Joint UKBTS Professional Advisory Committee ([1])

UKBTS General Information 07

Validation of Blood Component Quality Following Prion Removal Procedures for Red Cell Components

April 2015

Prepared by: Standing Advisory Committee on Blood Components (SACBC) and Immunohaematology (SACIH) Requirements

This document will be reviewed whenever further information becomes available. Please continue to refer to the website for in-date versions.

Background

A number of devices designed to remove prion protein from blood are in development. At the time of writing, the only CE marked devices for this purpose are specifically designed to remove prion protein from red cell concentrates, and are an additional step to leucocyte depletion. However, it is anticipated that in due course prion removal and leucocyte depletion may be a combined process, and that prion removal may be applied to whole blood.

Manufacturers are strongly advised to consult the UK Prion Working Group prior to commencing any validation studies, since the extent of validation may depend on the exact nature of the device to be validated.

Red Cells

a) In vitro studies

Red cells that have been subjected to a prion reduction process must be evaluated in vitro for the parameters listed in Table 1 as a minimum. The following variables in red cells production must be assessed:

- blood that has been stored at ambient or 4^{o}C prior to prion reduction
- blood that has been anticoagulated using CPD, due consideration may need to be given to alternative anticoagulants
- red cells stored in additive (SAG-M) or CPD plasma (if the device will be CE marked for use with red cells in plasma)

Table 1. In vitro assessment of red cell quality:

Variable	Day 0/1 Pre-removal	Day 0/1 Post-removal	Day6/7	Day 35	Day 42
Volume (ml)	Y	Y	Y	Y	Y
Leucocyte count (10^6/U)	Y	Y	N	N	N
RBC count (10^{12}/U)	Y	Y	N	N	N
RBC loss due to process (%)	NA	Y	N	N	N
Red cell microparticles	Desirable	Desirable	Desirable	Desirable	Desirable
Haemoglobin (g/U)	Y	Y	Y	Y	Y
MCV (fl)	Y	Y	Y	Y	Y
Haematocrit (l/l)	Y	Y	Y	Y	Y
Platelet count (10^9/l)	Y	Y	N	N	N
K+ (mmol/l)	Y	Y	Y	Y	Y
haemolysis (%)	Y	Y	Y	Y	Y
ATP (umol/gHb)	Y	Y	Y	Y	Y
Glucose (mmol/l)	Y	Y	N	Y	Y
2-3, DPG (umol/gHb)	Desirable	Desirable	Desirable	N	N
Lactate (mmol/l)	Y	Y	N	Y	Y
pCO_2/pO_2	Y	Y	N	Y	Y
pH	Y	Y	N	Y	Y

Data from units that have not been through a prion removal device should be included as suitable controls and a minimum of 10 test units for each component should be assessed.

b) irradiated and frozen red cells

Below outlines the minimum validation requirements if prion reduced red cells are then further processed by irradiation or freeze-thawing. It is important to understand whether the effects of prion reduction and either irradiation or freeze-thawing on red cell quality are more than additive. These studies will be performed as part of blood services phase 1 operational studies.

Red cells produced for IUT or exchange transfusion must be irradiated no later than 5 days following donation, and must be transfused within 24 hours of irradiation and no longer than 5 days following donation. Red cells in additive can be irradiated up to 14 days following donation, and must be transfused within 14 days of irradiation.

The main result of irradiation of red cells is an increase in haemolysis of red cells and potassium leakage into the supernatant. Both of these parameters increase during red cell storage and following irradiation.

As a minimum, supernatant levels of potassium and haemoglobin must be assessed in the

worst possible combination of shelf-life and irradiation for the above components (below). Since the haematocrit and storage media of the three types of red cell components listed varies considerably it is advisable to assess each type.

Component	Day of prion removal	Day of irradiation	Time points following irradiation to assess
Red cells for IUT	1	4	0, 24 hours
Red cells for exchange	1	4	0, 24 hours
Red cells in additive	1	14 (and possibly 4)	0, 7 days, 14 days and possibly 24 hours

Red cells are usually frozen within 5 days of donation and must be transfused within 24 hours of thawing if washed using an open system. However, closed systems may allow the shelf-life of thawed and washed red cells to be extended up to 14 days (depending upon the system). As with irradiation, the worst combination should be validated:

Washing system	Day of prion removal	Day of freezing	Time points following thawing to assess
Open system	1	5	0, 24 hours
Closed system	1	5	0, 24 hours, days 5, 7, 14

Data from units that have not been through a prion removal device should be included as suitable controls and a minimum of 10 test units for each component should be assessed.

c) red cells for exchange transfusion

Since the coagulation factor and albumin content in the plasma of red cells used for exchange transfusion is important, this must also be assessed in red cells stored in CPD plasma (in addition to the above tests on red cell quality), if the filter is to be used in this manner. This should be assessed on the day of production, and at day 4 prior to irradiation and day 5 (24 hours following irradiation). Essentially, those assays listed in Table 2 would be required, but a reduced data set may be applicable so manufacturers are advised to consult the UK Prion Working Group. If the filter significantly reduces coagulation factor activity, then re-manufacture of red cells in a suitable FFP product may need to be validated.

d) autologous recovery and survival studies on red cells

Recovery and survival of red cells that have been processed with the prion reduction device must be assessed in healthy volunteers. Red cells in additive and red cells stored in CPD plasma (if the device will be used on red cells in plasma) should be studied at the end of their shelf life, which would be expected to be 42 and 28 days respectively, unless in vitro data indicates that this should be otherwise. Red cells produced from whole blood that has been

stored for 24 hours at 22^{o}C prior to component production may need to be assessed at a reduced shelf-life of 35 days. Results will be considered acceptable if the mean 24 hour recovery is >75% with a SD of <9%.

e) Immunohaematology studies on red cells

Recent results with two red cell pathogen reduction technologies have raised concerns around neoantigen formation in red cells subjected to further processing. While these involve chemical treatments rather than chromatographic contact, there are also examples in the literature of increased immunogenicity arising as a result of chromatographic purification (e.g. as was seen with a high purity plasma derived factor VIII concentrate in the Netherlands). While there are no established
definitive tests in the field that are predictive of neoantigen formation, it might be wise to undertake some level of assessment aimed at this area. The SAC Immunohaematology of UKBTS has suggested the following possible approaches to determine whether or not the prion filtration process has led to any immunologically important changes.

1. Red cell antigen profile changes - it would be possible to look at changes in red cell antigen profile of some of the more labile blood group antigen systems, using defined monoclonal antibodies. However, a reduction in, for example, Duffy antigen presentation would not be expected to be of clinical consequence.
2. Detection of the neo-antigen associated with red cell ageing. Premature ageing of erythrocytes can result from alterations in band 3 protein interacting with the autoantibody present in all normal individuals. There are a number of assays available in the literature for the detection of this autoantibody and the membrane changes that occur in the red cells associated with the altered band 3 protein.
3. CD47 expression. Alterations to the red cell membrane may result in changes in CD47, which is important in self-recognition of red cells, and may protect the circulating red cells from phagocytosis. Again, there are assays available using flow cytometry and FITC conjugated CD47 monoclonal antibodies, which could examine this effect.
4. Compatibility testing against a panel of normal sera or plasma. Examination of neo-antigenicity by animal immunisation may not identify changes recognised only in the human. This would ultimately only be determined by decreased red cell survival in test transfusions, but it might be of value to test pre- and post-filtered red cells against a large number of donor plasmas or sera, to look for cross-reacting antibodies which pre-exist in normal individuals and might result in accelerated red cell clearance after transfusion. For example, such antibodies may have been the cause of accelerated clearance in PEG treated red cells due to unexpected pre-formed circulating antibodies in normal individuals.

Points 1 and 4 will be performed during operational assessment of the reduction device by the UKBTS. Points 2 and 3 are expected to be carried out by the manufacturer.

In addition, binding of red cells to the matrix could be assessed, which might also answer concerns that any PrPc on red cells might result in red cell binding to the matrix, and conformational changes under certain conditions, but needs careful consideration of the

appropriate controls.

However, the only true test is in vivo human studies, and immunohaematology will be assessed in early clinical studies, so any of the above approaches are for reassurance rather than being definitive or predictive.

Fresh-Frozen Plasma

This section is also applicable to red cells for exchange transfusion (see section page 3, section c).

Prion removal technology may also be applied to whole blood. If this whole blood is used to produce FFP, then as a minimum the tests listed in Table 2 would be required to approve the process in terms of effect on the component IN ADDITION TO STUDIES ON RED CELLS.

At least n=10, preferably 20 whole blood units would be required to be studied. Ideally these should be an equal mix of O and A groups due to ABO variability in coagulation factors.

If the prion removed plasma is used as a starting material for other pathogen reduction technologies (e.g. methylene blue treatment), then the combination of prion reduction and pathogen inactivation should be validated.

UKBTS General Information 07

Validation of Blood Component Quality Following Prion Removal Procedures for Red Cell Components

April 2015

Table 2. In vitro assessment of FFP

Variable	Before device	After device	After 12 months of plasma storage*	After 24 months of plasma storage*
Volume	Y	Y	N	N
WBC content (10^6/U)	Y	Y	N	N
Platelet content (10^9/l)	Y	Y	N	N
PT/APTT ratio	Y	Y	N	N
Fibrinogen (g/l)	Y	Y	N	N
Factor VIII	Y	Y	Y	Y
Factors II, V, VII, IX, X, XI, XIII	Y	Y	N	N
vWf antigen	Y	Y	N	N
vWf activity	Y	Y	N	N
vWf multimers	Y	Y	N	N
vWf cleaving protease	Y	Y	N	N
ATIII, protein C, protein S	Y	Y	N	N
TAT complexes or Fragment 1+2/ FPA	Y	Y	N	N
FXIIa	Y	Y	N	N
FVIIa	Y	Y	N	N
C3a and C5a**	Y	Y	N	N
C1-esterase inhibitor	Y	Y	N	N
$Alpha_2$-antiplasmin	Y	Y	N	N

*need not prevent use of component clinically provided data keep ahead of age of product issued. Coagulation assays must be based on the biological activity of the protein where possible. In addition to assessing plasma the volume of blood lost due to the device should be quantified.
** does not apply if device is used pre-storage

([1]) **Joint U**nited **K**ingdom **B**lood **T**ransfusion **S**ervices Professional **Advisory Committee**