



## METHODS TO EVALUATE THE EFFICACY OF PRION REDUCTION FILTERS

### ISSUE

1. The UK Blood Services (UKBS) is currently undertaking an evaluation of prion reduction methodologies developed to reduce the potential for vCJD transmission via blood transfusion. The UKBS Prion Reduction Group have asked SEAC to comment on a proposed approach and experimental models to evaluate whether prion reduction filters reduce vCJD infectivity in blood sufficiently to have an impact on transmission risks.

### BACKGROUND

#### TSE transmission via blood

2. There is compelling evidence that vCJD infection can be transmitted via blood transfusion, including:
  - Hunter and colleagues<sup>1</sup> demonstrated using a transfusion model in sheep that BSE and scrapie could be transmitted via blood transfusion from BSE infected donor sheep (both at the clinical and asymptomatic stages of infection) to uninfected recipient sheep.
  - Two cases of probable human transmission of vCJD via blood transfusion of non-leucodepleted red blood cells (RBC) have been reported<sup>2,3</sup>.
3. A recent review on the risk of vCJD transmission via blood transfusion is included as Annex 1 for further information.

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<sup>1</sup> Hunter *et al.* (2002) Transmission of prion diseases by blood transfusion. *J Gen Virol.* 83, 2897-905.

<sup>2</sup> Peden *et al.* (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 364, 527-529.

<sup>3</sup> Llewelyn *et al.* (2004) Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion *Lancet.* 363, 411-412.

## Prion reduction filters

4. A number of commercial companies are developing technologies for use by blood services to reduce the risk of vCJD transmission via transfusion of blood and blood components. UKBS are currently commissioning an independent evaluation of the efficacy of a prion reduction filtration method developed by the Pall Corporation. The evaluation will inform consideration of the possible introduction of these filters in addition to the measures already undertaken to improve the safety of blood.
5. UKBS is evaluating whether use of these filters will adversely affect the quality of blood<sup>4</sup>, this aspect is not considered in this paper.
6. At SEAC 87 (April 2005), SEAC was asked by UKBS for advice on the use of prion reduction filters for blood. The committee noted it was not the role of SEAC to validate commercial products or endorse their use. However, it strongly recommended that the UKBS should commission an independent validation of such products. Once such a validation had been completed and a risk assessment conducted to examine the efficacy of the filters in reducing transfusion associated prion disease transmission, the committee could comment on the data presented.
7. UKBS is taking forward a programme of work to evaluate prion reduction filters and has asked SEAC to advise on its proposed approach to this evaluation.

## Pall Corporation filter

8. Performance characteristics of the Pall prion reduction filter have been published<sup>5</sup>. It is reported that filtration of human RBC spiked with hamster scrapie brain homogenate reduced infectivity by 3.7 log<sub>10</sub> LD<sub>50</sub>/ml (from 9.2 to 5.5 log<sub>10</sub> LD<sub>50</sub>/ml) as measured by hamster bioassay. Western blot analysis showed that a strong signal which was detected in the pre-filtration sample was reduced to below detectable levels post-filtration (see Annex 2). In a further experiment, infectivity in pooled blood from hamsters with clinical hamster scrapie following intracranial inoculation was measured by hamster bioassay pre- and post filtration. Infection was transmitted to 6 out of 43 animals inoculated with pre-filtration blood. In

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<sup>4</sup> Saunders *et al.* (2005) In-vitro evaluation of the PALL Leukotrap Affinity Prion Reduction Filter as a secondary device following primary leucoreduction. *Vox Sang.* 89, 220-228.

<sup>5</sup> Sowemimo-Coker *et al.* (2005) Removal of exogenous (spiked) and endogenous prion infectivity from red cells with a new prototype of leukoreduction filter. *Transfusion.* 45, 1839-1844.

contrast, none of the 35 animals inoculated with post-filtration blood succumbed to disease after 300 days as assessed by clinical signs and the absence of detectable PrP<sup>Sc</sup> in the brain (see Annex 2).

## **UKBS APPROACH TO EVALUATION OF FILTERS**

9. The UKBS Prion Reduction Group propose to investigate the potential of the Pall filters to reduce infectivity in human leucodepleted RBC using a three stranded approach (the study specification developed by the Group is given at Annex 3):

(i) replication of the key experimental data from the company using the same prion strain/animal system.

(ii) evaluation in a different experimental system that could be applied on similar technologies produced by other companies to provide comparable data.

(iii) evaluation of the filter using a practicable system that replicates as closely as possible the human blood transfusion situation.

10. To achieve objectives (i) and (ii) the Group is planning:

(i) a study of the efficacy of the filters to reduce the infectivity in human leucodepleted RBC spiked with hamster scrapie (strain 263K) brain in three forms: crude brain homogenate, microsomal fraction and sonicated microsomal fraction.

(ii) a study of the efficacy of the filters to reduce the infectivity in human leucodepleted RBC spiked with mouse adapted BSE (strain 301V) spleen (or brain).

11. It is recognised that blood spiked with spleen or brain homogenate may not closely reflect infectivity in blood from an individual infected with vCJD. However, such spiking experiments can be used to evaluate the efficacy of filters over a very wide range of infectivity titres. Currently, spiking experiments of blood with high titre infectivity brain homogenate, has been used to measure a large reduction in infectivity.

12. There is a great deal of uncertainty about the level of infectivity in blood, its distribution in blood components and the effectiveness of the existing infectivity reduction method used (leucodepletion). Consequently, there is much uncertainty regarding the level of

infectivity reduction that filters would need to achieve to have a significant affect on transmission risks. However, on the basis of two plausible scenarios for infectivity in blood and the distribution of infectivity in blood components, a 3-4 log<sub>10</sub> reduction in infectivity will be required to significantly reduce transmission risk. Both of these scenarios are based on a large number of assumptions about the infectivity of blood and the distribution of infectivity in blood components. Details on these scenarios are not provided (one is based on the DNV assessment of infectivity in blood<sup>6</sup> which was considered by the committee at SEAC 90) and are illustrative of the sort of efficacy that might be required to reduce infectivity of blood.

13. Given the uncertainty in the required efficacy of filters to reduce in infectivity levels in blood to have a significant effect on transmission risks, experiments to measure the efficacy of filters may need to be conducted over a wider infectivity range than 3-4 log<sub>10</sub> suggested by these scenarios.
14. The UKBS Prion Reduction Group also wishes to undertake a study to evaluate the efficacy of filters using a model that replicates as closely as possible the human blood transfusion situation (i.e. using blood with endogenous infectivity). The Group would welcome SEAC advice on the most appropriate model(s) to use.

### **Experimental models for endogenous infectivity in blood**

15. Blood from clinical cases of vCJD that could be used to measure the efficacy of infectivity removal of filters is in very short supply. Thus, it is necessary to consider blood collected from TSE infected animals as alternatives to model human blood that could be used for such evaluations. Key considerations for such models would be:
  - blood donor species.
  - prion strain (somewhat dependent on test species).
  - method to detect infectivity (somewhat dependent on donor species) or PrP<sup>Sc</sup>.

### Donor species

16. Blood could be collected from a number of different animal species experimentally infected with a TSE. Non-human primates, sheep,

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<sup>6</sup> <http://www.dnv.com/consulting/news/riskofinfectionfromvariantcjdinblood.asp>

and rodents have been used previously to study the distribution and pathogenesis of TSEs.

17. Infectivity in blood from non-human primates with TSE may mimic the human situation most closely. However, practical, ethical, time and resource implications may make use of these species unfeasible.
18. Sheep have been used to model vCJD infectivity in human blood<sup>7</sup> as relatively large volumes of blood can be obtained and the pathogenesis of BSE in sheep is similar to that of vCJD in humans.
19. One alternative is the use blood from rodents (mice or hamsters) infected with a TSE. However, the small amounts of blood available from each animal would require pooling of blood from many animals. Furthermore, a rodent model may be less applicable to the human situation by comparison with non-human primates and sheep. Recently developed transgenic mouse lines that express the human prion protein gene could be used. However, these models have not been in widespread use.
20. It is possible that the level of endogenous infectivity in blood from animals may not be high enough to test the filters over the 3-4 log<sub>10</sub> reduction range thought necessary on the basis of at least two scenarios for infectivity in human blood. However, it would provide a more realistic model for infectivity human blood than blood spiked with TSE infectivity.

### Prion strain

21. The choice of prion strain to evaluate endogenous infectivity is important. The most appropriate strain might be human vCJD. However, the properties of this strain in a non-human host could be somewhat altered. Studies on infectivity in blood have used rodent adapted scrapie, CJD or vCJD<sup>8</sup>. BSE and scrapie have been used in experimental model in sheep of TSE transmission via blood transfusion<sup>9</sup>. Thus, depending on the donor species, consideration would have to be given to whether a species adapted strain, non-

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<sup>7</sup> Hunter *et al.* (2002) Transmission of prion diseases by blood transfusion. *J Gen Virol.* 83, 2897-2905.

<sup>8</sup>e.g Cervenakova *et al.* (2003) Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. *Transfusion* 43, 1687-1694. Yakovleva *et al.* (2004) Effect of protease treatment on plasma infectivity in variant Creutzfeldt-Jakob disease mice. *Transfusion* 44, 1700-1705

<sup>9</sup> Hunter *et al.* (2002) Transmission of prion diseases by blood transfusion. *J Gen Virol.* 83, 2897-2905.

species adapted or the human strain should be used. Similarities in the pathogenesis and distribution of infectivity of a TSE strain in the donor species and vCJD in humans is possibly the most important factor to consider.

#### Method to detect infectivity/PrP<sup>Sc</sup>

22. Detection of TSE infectivity requires a bioassay. To achieve the optimal sensitivity of the bioassay the recipients of blood or blood components pre- and post-filtration should be the same species as the blood donor(s). Thus, the choice of bioassay is somewhat dependent on the choice of blood donor. The route of administration is a further consideration. Intracerebral inoculation is generally considered to be the most efficient route of transmission. However, the amount of blood that can be administered by this route is limited. Larger quantities of blood or blood components can be administered by the intravenous route which also mimics the human situation more closely. The choice of bioassay will also determine to some extent the length of time that results from experiments would be expected. Rodent bioassays would provide results in a shorter period of time than experiments in non-human primates and sheep. A number of humanised mice with increased susceptibility to human prion diseases may be useful in this regard<sup>10</sup>.
23. Sensitive analytical tests for PrP<sup>Sc</sup> (e.g. Western blot, conformation dependent immunoassay (CDI) and paraffin-embedded tissue (PET) blot) can also be used to measure the efficiency of filters. However, as noted previously by SEAC (SEAC 90)<sup>11</sup>, PrP<sup>Sc</sup> may not always be a good surrogate marker for TSE infectivity.

#### **ADVICE SOUGHT FROM THE COMMITTEE**

24. The committee is asked to:
  - advise on the suitability of experiments and animal models used in the three stranded approach proposed by UKBS to evaluate the efficacy of prion reduction filters.

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<sup>10</sup> e.g. Wadsworth *et al.* (2004) Human prion protein V129 prevents expression of variant CJD phenotype. *Science*. 306, 1793-1796.

<sup>11</sup>[http://www.seac.gov.uk/summaries/seac89\\_summary.pdf](http://www.seac.gov.uk/summaries/seac89_summary.pdf)

- consider whether the additional experiments to evaluate prion reduction filters using a model that more closely replicates the human blood transfusion situation are needed and, if so, advise on the most appropriate model to use.
- comment on whether any additional experiments should be undertaken that the committee consider would provide important additional data.



**Risks of transmission of variant Creutzfeldt-Jakob disease by blood transfusion**

Peden *et al.* (2005) *Folia Neuropathol.* 43, 271-8.



**Removal of exogenous and endogenous prion infectivity from red cells with a new prototype of leucoreduction filter.**

Sowemimo-Coker *et al.* (2005) *Transfusion* 45, 1839-1844



**Research specification developed by UKBS Prion Reduction Group for studies to evaluate prion reduction filters**

The following specification was developed by the UKBS Prion Reduction Group as an outline of the independent evaluation of prion reduction filters. The specification only includes experiments using blood spiked with TSE infectivity.