Guidelines for the Blood Transfusion Services

8.2: Evaluation of new red cell components for transfusion

http://www.transfusionguidelines.org/red-book/chapter-8-evaluation-of-novel-blood-components-production-processes-and-blood-packs-generic-protocols/8-2-evaluation-of-new-red-cell-components-for-transfusion

8.2: Evaluation of new red cell components for transfusion

8.2.1: Introduction

In establishing any novel component, the development process is expected to involve three stages (see Table 8.1d):

- **Investigation (Phase 0):** Initial intensive investigation of a range of parameters on a relatively small number of units (e.g. 10 -16) to establish concepts. This should involve *in vitro* studies with serial sampling, and may also involve *in vivo* studies. Components produced during this phase should not be used for transfusion. For clarity the guidance on which tests need to be performed is as shown in Table 8.2.

- **Validation (Phase 1/Phase 2):** Operational validation on a larger number of units (e.g. 125) to establish routine operation of the technique, normally testing for those parameters listed in the current edition of the Red Book. These tests may be supplemented by a limited set of assays selected from the investigational phase to allow setting of routine quality parameters. This may involve *in vivo* studies and normally would involve sampling at the times shown below for routine testing.

- **Routine (Local process validation):** Ongoing routine validation using a small set of parameters selected on the basis of the above studies. This will not normally involve *in vivo* studies. Advice may be sought from SACBC on the validation requirements for red cells produced from automated processing of whole blood or other technologies that are not specified in Table 8.2.

Red cell components may be derived either from whole blood or collected by apheresis and, in either case, the standard requirements for donor selection and for mandatory donation microbiological testing should be fulfilled. When well prepared, there is no evidence that the clinical performance of any of these products is different, and the guidance provided below applies equally to the various approaches.

In vitro assays should be performed on samples representative of the pack contents taken by an aseptic technique that does not appreciably alter the gross volume of the pack contents (must be kept to a minimum, but in any case no greater than 10%). Parallel testing of units prepared by a well-established method is recommended, and the use of a split-pool or crossover design will increase the power of such comparisons. If required, *in vivo* studies, preferably with parallel testing of ‘standard’ components, should be performed on the last day of the proposed storage period. The number of units studied should be determined by statistical analysis based on the difference between test and control units to be detected. A sample size of at least 12 tests or controls would be required to detect a 30% difference in ATP and potassium at Day 35 of storage using an unpaired study. Fewer units will be required if a pooled and split study design is used, but should not be less than four.

Red cell components will be stored for the recommended storage period or longer in the case of experimental additive solutions (AS) that are designed to extend the shelf life of red blood cells (RBC). Samples will be taken weekly (or minimally at Days 1, 21, 35 and at the end of storage if this is >35 days) for *in vitro* studies. If required, autologous *in vivo* recovery studies should be undertaken at the end of the storage period.

8.2.2: In vitro studies

The measurements described below and in Table 8.2 will be made at the time of component production (Day 0/Day 1) or other relevant stages of component preparation. An equal number of appropriate control components (e.g. standard AS RBC) should be tested in parallel. Greater consistency of information may be obtained if two or more group-compatible components are pooled and divided prior to processing for *in vitro* studies only. The number of units to be studied should be based on the study objectives and design.

8.2.3: On the day of component production/collection

Weight, volume, haematocrit (L/L), haemoglobin (Hb, g/unit), platelets (x 10^9/unit), red cell loss* (%), platelet loss* (%), leucocyte depletion (given as residual WBC × 10^6/unit) and log depletion*. These results should be obtained by validated test procedures and be within the limits defined by the preliminary component specification.

* Relevant to procedures involving integral filtration or other methods that are likely to result in loss of cellular components during production. Validated techniques using flow cytometry or cell counting chambers should be used to count leucodepleted components and would currently be expected to exhibit a sensitivity of less than or equal to 1 leucocyte per microlitre.

Table 8.2 Evaluation of new red cell components for transfusion: recommended tests

New characteristic parameter	New pack	Leuco-depletion	New centrifugation / component extractor (e. g. Optipress, Compomat etc.)	Novel anti-coagulant	Novel apheresis system	Novel additive solution	Novel plasticiser/ plastic	Irradiation	Pathogen reduction
Unit volume (mL)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Haematocrit (L /L)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Haemoglobin (g/unit)	✓	✓	✓	✓	✓	✓	✓	✓	✓
MCV	✓	✓	✓	✓	✓	✓	✓	✓	✓
		✓			✓		✓		

WBC (x 10^6 /unit) (post-leucodepletion)									
Leucocyte subsets (%) (post-leucodepletion)		?							
Residual platelets (x 10^9/unit)			✓	✓					
Hb loss (g) (post-filter)		✓	✓						
K^+ (mmol/L)	✓	✓	✓	✓	✓	✓	✓†	✓	✓
Haemolysis (%)	✓	✓	✓	✓	✓	✓	✓†	✓	✓
pH				✓		✓	✓	✓	✓
Lactate (mmol /L)				✓		✓	✓	✓	✓
Glucose (mmol /L)				✓		✓	✓	✓	✓
ATP (µmol/g Hb)	✓	✓		✓	✓	✓	✓	✓	✓
2,3-DPG (µmol/g Hb)				✓		✓	✓	✓	✓
Na^+ (mmol/L)				✓		✓	✓	✓	✓
pCO_2 (kPa)				✓		✓	✓	✓	✓
pO_2 (kPa)				✓		✓	✓	✓	✓

Pathogen reduction*	?	✓							
Prion protein (PrPc) and microvesicles		?							?
24-hour recovery (%)				?		✓	✓	?	✓
Recovered plasticiser in supernatant and cells							✓†		
Osmotic fragility						✓	✓		
Microvesicles						✓	✓		
Some components may need to be tested for a combination of parameters, e.g. apheresis red cells in a novel /experimental additive solution (AS) that are also leucodepleted. In this case the sampling requirement includes that of a leucodepleted red cell component and that of an experimental AS component. Where novel plasticiser and additive solution are combined, the requirements for novel plasticiser are sufficient to cover both elements.									
Key:✓ = recommended; ? = optional; other tests are not excluded; *= normally undertaken by the manufacturer; †= also consider the effects of irradiation									

At the end of the storage period components should be checked for sterility and a representative sample tested for 24-hour red cell recovery by a validated technique yielding equivalent results to ^{51}Cr labelling methods. Results will be considered acceptable if the mean 24-hour recovery is >75% with a standard deviation of <9%.

8.2.4: During storage

Parameters to be studied during storage of red cells include: haemoglobin, haematocrit, MCV, ATP, 2,3-DPG, glucose, lactate, potassium, haemolysis (soluble haemoglobin as a percentage of total haemoglobin per mL of whole product), pH, pO_2, pCO_2, cytokines. These may include interleukin-1, IL-1, IL-6, IL-8, TNF- and TGF-. Measurements should, wherever possible, be by bioassay (seek advice from SACBC). Cytokine measurements are complex and may be considered optional. As red cell components are leucocyte depleted, measurement of leucocyte-derived cytokines is probably not informative. Advice should be taken from SACBC on the selection of cytokine tests.

These results should be obtained by validated test procedures. Where manipulation of components during processing might increase the risk of bacterial contamination, microbiological sterility testing should be performed at the end of storage.

Consideration should be given when performing *in vitro* studies to including periods where red cells are removed from controlled temperature environment to reflect current practice where red cells may be temporarily stored out with their designated storage temperature. This might include transport of red cells, equipment breakdown or when red cells are issued and collected for transfusion to a patient.

Novel Plasticisers (please also refer to Table 8.2 and section 8.8 for further guidance):
Where novel plasticisers are used, the levels of recovered plasticiser should be monitored over shelf life to assess the levels of leaching into the blood component. Blood bag manufacturers or external laboratories may be required for chemical analysis of plasticisers. Methodology will be specific to the plasticiser under investigation, but likely to be by liquid chromatography-mass spectrometry. Advice can be sought from manufacturers, SACBC and peer-reviewed literature. It is also important to consider metabolites that may also influence product quality and may have toxicological effects. Concentrations in the supernatant and red cells should be measured at the beginning, during and end of storage to assess leaching and potential patient exposure. Consideration must be given to the effects of irradiation on the bag and subsequent leaching potential. Suppliers must undertake toxicology studies as part of CE/UKCA/UKNI marking. Suppliers must provide evidence of an independent review of toxicology data; this data will then be reviewed by SACBC.

Novel Additive Solutions (please also refer to section 8.8 for further guidance):
Where novel additive solutions are used, effects on storage must also be taken into account. Table 8.2 provides a list of recommended tests. Where there are combinations of novel elements to a blood bag system (e.g. novel plasticiser and additive solution), then Table 8.2 should be used to ensure requirements for each element is included within the minimum recommended tests. There will likely be overlap in requirements and SACBC can provide advice on this if required.

8.2.5: Autologous in vivo studies

See Table 8.2 for details of testing. An equal number of appropriate control components obtained from healthy volunteer donors with ethical approval (e.g. standard AS RBC) should be tested in parallel. The number of components transfused should be justified based on the study objectives and design.