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ORIGINAL ARTICLE

In vitro assessment of red-cell concentrates in SAG-M filtered through the MacoPharma^{TM} P-CAPT prion-reduction filter

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SUMMARY. This study investigated whether filtration of leucodepleted red cells in SAG-M through the P-CAPT^{TM} filter in order to prevent the potential risk of vCJD infection associated with prion transmission through transfusion has any deleterious effect on red-cell quality. Bottom-and-top SAG-M leucodepleted red-cell concentrates (24 units) were prion-reduction filtered on the day following collection, with half of the units undergoing irradiation on day 14. A control group (12 units) was not prion filtered. Units were sampled at 7-day intervals up to day 35 and tested using standard measures of red-cell quality as well as prothrombin content (to examine prion filter efficacy). Haemoglobin loss per unit was ~9 g and in some cases levels were below standard specification (40 g). Haemolysis increased significantly after filtration [0.01 (0.00-0.05) vs. 0.23 (0.07-0.52, $p<0.001$)]. Prothrombin levels were reduced 41.6-fold compared to leucodepleted red-cell units. Product specifications were within or close to routine acceptable levels. Owing to the reduction in haemoglobin levels below those specified, it may be preferable to reduce haemoglobin specification levels and transfuse more prion-filtered units rather than transfuse potentially unsafe blood product. The risk of transfusing more units with less haemoglobin should be offset against the risk of transfusing unfiltered blood.

Key words: haemoglobin, prion-reduction filter, red-cell concentrate, vCJD.

Prion protein (PrP^{C}) normally occurs in a harmless form on cell membranes. Abnormally folded prions (PrP^{Sc}) may cause disease by inducing normal counterparts within the cell to fold in an abnormal manner and aggregate (PrP^{res}). Transmissible spongiform encephalopathies (TSE) are neurodegenerative diseases that are thought to be transmitted by these misshaped prions in humans and mammals. They are transmissible within and between species by inoculation of infected material in the brain, via blood and by ingestion of infected material. Creutzfeldt–Jakob disease (CJD), the most common form of human TSE, is a rare, ultimately fatal disease that has been passed on person to person through medical instruments and organ/tissue transplantation (Bernoulli *et al*., 1977, Ironside & Head, 2003), but not through transfusion (Esmonde *et al*., 1993, Vamvakas, 1999, Wilson *et al*., 2000).

A variant form of CJD (vCJD) appeared in the UK in the mid-1990s as a result of the consumption of tissue or meat, which had been infected with bovine spongiform encephalopathy (BSE). There have so far been over 200 confirmed cases of vCJD worldwide (National Creutzfeldt–Jakob Disease Surveillance Unit, 2008), with over 80% of cases arising in the UK. Three cases of probable transmission of vCJD by transfusion (Llewelyn *et al*., 2004, United Kingdom Health Protection Agency, 2008) and one case of probable transmission of prion (Peden *et al*., 2004) have raised concerns about the transmission of abnormal prion by transfusion.

In order to reduce the risk of transmission of vCJD via blood transfusion, blood components in the Irish Blood Transfusion Service (IBTS) are currently filtered to reduce residual leucocyte content to $<1 \times 10^{6}$ per unit of red cells or platelets. This policy was implemented in the light of observations that PrP^{res} are found

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in the peripheral lymphoid tissue of vCJD patients (Hill *et al*., 1999), that TSE infectivity is associated with buffy coat experimentally (Brown *et al*., 1998), that B lymphocytes transfer infectivity from the periphery to the brain in mice (Klein *et al*., 1997) and that prion disease can be transmitted by transfusion in sheep (Houston *et al*., 2000, updated 2008). More recently a study raised doubts about the ability of leucodepleted blood to clear all infectivity from blood carrying TSE (Gregori *et al*., 2004). Leucodepletion is necessary to remove the infectivity associated with WBCs (cell associated); however, in order to provide prion free product, the remaining non-cell associated infectivity may also need to be removed. To address this problem, filter systems have been tested (Saunders *et al*., 2005, Sowemimo-Coker *et al*., 2006) and a new filter has been developed by Pathogen Removal and Diagnostic Technologies; a joint venture between the American Red Cross, Prometric Life Sciences (Montreal, QC, Canada) and MacoPharma (Tourcoing, France). The P-CAPT™ Prion Capture Filter is a prion specific filter incorporating prion specific ligands for the selective adsorption of prions in leucodepleted red-cell concentrates (RCC) suspended in additive solution or plasma. It has previously been shown that this technology reduces transmissibility of TSE in blood (Gregori *et al*., 2006a,b).

We investigated the quality of leucodepleted RCC after processing through the P-CAPT™ filter and the effect of irradiation on these prion-filtered RCC at day 14 (normal practice). As a marker of filter efficacy we measured supernatant prothrombin levels pre- and post-filtration. According to the manufacturers, prothrombin binds selectively to the filter resin via a mechanism that is different from, but closely related to, prion binding and provides a useful marker of protein binding. As it is not yet possible to detect or measure PrP^{res} in human blood, prothrombin measurement offers a useful surrogate approach.

MATERIALS AND METHODS

All chemicals used in this study were purchased from Sigma-Aldrich, Gillingham, UK unless otherwise stated.

Blood collection

Whole blood (405–495 mL) was collected from healthy donors in Fresenius (Grafelfling, Germany) and Baxter (Vienna, Austria) bottom-and-top quadruple packs according to standard IBTS procedures. Thirty-six units (18 of each type) were held at 22 °C overnight and processed by centrifugation, with red cells taken off the bottom, then leucodepleted and resuspended in additive solution (SAG-M) on day 1. Twenty-four RCC (12 of each) units were connected to and filtered through the P-CAPT™ Prion Capture Filter (MacoPharma, Tourcoing, France). All 36 units were then stored at 4 °C. Twelve filtered units (6 of each) and four unfiltered units (three Fresenius and one Baxter) were irradiated on day 14. One unfiltered Baxter unit was irradiated on day 13.

Sampling

Samples were taken from the red-cell units by sterile connection of a sampling pouch and subsequent transfer of a well-mixed sample to that pouch. Samples were taken prior to prion filtration (day 1 pre), immediately post-filtration (day 1 post), on day 8, day 15, day 28 and day 35 for all samples apart from two Baxter unfiltered samples (1 irradiated, 1 non-irradiated), which were sampled on days 1, 7, 14, 27 and 34 (results were included with those sampled a day later). Whole product (approximately 25 mL) was prepared and assayed for leucocyte (day 1 only), haematocrit, total haemoglobin and ATP. The remainder of the sample (approximately 20 mL) was centrifuged at $3000 \times g$ for 15 minutes and supernatants were stored either at 4 °C or −70 °C. Haemolysis (days 1, 15, 28 and 35), potassium, sodium and prothrombin were measured in the supernatant.

Whole product

White blood cell counts. White blood cell (WBC) counts were performed by flow cytometry (FACSCalibur; BD Biosciences, Oxford, UK) using the BD Leucocount kit™ (BD Biosciences, Oxford, UK). RCC samples were combined with lyophilised bead pellet in BD Trucount tubes before staining with propidium iodide. Samples were then acquired on the flow cytometer and absolute counts were calculated as described by Barclay *et al*., 2000.

Full blood count (erythrocytes, platelets, haematocrit, total haemoglobin). RCC were analysed using a Cell Dyn 4000 (Abbott Diagnostics, IL, USA).

Red-cell ATP

ATP levels were determined in neutralized perchloric acid extracts of the RCC stored at −70 °C. ATP was measured enzymatically using the glucose/hexokinase method, coupled to reduced nicotinamide adenine dinucleotide phosphate formation by glucose-6-phosphate dehydrogenase (Lamprecht & Trautschold, 1974).

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Readings were taken at 340 nm using a VersaMax™ plate reader (Molecular Devices, UK). ATP concentration was calculated using the molar extinction coefficient of NADPH and light path length correction was determined by reading standards at 340 nm in an acryl cuvette in a Jenway 6305 spectrophotometer (Keison Products, Essex, UK).

Supernatant

Potassium and sodium. Levels of potassium and sodium in the supernatant of RCCs were evaluated on days 1 (pre- and post-filtration), 8, 15, 28 and 35. Potassium and sodium were measured by potential difference across ion selective electrodes on an AU analyser 55400 (Olympus, Watford, UK) in supernatant stored at 4 °C.

Haemoglobin

Supernatant samples stored at −70 °C were thawed and assayed for haemoglobin using a Low Haemoglobin Photometer System (HemoCue AB, Angelholm, Sweden).

Haemolysis

Percentage haemolysis was calculated for days 1, 15, 28 and 35 using the following formula:

$$\%\text{Haemolysis} = [\text{supernatant haemoglobin}(\text{g dL}^{-1}) \div \text{total haemoglobin}(\text{g dL}^{-1})] \times [100 - \text{haematocrit}]\%$$

Prothrombin

Prothrombin was measured by ELISA. Supernatant samples stored at −70 °C were thawed and diluted in assay diluent (HEPES 0.1M, NaCl 0.1M, 1% BSA, 0.1% Tween-20, pH 7.4). Prothrombin levels were measured in the diluted samples using a paired set of antibodies (Affinity Biologicals, Ancaster, ON, Canada), one of which is labelled with peroxidase and which converts OPD substrate, at 490 nm. The absorbance was measured using a VersaMax™ plate reader.

Statistical analysis

Significance was determined using the unpaired two-tailed Student's t-test ($p<0.05$) by GraphPad Prism™ (San Diego, CA, USA) software. Groups were tested for normality using the d'Agostino–Pearson test.

RESULTS

Whole blood was processed into leucodepleted red-cell concentrate in SAG-M using Baxter and Fresenius bags, and subsequently filtered through the P-CAPT™ Prion Capture Filter. Prion-filtered units are referred to as test and leucodepleted unfiltered as control. A portion (half) of the test and control samples was subjected to irradiation on day 14 (with the exception of 1 Baxter control on day 13). A single Baxter control was found to be haemolysed (4.8%) after leucodepletion at the outset and was not used in this study. Results below are written as mean (range).

Filtration

Volume pre- and post-filtration was 252 mL (228–274) and 244 mL (221–261) respectively for Fresenius packs, with a mean volume loss during filtration of 8.03 mL, 258 mL (238–275) and 254 mL (228–271) respectively for Baxter packs with a mean volume loss of 4.21 mL (Table 1). This difference was not statistically significant. Haemoglobin levels pre-filtration for red-cell packs were 49.3 g unit^{-1} (39.7–53.0) and 40.5 g unit^{-1} (32.3–43.2) post-filtration (Table 1). Unfiltered control levels were 49.5 g unit^{-1} (41.7–59.4). Leucocyte counts for units pre-filtration were 0.09×10^6 WBC unit^{-1} ($0.00–0.51 \times 10^6$) and for post-filtration 0.02×10^6 WBC Unit^{-1} ($0.00 - 0.10 \times 10^6$). Prion filtration reduced prothrombin levels in leucodepleted red cells from 5.62 μg mL^{-1} (2.63–12.61) to 0.15 μg mL^{-1} (0.05–0.38), a 41.6-fold reduction (Table 2) across the filter. This reduction was similar in both pack types.

Red-cell storage

Haemolysis. Prion filtration resulted in a significant increase in haemolysis in both pack types compared

Table 1. Volume [mean (range)] and haemoglobin content during red-cell storage

Bag type/ Treatment	Test/Control	Pre-P-C apt™ filtration unit volume (mL)	Final unit volume (mL)	Pre-filtration haemoglobin/ unit (g)
Fresenius	Test (n = 12)	252 (228–274)	244 (221–261)	47.2 (39.7–53.1)
	Control (n = 6)	249 (230–268)	n/a	49.5 (41.7–59.4)
Baxter	Test (n = 12)	258 (238–275)	254 (228–271)	51.5 (43.7–57.3)
	Control (n = 5)	254 (239–262)	n/a	49.5 (44.2–51.9)

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Table 2. Prothrombin levels [mean (range)] before and after filtration

Bag type	Test/Control	Prothrombin levels (µg/mL)		Fold reduction across prion filter
		Day 1 Pre	Day 1 Post	
RCC	Test (n = 24)[a]	5.83 (2.63-8.13)	0.14 (0.05-0.38)	41.6
	Control (n = 6)	6.74 (3.70-12.61)	n/a	n/a

a n = 23 for Day 1 Pre owing to sampling error

to non-filtered controls (Fig. 1). This difference was apparent immediately after filtration, and persisted, though did not worsen throughout the storage period in non-irradiated units. There was also a significant increase in irradiated units. One Baxter control unit was haemolysed (4.8 %) from day 1 and was excluded from this study. All the remaining units were within specification for haemolysis (<0.8%) at day 28 and up to day 35 for non-irradiated units. Two Baxter irradiated units showed greater than 0.8% haemolysis on day 35.

ATP. There was no significant difference between filtered and non-filtered units throughout storage. There was an overall reduction in ATP levels after day 8, but this was not affected by pack type or irradiation (Fig. 2).

Potassium and sodium. Potassium levels did not differ significantly between filtered and control units. There were significant differences between irradiated and non-irradiated units (Fig. 3). Filtration and irradiation affected sodium levels significantly. There was an increase in sodium ion concentration immediately after filtration, and a reduction after irradiation, which persisted until day 35. The type of pack had no influence on sodium levels.

DISCUSSION

Three clinical cases of vCJD (Llewelyn *et al.*, 2004, United Kingdom Health Protection Agency, 2008) in patients who were homozygous for methionine at codon 129 of the PrP gene, have been ascribed to transfusion of prions from asymptomatic donors. In addition, transmission of prions without subsequent clinical disease by the time of death occurred in a fourth case who was heterozygous for methionine at codon 129 of the PrP gene, indicating that susceptibility may

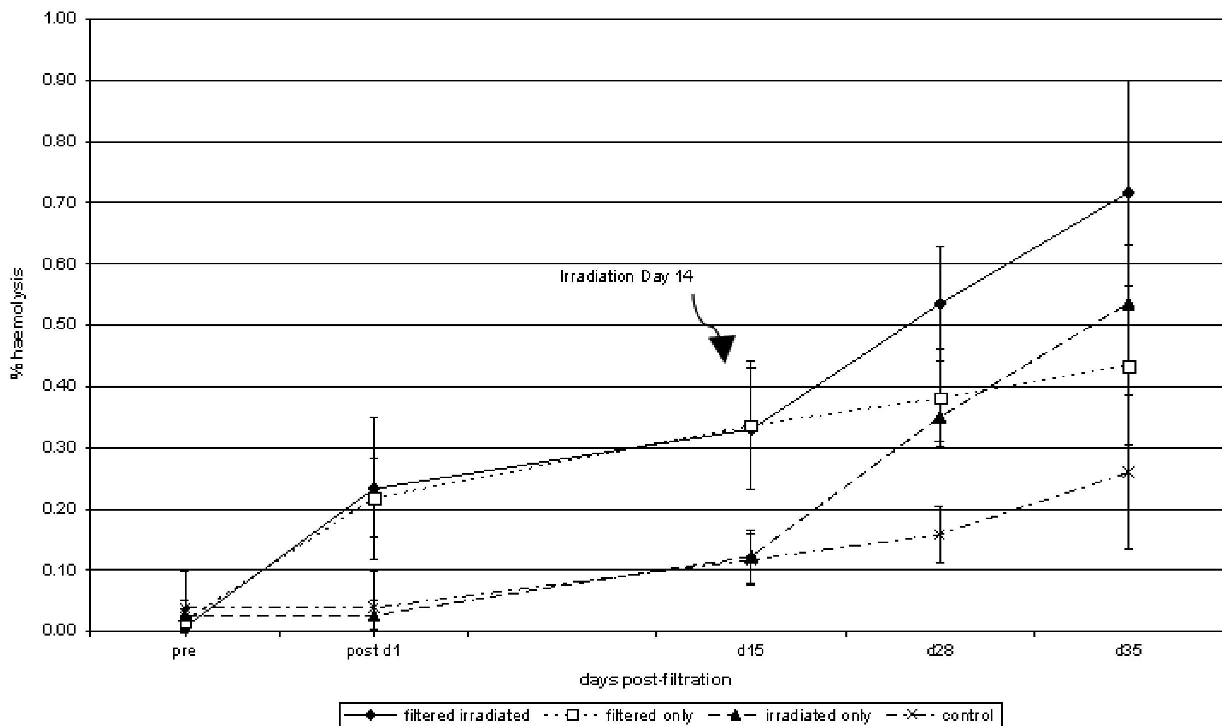

Fig. 1. Percentage haemolysis in red-cell packs after prion filtration and irradiation (mean ± standard deviation).

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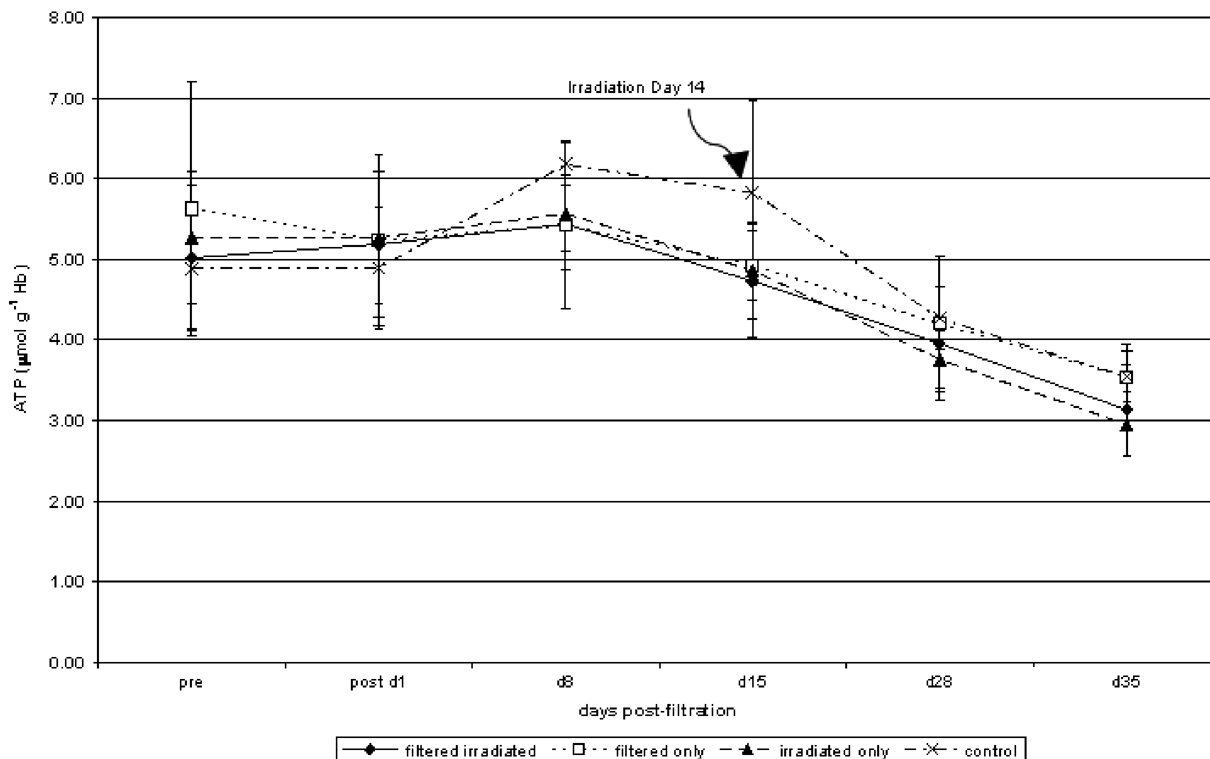

Fig. 2. ATP concentration [μmol g^{-1} haemoglobin (Hb)] after filtration and irradiation (mean ± standard deviation).

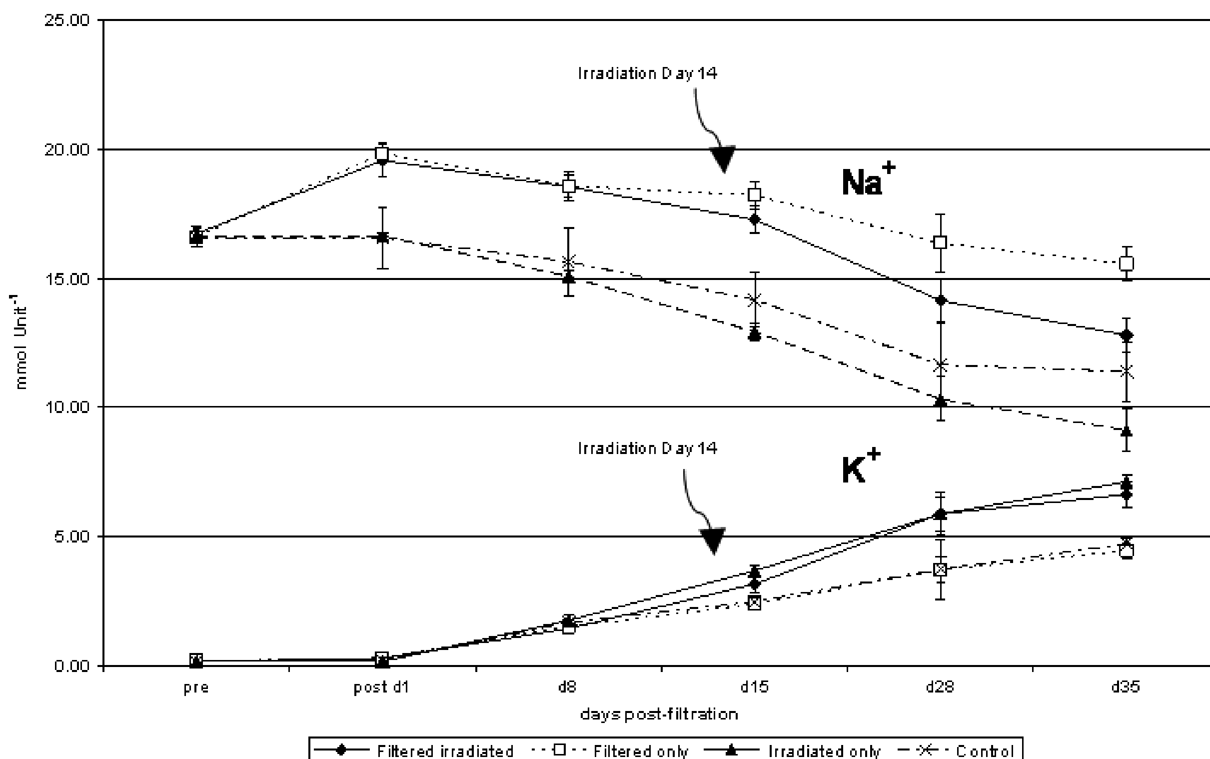

Fig. 3. Potassium and sodium concentration (mmol Unit^{-1}) after prion filtation and irradiation (mean ± standard deviation).

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not be limited to the methionine homozygotes as was previously thought (Peden *et al*., 2004). In the absence of any screening methods for this lethal disease, these probable transmissions of vCJD through blood transfusion have made the removal of potentially infective material from blood concentrates prior to transfusion an attractive option in countries where there may be significant numbers of infected donors (Klein *et al*., 1997, Brown *et al*., 1998, Houston *et al*., 2000). Current practice in Ireland to reduce the risk of transmission of vCJD by transfusion includes the removal of leucocytes; however, leucoreduction may not be a wholly effective method of preventing prion transmission by blood transfusion, as residual infectivity may persist in the plasma fraction (Brown *et al*., 1999, Gregori *et al*., 2004).

This study describes the *in vitro* assessment of red cells filtered using the P-CAPT^{TM} prion filtration system manufactured by MacoPharma. The filter system is adapted for red-cell units that have been leucodepleted and suspended in additive solution containing less than 120 mL of residual plasma. Standard measures of leucodepleted RCC in additive solution were analysed at 7-day intervals (apart from day 22) from day 1 to day 35. Volume, haemoglobin and residual leucocyte counts were measured before and after filtration. ATP, haemolysis, sodium and potassium during storage were measured. Upper and lower limits were specified by the Council of Europe Guidelines (Council of Europe Publishing, 2004) and Guidelines for the Blood Transfusion Service in the UK, 2005, Table 9.2. In addition the red-cell loss during the filtration process was measured. Separately, we studied the applicability of measuring residual prothrombin levels across the filter as a method of validating filter integrity. Prothrombin is removed by the prion filter via a parallel but distinct method to that of prion removal. Reduction in prothrombin levels should therefore, at least partially, mirror potential prion removal.

Filtration using the P-CAPT^{TM} device resulted in significant changes in the filtered units. Most marked was the loss of approximately 9 g of haemoglobin from the unit, because of a hold up of red cells in the dead space of the filter. This is an intractable problem, resulting in approximately 22.5% loss of haemoglobin per unit. The filter process also caused haemolysis of the red cells. Haemolysis levels did not breach the allowable threshold of the European technical specifications in any of the units studied (Council of Europe Publishing, 2004) (Fig. 1). Increased plasma haemoglobin levels were detectable immediately following filtration, and persisted throughout the shelf-life of the product. ATP levels did not vary significantly from control levels and were acceptable ($>2.5 \mu\text{mol g}^{-1}$ haemoglobin) at the end of storage (Fig. 2). Potassium consistently leaked from red cells into storage buffer during storage regardless of pack type. Sodium levels in storage buffer decreased at a rate comparable to potassium increase, apart from post-filtration samples, which showed an initial increase (Fig. 3). Prothrombin levels were reduced 41.6-fold compared to leucodepleted samples (Table 2), indicating that this parameter may provide a useful indicator of filter integrity and function for quality assurance and control purposes.

Red cells filtered using the P-CAPT^{TM} device meet with Council of Europe standard specifications for red-cell units leucocyte depleted in additive solution, with the exception of the haemoglobin content of the final product, though the increased haemolysis observed in this study emphasises the need for careful evaluation of patients receiving red cells filtered using this device in Phase 1 studies. We propose to address the haemoglobin loss by introducing a new specification for haemoglobin content for prion-filtered red cells of 35 g haemoglobin per unit, by assessing red-cell requirements in multi-transfused patients in subsequent clinical studies, and by urging manufacturers to expedite development of an integrated leucodepleting prion-reduction filter. Prion filtration is a very serious measure, taken in response to the present risk of transmission of vCJD through blood transfusion. Although reducing the specification for haemoglobin content of a red-cell unit is not ideal, we consider that it may be justified on the balance of risk for the time being. The target blood volume collected for whole blood donors in Ireland is 470 mL; raising the target blood volume to 500 mL will help address this issue. Some patients may need to be transfused with additional units of blood because of this device, though adopting the approach of Arslan *et al*. (2004) may reduce this possibility. It is reasonable to consider that in the light of current understanding of the present risks of transfusion, particularly the unquantifiability of the risk of transmission of vCJD, that transfusion of five units of prion-filtered blood is preferable to transfusion of four units of non-prion filtered blood. It is important to note that the basis of the 40 g specification has no systematic foundation. This is based entirely on pragmatic grounds defined by the producers, and not on any basis of patient dose or delivery. Patient dosing with red cells according to current guidelines and best practice requires defining a target haemoglobin level in the patient, and titrating the number of units transfused against that target. Thus the haemoglobin content of the transfused units may alter the number of units required to achieve the desired level, but should not generally affect the final level achieved.

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In addition we may be able to improve the yield from bottom top and units, that is, abandon the necessity to recover buffy coats from red-cell units for platelet manufacture and replace the platelet supply through apheresis. Lastly future developments by filter manufacturers should address the issue of haemoglobin loss, for example by combining leucodepletion and prion filtration in a single devise. For the time being, reducing the specification for haemoglobin content in the final product, and fully informing the product users of the situation, represents in our view a practicable method of ensuring that this important safety measure can be introduced in a controlled manner.

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REFERENCES

Arslan, O., Toprak, S., Arat, M., Kayalak, Y. (2004) Hb content-based transfusion policy successfully reduces the number of RBC units transfused. *Transfusion*, **44,** 485–488.

Barclay, G.R., Walker, B., Gibson, J., McColl, K., Turner, M.L. (2000) Quality assurance by a commercial flow cytometry method of leucodepletion of whole blood donations: initial application of universal testing and proposals for a batch-release sampling plan. *Transfusion Medicine*, **10,** 37–48.

Bernoulli, C., Siegfried, J., Baumgartner, G., Regli, F., Rabinowicz, T., Gajdusek, D.C., Gibbs, C.J. (1977) Danger of accidental person-to-person transmission of Creutzfeldt–Jakob disease by surgery. *Lancet*, **1,** 478–479.

Brown, P., Cervenáková, L., McShane, L.M., Barber, P., Rubenstein, R., Drohan, W.N. (1999) Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt–Jakob disease in humans. *Transfusion*, **39,** 1169–1178.

Brown, P., Rohwer, R.G., Dunstan, B.C., MacAuley, C., Gajdusek, D.C., Drohan, W.N. (1998) The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. *Transfusion*, **38,** 810–816.

Council of Europe Publishing (2004) *Guide to the Preparation, Use and Quality Assurance of Blood Components*, 10th edn. Strasbourg, Council of Europe Publishing.

Esmonde, T.F., Will, R.G., Slattery, J.M., Knight, R., Harries-Jones, R., de Silva, R., Mathews, W.B. (1993) Creutzfeldt–Jakob disease and blood transfusion. *Lancet*, **341,** 205–207.

Gregori, L., Gurgel, P.V., Lathrop, J.T. *et al*. (2006a) Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins. *Lancet*, **368,** 2226–2230.

Gregori, L., Lambert, B.C., Gurgel, P.V.*et al*. (2006b) Reduction of transmissible spongiform encephalopathy infectivity from human red blood cells with prion protein affinity ligands. *Transfusion*, **46,** 1152–1161.

Gregori, L., McCombie, N., Palmer, D., Birch, P., Sowemino-Coker, S.O., Giulivi, A., Rohwer, R.G. (2004) Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encaphalopathies from blood. *Lancet*, **364,** 529–531.

Guidelines for the Blood Transfusion Services in the United Kingdom, 7th Edn. (2005) The Stationery Office Ltd. http://www.transfusionguidelines.org.uk/ (last accessed 10/7/2008).

Hill, A.F., Butterworth, R.J., Joiner, S.*et al*. (1999) Investigation of variant Creutzfeldt Jakob disease and other human prion diseases with tonsil biopsy samples. *Lancet*, **353,** 183–189.

Houston, F., Foster, J.D., Chong, A., Hunter, N., Bostock, C.J. (2000) Transmission of BSE by blood transfusion in sheep. *Lancet*, **356,** 999–1000.

Houston, F., McCutcheon, S., Goldmann, W.*et al*. (2008) Prion diseases are efficiently transmitted by blood transfusion in sheep. *Blood*, **112,** 4739–4745.

Ironside, J.W., Head, M.W. (2003) Variant Creutzfeldt–Jakob disease and its transmission by blood. *Journal of Thrombosis and Haemostasis*, **1,** 479–486.

Klein, M.A., Frigg, R., Flechsig, E.*et al*. (1997) A crucial role for B cells in neuroinvasive scrapie. *Nature*, **390**, 687–690.

Lamprecht, W., Trautschold, I. (1974) Adenosine-5'-triphosphate. Determination with hexokinase and glucose-6-phosphate dehydrogenase. In: *Methods of Enzymatic Analysis* (eds Bergmeyer, H.U.). 2102–2110. Academic Press, New York.

Llewelyn, C.A., Hewitt, P.E., Knight, R.S.G., Amar, K., Couesens, S., Mackenzie. J., Will, R.G. (2004) Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. *Lancet*, **363,** 417–421.

National Creutzfeldt–Jakob Disease Surveillance Unit (NCJDSU), UK http://www.cjd.ed.ac.uk/vcjdworld.htm 4 July (2008) (Accessed 10/7/2008).

Peden, A.H., Head, M.W., Ritchie, D.L., Bell, J.E., Ironside, J.W. (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet*, **364,** 527–529.

Saunders, C., Herbert, P., Rowe, G.*et al*. (2005) In-vitro evaluation of the PALL Leukotrap Affinity Prion Reduction Filter as a secondary device following primary leucoreduction. *Vox Sanguinis*, **89,** 220–228.

Sowemimo-Coker, S.O., Pesci, S., Andrade, F., Kim, A., Kascsak, R.B., Kascsak, R.J., Meeker, C., Carp, R. (2006) Pall leukotrap affinity prion-reduction filter removes exogenous infectious prions and endogenous infectivity from red cell concentrates. *Vox Sanguinis*, **90**, 265–275.

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United Kingdom Health Protection Agency (HPA) *Variant CJD and blood donors and recipients* (updated 9 April 2008). http://www.hpa.org.uk/infections/topics_az/cjd/menu.htm.

Vamvakas, E.C. (1999) Risk of transmission of Creutzfeldt–Jakob disease by transfusion of blood, plasma, and plasma derivatives. *Journal of Clinical Apheresis*, **14**, 135–143.

Wilson, K., Code, C., Ricketts, M. (2000) Risk of acquiring Creutzfeldt–Jakob disease from blood transfusion: systematic review of case-control studies. *British Medical Journal*, **321,** 17–19.

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