sterility and the integrity of red cell and plasma fractions required for future testing. The requirement for washing procedures associated with reagents that are not approved for human infusion (eg hydroxyethyl starch) is averted. This method has significantly improved the processing outcomes in our laboratory and will translate into a higher success rate of achieving bankable units and ultimately cost efficiency. The application of this method in the processing of paediatric bone marrow harvests is to be explored.

The Blood Supply Of The Future: Establishing Donation Patterns Through A School Based Youth Program

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Background: It is difficult to recruit and retain regular blood donors. The future blood supply depends on well developed altruistic and community centred behaviour in young people. Some five years ago, based on this principle, the Geelong Secondary Schools Blood Challenge commenced as an initiative of a Geelong teacher (who is also a plasma donors) and supported by the Geelong Blood Centre. In this year, the International Year of the Volunteer, this program has become well established and is now bearing fruits.

Aim: To present the school based blood donor program as a means of building a young committed donor base using the Blood Challenge as a fun competitive way of achieving this.

Method: Target Group: 16-19 year old students in years 11 and 12 and teachers - Parental consent required for those under 18years. Teacher and school council approval also needed as part of duty of care.

Length of the Challenge: February to December each year.

Marketing: done locally

- Direct mail out at the commencement of the school year reporting the Blood Challenge Results from previous year and encouraging year 11 and 12 coordinators to get involved with the Blood Challenge for the new year.
- Local Newspaper - photo opportunities and newspaper articles on the Blood Challenge
- Lecture Presentations to school groups by the Geelong Blood Centre Manager and Charge Nurse delivered either in a state of the art tiered lecture facility in the Geelong Hospital or within the schools themselves. The topic - Blood Transfusion and Blood Products is now a part of the Victorian Certificate of Education (VCE) Biology Course and can also in other subjects such as Communication, Community Service and Religious Education
- Video presentations are also shown: Vital Factor and Plasma Donating
- Award: A perpetual shield and a certificate of thanks to the students of school who donate the most blood and to student leaders who have been directly involved.

Results: So far this year the students of Geelong have collected 730 units of blood and at the rate the program is progressing more than 1000 units will be collected this year.

Year	1997	1998	1999	2000	2001
Donations	92	180	185	428	730 (YTD) (1398 Projected YE)

Conclusion: The enthusiasm of the young donors is wonderful to see in the context of the Blood Collection Centre. There is great potential for recruiting committed youth. At this stage of their busy lives, it gives them the idea that they still need to take time out to think of others and that they can help the community (and in particular very ill patients) in a very positive way. It provides the school with a community project to focus on and gives students an opportunity to adopt the Blood Service as a leadership or community service based activity.

Optimising plateletpheresis procedures using the Gambro® Trima™ Outcome Review Process.

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The Gambro® Trima™ Automated Blood Component Collection System can be connected via a router to the Gambro® Trima™ Outcome Review Process which enables collation of the stored plateletpheresis procedure information, allowing review on a monthly basis. Since early 2001 Australian Red Cross Blood Service- Southern Region have used this facility to evaluate the plateletpheresis procedure data with a view to improving productivity and identifying training issues.

Monthly reports provide information such as:

- Average donation time
- Number of procedures per platelet dosage
- Blood group of donor
- Alarms by type and frequency
- Productivity by month
- Preferred procedure vs Actual procedure (selected by operator)

Assessment of the data has allowed staff to monitor platelet productivity and identify opportunities for improvement. Monthly collation of Blood group data allows targeted recruitment of donors with a specific blood group as required, with information directly available to the apheresis team. Correlation between preferred machine procedure and actual procedure provides useful review data for training purposes. Evaluation of product distribution and platelet doses has also identified the opportunity for increasing double dose Platelets where possible.

As part of its strategic plan, ARCBS-SA aims to increase the number of plateletpheresis collections. Monthly assessment of machine data identifies areas for continuous improvement with measurable outcomes.

Use of DNA characterisation of the antigenic polymorphism of MART to confirm an Alloimmnue Neonatal Neutropenia

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Background

Investigation of a classic, clinical case of alloimmune neonatal neutropenia (ANN) indicated a probable anti-MART, but we could not confirm this because we had no panel cells negative for the MART antigen in Australia. We worked toward confirmation of the antibody specificity by genotyping the patient.

The MART^{a} antigen occurs in large quantities on granulocytes and monocytes and in smaller quantities on T lymphocytes, and the frequency in the United States is 99.1% with a gene frequency of 0.91. The MART polymorphism is located on the αM subunit (CD 11b) of the ß2 integrin family, and is associated with a single nucleotide substitution (G302A).

Results and Conclusion

Exon 3 of the αM subunit, a 124 bp fragment which contains the polymorphic site, was amplified from genomic DNA using PCR. The PCR product was bi-directionally sequenced and found to contain the predicted G302A substitution, making her MART^{a-}. This confirms our serological findings of an anti-MART antibody. As sequencing is impractical as a routine typing technique, efforts are currently underway to develop a SSP-PCR method.

The Fall of the House of Usher; Blood Safety in the Third Millennium.

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The Scottish National Blood Transfusion Service (SNBTS) had achieved national self sufficiency for blood components and plasma products in the early 1980's, if not before, from volunteer un-remunerated donors. This status was the basis for a safe blood supply in Scotland. Despite the usual transfusion transmitted infections SNBTS, as did other blood services, continued to emphasise the safety of blood transfusion. In the UK, responding to the possibility that variant CJD (vCJD) might be transmitted by blood has led to fundamental changes in the provision of blood (imported plasma and leucocyte depletion of components), and yet the risk is still described as 'theoretical' and blood transfusion remains safe. The European Commission espouses the aim of zero risk for blood transfusion, but this remains an impossible dream, as it does for any medical intervention. Despite the lure of NAT and pathogen inactivation systems, chasing the pathogen no longer seems an appropriate response to new threats, especially when these are from agents (vCJD) that defy previously held tenets for the exclusion or inactivation of pathogens in blood. The more tests that are applied, with ever greater sensitivity, the less secure we seem to be that blood is or ever can be safe. This experience, together with the recent UK ruling that the EU Consumer Protection Act applies to cellular blood components as well as to plasma products, suggests that a reappraisal of how to market "blood" is required. Clearly, it would not be difficult for Blood Services, working with their Governments, to frighten users and recipients into believing that blood is not safe, but this would risk upsetting donors who then might reasonably feel that their own blood was tainted. The challenge for Blood Services will be to educate recipients, clinicians and donors that risks will remain whatever we do to reduce them, and to achieve this educational process without frightening patients or alienating donors by implicating them in the uncertainty that we all share. Governments should accept this reality and support those patients who are harmed despite the best efforts of the Blood Services. In the future, we may have to accept that the only truly safe blood transfusion is the transfusion safely avoided.