RhD grouping of Fresh-Frozen Plasma (FFP)

Paper from SACBC/SACIH for consideration by JPAC

The question

Is it necessary to label standard frozen components (FFP and cryoprecipitate) with RhD group?

This question originally arose as a result of problems encountered in importation of sufficient RhD negative plasma units from the USA for production of methylene blue (MB) treated FFP. The MB system employs several filtration steps designed to remove particulate material from plasma. A piece of work was conducted by the NBS Component Development Laboratory (CDL) to examine the capability of these filters in removing red cells and red cell fragments from plasma. A summary of this data was submitted to JPAC in June 2004, resulting in the recommendation that MB FFP can be transfused without regard to RhD status (Appendix 1).

However, the question has arisen as to whether we need to RhD group other plasma components. The level of red cell contamination in FFP is dependent upon how plasma is processed after blood collection and therefore a second piece of work was undertaken by CDL to examine levels of red cell contamination in 'standard' plasma components (Appendix 2).

How many red cells are present in FFP?

a) MB FFP

The capability of steps in the MB process to remove red cells and the amount of red cells present in the final MB treated plasma is given in appendix 1. The dose from MB treated plasma is likely to be $<0.05 \times 10^9$ /L (= 0.001 ml RBC) per unit. Obviously where larger volumes of plasma are used, such as for plasma exchange, the dose could be significantly higher (although MB FFP is not recommended as the treatment of choice for plasma exchange).

b) standard FFP

Assuming an average FFP unit volume of 300ml and red cell volume of 95 fl, then the maximum red cell contamination measured (2.28×10^9 /L, appendix 2) equates to 0.06ml red cells prior to freezing, and would be expected to be <0.001 ml after thawing. The average patient who is transfused with FFP will receive 3 units and thus the dose of intact red cells transfused would not exceed 0.003 ml.

Clinical significance of findings

1) Previous reports of immunisation in RhD negative individuals following transfusion of RhD positive plasma:

Plasma transfusions have been shown to be capable of causing both primary and secondary responses to red cell alloantigens (Mollison, 1997), but the evidence of primary immunisation is suspect (see below).

a) primary immunisation

Only one case of possible primary immunisation has been reported in a female recipient following plasma exchange (McBride et al, 1983). The recipient received 120 units of plasma, the majority of which were RhD positive and were a mix of fresh (liquid) and fresh-frozen plasma. They estimated that a total of 10^{10} red cells (equivalent to 1ml of packed red cells) were transfused. The patient received this dose over 8 days, and six weeks later anti-D at a titre of 1/16 by IAGT was observed. It was claimed that this patient had not been previously transfused nor had a previous pregnancy. However, such a rapid response to a small dose of red cells, with a significant IgG titre, is highly likely to be a secondary response and the patient may have concealed evidence of a previous pregnancy. Even if this is considered a true primary response, it would be exceptional given the experience with deliberate immunisation of volunteer donors de novo, and would reflect the very high non-responder. The authors recalled 5 other RhD negative patients who received extensive plasma exchange without regard to the RhD group of the FFP, none of which had developed red cell alloantibodies to commonly tested antigens.

b) secondary immunisation

A case of secondary alloimmunisation has been reported following transfusion of 12 units of FFP from a RhD positive donor to a RhD negative recipient (Wolfowitz, 1984). Another case of secondary alloimmunisation has been reported following transfusion of 4 units of FFP (de al Rubia et al, 1994). In a further 5 RhD negative recipients that received FFP from RhD positive donors, 1 developed an anti-D antibody. In neither of these reports, do the authors state what the red cell contamination of plasma was. It is possible that contamination of plasma with red cells is worse in these cases of alloimmunisation than would be expected using current processing methodologies.

Red cell contamination of plasma may have also been a contributory factor to a secondary response in a female receiving intensive plasma exchange to reduce maternal anti-D concentrations (Barclay et al, 1980).

2) Is the quantity of red cells in FFP sufficient to elicit a response?

The literature seems very mixed on what dose of RhD positive red cells is required to immunise a RhD negative recipient, and many studies have been performed in normal subjects whose immune response may be different to various patient populations. In general however, the smaller the dose of red cells given, the fewer immunisations are induced. In addition, many of the recipients of FFP either have concomitant disease that predisposes them to suppression of the immune response, or receive immunosuppressive chemotherapy or other treatment that would be expected to make them less responsive to RhD positive red cells than normal donors. Recent data from the NBS/MRC Clinical Studies Unit suggests that approximately 50% of FFP recipients are immunocompromised (Dr Charlotte Llewelyn, personal communication).

It is of note that of course most cellular contamination of FFP will be fragments of membrane rather than intact cells and red cell stroma have been shown to be less immunogenic than intact red cells (Mollinson, 1972). D antigen has been shown to be present on red cell microparticles (Victoria et al, 1987; Oreskovic et al, 1992), but the immunogenicity of this material has never been demonstrated. The fact that FFP seems capable of causing a secondary response after very large infusions in some individuals

seems to suggest that some of the D antigen present in FFP remains antigenic, but the question is whether this is of sufficient clinical significance.

Current guidelines for labelling of FFP

Guideline	Require D group?	Comments
UK BTS Red Book 6th edition (2002)	yes	
BCSH Guidelines for the use of FFP	no	Sensitisation following the administration of RhD positive FFP to RhD negative patients is most unlikely as stroma is less immunogenic than intact red cells.
EU Directive	yes	
CoE guide to the preparation, use and QA of blood components 10th edition (2004)	no	
AABB Standards for Blood Banks and transfusion services 21st edition (2002)	no	RhD type not required for cryoprecipitate or fresh frozen plasma

Current practice among members of the EBA

Country	Label with RhD group?	Comments
Austria	yes	Mostly use SD FFP. Plan to remove RhD group shortly
Belgium	no	Leucocytes removed or destroyed in treatment for viral reduction (SD or MB) Probably also no need to label non-virus inactivated
Denmark	yes	Easier for wards if all components labelled the same
Finland	yes	
France	yes	Thinks risk of sensitisation is low but present
Germany	no	
Ireland	yes	For standard FFP, but mostly use SD
Netherlands	yes	But allow the wards to ignore!
Norway	no	
Portugal	yes	Easier to label all components the same, and mandated in EU Directive
Switzerland	no	Consider product is cell-free after filtration

In summary, 4/11 services do not label with RhD group. An additional 2 either plan to remove the RhD group information from the label or allow the users to ignore it. 2 continue to label because they feel it will cause less confusion at ward level rather than because of any scientific reason.

Summary

1. Guidelines vary as to their requirement for RhD labelling of FFP. Several countries / services have ignored the RhD group of frozen components for many years without any apparent reporting of adverse events, during which time many thousands of units of FFP have been transfused.

2. The process used for manufacture of MB FFP removes red cell contamination at the equivalent of 6 mL red cells per unit (10 x the maximum likely level) to below the level of detection, and there is sufficient data to conclude that MB FFP does not require labelling with RhD group to prevent alloimmunisation.

3. There is evidence that standard FFP can very rarely induce a secondary immunisation if given in sufficient quantities, with unfiltered product. The one case of primary immunisation to RhD reported is unconvincing.

4. Levels of red cells in 'fresh' LD plasma do not exceed the EU directive limit of 6 x 10^9/l. The maximum red cell contamination per unit of fresh plasma was 0.06ml. Previous experiments have shown that 6 x 10^9/l red cells in fresh-plasma, will be undetectable after freeze-thawing (<0.001ml) due to fragmentation of red cells.

5. There is sufficient preliminary experimental data to confirm the BCSH recommendations that FFP manufactured by current, quality controlled processes, including leucodepletion, may be transfused without regard to RhD group, and that all types of FFP (and components manufactured from it) do not require labelling with the RhD group to prevent alloimmunisation.

6. Since it is a legal requirement of the EU directive to monitor levels of red cells in FFP, this will need to be performed on an ongoing basis. However, it is not clear on what scientific and clinical basis the current limit of 6 x 10^9/l has been set.

Recommendation

All types of FFP produced by the UKBTS (and components manufactured from it) can be transfused without regard to RhD group. This supports the recommendations made by the British Committee for Standards in Haematology (BCSH) in the Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant (O'Shaunessy 2004).

An approach should be made through relevant committees to change the requirement for labelling these components with RhD group in the EU Directive, supported by the evidence provided in this paper.

R. Cardigan, S. MacLennan, S. Urbaniak
11 May 2005

References

Barclay GR, Ayoub Greiss M, Urbaniak SJ. Adverse effect of plasma exchange on anti-D production in Rhesus immunisation owing to removal of inhibitory factors. BMJ 1980;ii: 1571.

Krailadsiri P, Seghatchian J. Residual red cell and platelet content in WBC-reduced plasma measured by a novel flow cytometric technique. Trans Apheresis Sci 2001; 24: 279-286.

Jilma-Stohlawetz P, Marsik C, Horvath M, Siegmeth H, Hocker P, Jilma B. A new flow cytometric method for simultaneous measurement of residual platelets and RBCs in plasma: validation and application for QC. Transfusion 2001; 41: 87-92.

McBride JA, O'Hoski P, Barnes CC, Spiak C, Blajchman MA. Rhesus alloimmunisation following intensive plasma exchange. Transfusion 1983; 23: 352-354.

Mollison PI, Englefreit CP, Contreras M (Eds). Blood Transfusion in clinical medicine (1997), 10th edition, pp174-175. Blackwells Science, Oxford.

Oreskovic RT, Dumaswala UJ, Greenwalt TJ. Expression of blood group antigens on red cell microvesicles. Transfusion 1992; 32: 848-849.

O'Shaunessy DF, Atterbury C, Bolton-Maggs P, Murphy M, Thomas D, Yates S, Williamson LW. Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant. Brit J Haematol 2004;126:11-28.

De al Rubia J, Garcia R, Arriaga F, Guinot M, Lopez F, Marty ML. Anti-D immunisation after transfusion of 4 units of fresh frozen plasma. Vox Sanguinis 1994; 66: 297-298.

Victoria EJ, Branks MJ, Masouredis SP. Immunoreactivity of the Rh0 (D) antigen in cytoskeleton-free vesicles. Transfusion 1987; 27: 32-35.

Wolfowitz E, Shechter Y. More about alloimmunisation by transfusion of fresh-frozen plasma. Transfusion 1984; 24: 544.

APPENDIX 1

Determination of residual reds and red cell microparticle removal by processing steps used in methylene blue treatment of frozen plasma

Aim

To determine the efficiency of the PLAS4 filter in removing red cells and red cell microparticles in plasma spiked with known levels of red cells. Plasma was frozen-thawed and filtered as per current standard processes for production of MB plasma.

Method

Six ABO and RhD matched fresh plasmas were filtered using the MacoPharma PLAS4 filter to reduce cellular contamination to as low as possible and then pooled. The pool was then split in to six units. One unit of each pool was spiked as described in Table 1. Units were blast frozen and stored at -40°C overnight. This process was repeated three times.

Table 1 Levels of red cells to be added to each plasma unit

Unit	A	B	C	D	E*	F*
Red Cells ($x10^9$/L)	60	6	3	0.6	0.3	0.06

*** The level of red cells spike into units E and F will depend of the initial level of red cell contamination after double LD.**

Units were thawed by incubation in a waterbath (37°C, 30 min) and connected to the MacoPharma PLAS-4 LD filter system, methylene blue was added and the units mixed. Finally MB was removed by filtration of plasma through a Blueflex filter (MacoPharma).

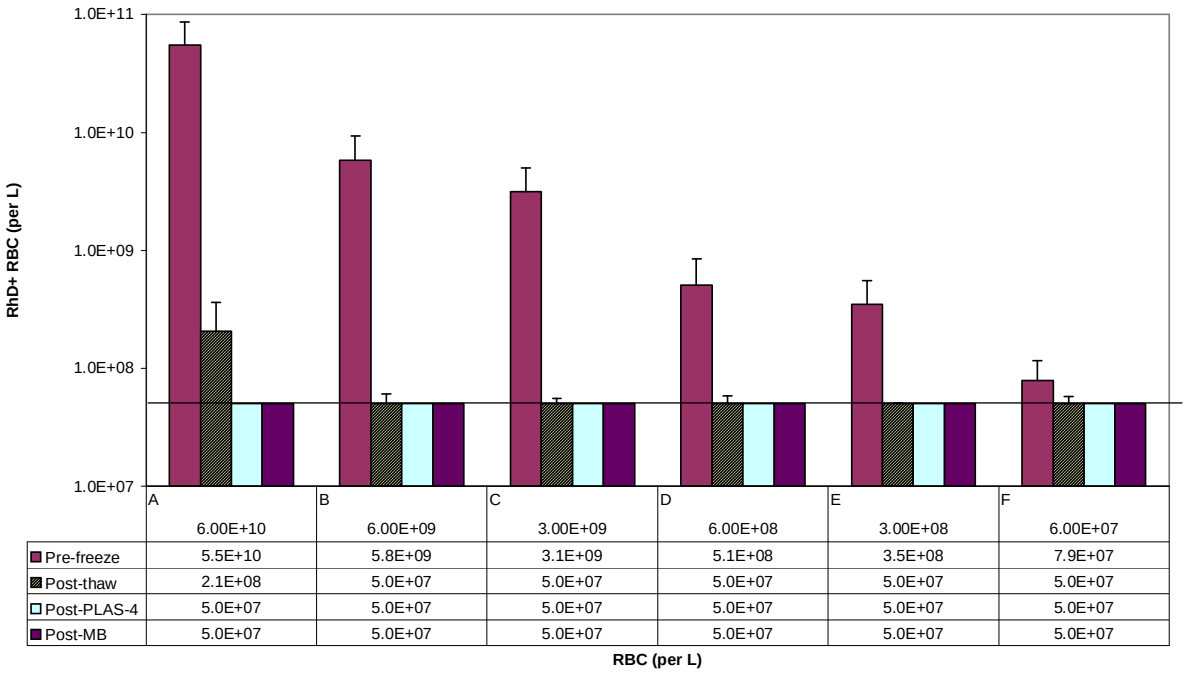
Samples were taken 1) pre-freeze, 2) post-thaw, pre PLAS4, 3) post-PLAS4, pre-Blueflex and 4) post Blueflex. The following tests were performed; full blood count using a Sysmex SE-9000, red cells and red cell microparticles were determined by flow cytometry using monoclonal antibodies to glycophorin A and RhD (dual labelling) and fluorescent calibrant beads.

Results

The haematology analyser was only able to detect red cells spiked at a level of 60 x 10^9/l. Red cell counts as determined by flow cytometry were very close to expected in samples prior to freezing (Figure 1). As expected freezing and thawing of plasma reduces the intact red cell content. Following filtration of plasma with the PLAS4 filter, red cells concentrations were below the limit of detection (<5x10^7/L) even when plasma was spiked with red cell concentrations 1 log higher than would be expected in plasma. As expected, freeze-thawing resulted in an increase in red cell microparticles (Figure 2). At 6 x 10^9/l spiked red cells or below (levels expected in plasma), these were removed below the limit of detection by the PLAS4 filter. At the highest concentration of spiked red cells (60 x 10^9/L), the capability of the PLAS4 filter to remove red cell microparticles appeared to be exceeded. However, the filter used to remove MB also appears to remove microparticles so that these were below the level of detection (5 x 10^7/L) at the end of the process even in the highest spiked sample.

We have also assessed levels of red cells and red cell microparticles in units of plasma that have been imported from the USA and then been treated with the MB system. Levels of red cells in the final component are on average <0.05 x 10^9/l (range <0.05-0.11) and red cell microparticles 0.06 x 10^9/l (range <0.05-0.09).

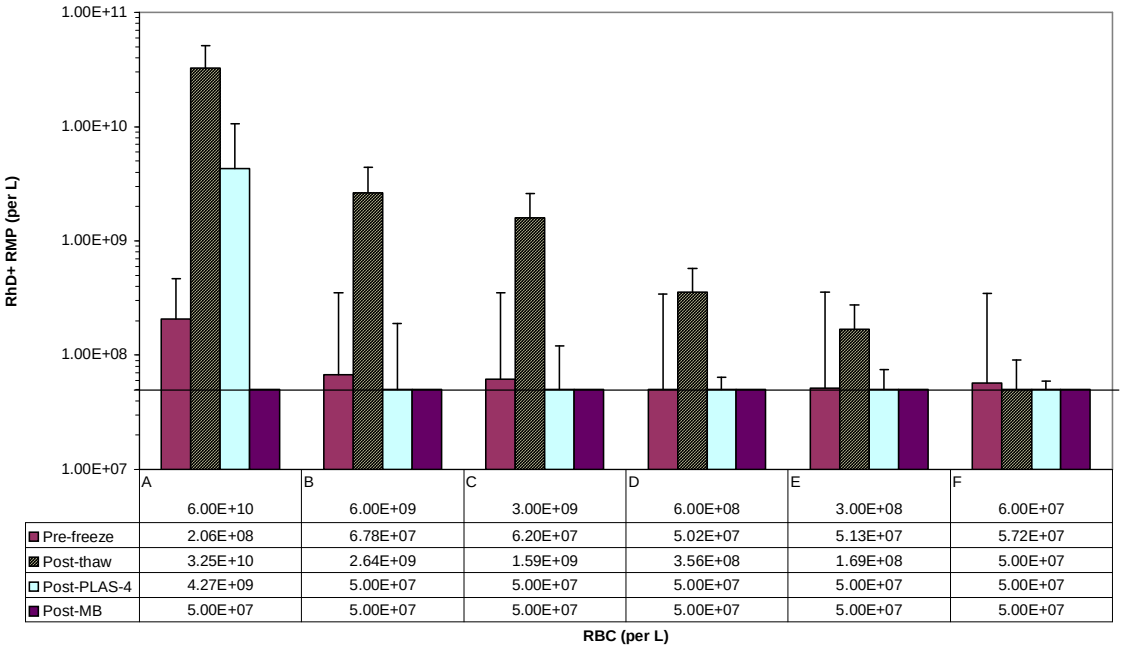
Figure 1 Efficiency of red cell removal by various stages of methylene blue processing



	A	B	C	D	E	F
	6.00E+10	6.00E+09	3.00E+09	6.00E+08	3.00E+08	6.00E+07
Pre-freeze	5.5E+10	5.8E+09	3.1E+09	5.1E+08	3.5E+08	7.9E+07
Post-thaw	2.1E+08	5.0E+07	5.0E+07	5.0E+07	5.0E+07	5.0E+07
Post-PLAS-4	5.0E+07	5.0E+07	5.0E+07	5.0E+07	5.0E+07	5.0E+07
Post-MB	5.0E+07	5.0E+07	5.0E+07	5.0E+07	5.0E+07	5.0E+07

Data shown is the mean +1SD (n=3). Bar represents the lower limit of sensitivity (<5 x 10^7/L).

Figure 2 Generation of red cell microparticles and their removal during methylene blue processing.



	A	B	C	D	E	F
	6.00E+10	6.00E+09	3.00E+09	6.00E+08	3.00E+08	6.00E+07
Pre-freeze	2.06E+08	6.78E+07	6.20E+07	5.02E+07	5.13E+07	5.72E+07
Post-thaw	3.25E+10	2.64E+09	1.59E+09	3.56E+08	1.69E+08	5.00E+07
Post-PLAS-4	4.27E+09	5.00E+07	5.00E+07	5.00E+07	5.00E+07	5.00E+07
Post-MB	5.00E+07	5.00E+07	5.00E+07	5.00E+07	5.00E+07	5.00E+07

Data shown is the mean +1SD (n=3). The Bar represents the lower limit of sensitivity (<5 x 10^7/L).

Appendix 2. Quantitation of red cells in 'standard' plasma

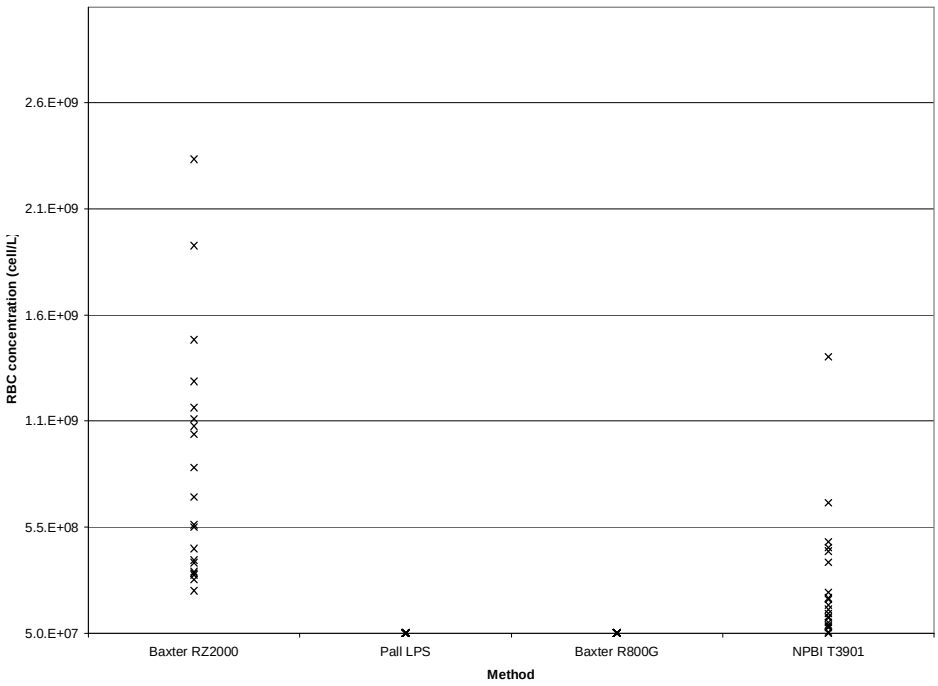
Methods:

The two basic methods used to produce leucocyte depleted (LD) plasma were investigated: 1) plasma produced from filtration of whole blood (top-top processing) and 2) plasma produced from filtration of plasma that had already been separated from whole blood (bottom and top processing). In top-top processing, red cell contamination of plasma is a feature of centrifugation and separation of whole blood and is not dependent on the type of whole blood filter use. Therefore only one type of whole blood filter was assessed (Baxter RZ2000). However, for bottom and top processing, red cell contamination may also be determined by the type of LD filter used, since for plasma these remove red cells to varying degrees. Therefore three different types of plasma filter were assessed (Pall LPS1, Baxter R800G, NPBI T3901). Since we know that levels of red cells in plasma below the current EU specification of 6 x 10^9/l will be undetectable once plasma is frozen (as RBC fragment), plasma was tested prior to freezing as a worse-case scenario. In addition, Council of Europe Guidelines state that residual red cells should be measured in plasma prior to freezing. Levels of red cells were quantified by flow cytometry using monoclonal antibodies to glycophorin A and RhD (dual labelling) and fluorescent calibrant beads.

Results:

Results are shown in Figure 1. As expected, levels of red cells were higher in plasma produced from whole blood filtration with a median of 0.63 x 10^9/l (range 0.25-2.28). Plasma produced by bottom and top processing and filtered using either the Pall or Baxter LD filters had levels of red cells below the detection limit (0.05 x 10^9/l). However, plasma filtered using the NPBI filter did contain detectable levels of red cells with a median of 0.17 x 10^9/l (range <0.05-1.40). None of the units exceeded the EU directive specification of 6 x 10^9/l. For all units it would be expected that freezing of plasma would result in no detectable red cells being present, since we have previously demonstrated this (Appendix 1).

Figure 1. Levels of red cells in fresh plasma processed by four different LD methods



Data points are individual units (n=20 for each method). Baxter RZ2000 = whole blood filter, all other methods are plasma filters. The lower limit of detection is 5 x 10^7/l.