



vCJD INFECTIVITY AND PLASMA DERIVATIVES

ISSUE

1. The Department of Health (DH) has requested that SEAC:
 - (i) assesses the timing of possible infections from plasma derivatives that may have been contaminated with the variant Creutzfeldt-Jakob Disease (vCJD) agent when derivatives were prepared using plasma sourced in the United Kingdom (UK).
 - (ii) re-examines the likely removal of vCJD infectivity during the production of plasma derivatives when prepared using plasma sourced in the UK.

BACKGROUND

2. Very few blood donations are transfused as whole blood. Most are separated into blood components (red blood cells, platelets and plasma) for transfusion. Plasma from donors is also pooled and separated into a number of different plasma derivatives to treat specific blood disorders.
3. Transfusions of non-leucodepleted¹ red blood cell concentrates, derived from blood from asymptomatic individuals that later developed clinical vCJD, have transmitted vCJD^{2,3,4,5}. There are concerns that transfusions of other blood components and plasma derivatives from the blood of asymptomatic individuals with vCJD, may also lead to infections. Therefore, a number of precautionary

¹ In the United Kingdom since 1998, blood components have been processed to reduce their white blood cell content (leucodepletion) to reduce the risk of transmission of vCJD. The effectiveness of this measure is uncertain.

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

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

⁴ Wroe *et al.* (2006) Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. *Lancet*. 2006 368, 2061-2067.

⁵ Editorial team. (2007) Fourth case of transfusion-associated vCJD infection in the United Kingdom. *Eurosurveillance* 12, 1.

measures have been introduced in the UK to reduce the potential for transfusion associated transmission of vCJD. These have included the importation, since 1998, of plasma used to make plasma derivatives from countries considered to have little or no Bovine Spongiform Encephalopathy (BSE). No clinical cases of vCJD infection associated with the use of plasma derivatives have been identified. However, as the possible incubation period of such infections is unknown, if infections have occurred, it may be too early for any to have developed into clinical disease.

4. SEAC has considered the potential infectivity of blood from individuals infected with vCJD on numerous occasions, most recently at SEAC 92 (April 2006). The committee assessed the likely infectivity of whole blood, red blood cells, white blood cells, platelets and plasma and published a position statement (Annex 1).
5. The committee last considered the possible infectivity of plasma derivatives at SEAC 74 (June 2002) when reviewing a draft analysis of the possible infectivity of whole blood, blood components and plasma products by Det Norske Veritas Consulting (DNV)⁶.
6. SEAC agreed at SEAC 97 (May 2007) to a DH request to re-examine the likely timing of possible infections, and the removal of infectivity, from plasma derivatives prior to 1999. The basis of, and background to, the DH request is explained at Annex 2.

SEAC CONSIDERATION

Timing of infectivity

7. DH has asked the committee to consider the likely time period when infections from plasma derivatives from UK-sourced plasma, if they occurred, may have happened. Thus, the timing of such infections is dependent on:
 - (i) the timing of the UK BSE epidemic.
 - (ii) the timing of use of plasma derivatives from UK-sourced plasma.
8. Back-calculation models of BSE infected carcasses entering the human food chain suggest that almost all dietary exposure to BSE

⁶ DNV Consulting. (2003) Risk assessment of exposure to vCJD infectivity in blood and blood products. <http://www.dnv.com/consulting/news/riskofinfectionfromvariantcjdinblood.asp>

occurred during the period 1980 to 1996 with a peak in exposure between 1987 and 1991⁷ (see Annex 3). Thus, human dietary BSE infections may have begun to accumulate in the UK population from 1980 onwards, possibly increasing in rate until after the BSE epidemic started to decline.

9. Following concerns about the possible risk of transmission of vCJD via blood transfusion, plasma for the production of plasma derivatives was from 1998 sourced from outside the UK. Thus, transmissions of vCJD via plasma derivatives, if they occurred from UK blood donations, should have ceased from 1998/1999 onwards.
10. Given the timing of these events, the risk period for vCJD infections from plasma derivatives spans from 1980 (the start of the BSE epidemic) to 1998/99 (the introduction of use of non-UK sourced plasma for production of plasma derivatives). However, given the dynamics of the BSE epidemic and consequent variation in dietary BSE exposure in the UK, the risk of vCJD infection from use of plasma derivatives will not be constant during this period. DH propose, therefore, that the time window of greatest risk will be narrower if the following factors are taken into consideration:
 - (i) as dietary BSE exposure was relatively low in the early years of the BSE epidemic (see Annex 3), it is possible only a small proportion of human BSE infections may have occurred in the early 1980s. Thus, it may be reasonable to assume that period of greatest risk from vCJD infection from plasma derivatives occurred later into the BSE epidemic.
 - (ii) blood may not be infectious during the early part of the incubation period. Thus, it may be reasonable to assume that following the accumulation of dietary BSE infections in the blood donating population, there would be a delay in the rise of the risk of secondary vCJD infections from use of plasma derivatives compared with the timing of the BSE epidemic.
11. When considering how the level of infectivity may change over the course of the incubation period SEAC concluded (see Annex 1) that *“the available data show that blood is infectious during the preclinical stage of vCJD. Although the precise time in the incubation period of vCJD at which blood becomes infectious is unclear, data from animal models suggest it may be infectious from at least, if not before, the middle of the incubation period.”* This

⁷ Donnelly *et al.* (2002) Implications of BSE infection screening data for the scale of the British BSE epidemic and current European infection levels. *Proc. R. Soc. Lond. B.* 269, 2179-2190.

conclusion is largely based on unpublished experiments⁸ that examined infectivity in the blood of hamsters at around half and three quarters of the way through the incubation period and at clinical stage of disease following intracerebral or oral inoculation with hamster scrapie. Infectivity was first detected at the mid-point of the incubation period with the level of infectivity increasing relatively linearly towards the clinical stage of infection. Extrapolation of these data suggested infectivity could have first appeared in blood at around a third of the way into the incubation period (see Annex 1).

12. The mean incubation period for primary vCJD is uncertain. Two estimates of the mean incubation period for primary vCJD from analyses based on clinical vCJD case data both suggest it may be around 12.6 years (95% confidence intervals of 8 – 35⁹ and 10.5 - 14.7 years¹⁰) for the MM genotype (see Annex 3). Given the lack of clinical vCJD cases of the MV or VV genotype observed to date, the mean incubation period of primary vCJD in these genotypes must be longer than that of the MM genotype.
13. Given the estimated incubation period and an assumption that vCJD infectivity does not appreciably accumulate in blood during the first third of the incubation period, it may be reasonable to assume that plasma from a large proportion blood donors infected with primary vCJD may not be infectious for several years following infection.
14. If the assumptions above about dietary BSE exposure and/or the accumulation of vCJD infectivity in blood are accepted, the start of the period of greatest/appreciable risk of infection from UK sourced plasma derivatives could be significantly narrowed. This could allow a re-assessment by DH of exposure to potentially contaminated plasma derivatives.

Removal of infectivity during plasma derivative production

15. DH has asked the committee to re-examine estimations of the removal of vCJD infectivity during the production processes used to prepare plasma derivatives prior to the importation of plasma into the UK. Currently the infection risk to recipients of plasma derivatives prior to importation of plasma for fractionation, uses the

⁸ Unpublished data from the VA Medical Center, University of Maryland, Baltimore, USA presented by Dr R Rohwer.

⁹ Ghani *et al.* (2003) Updated projections of future vCJD deaths in the UK. *BMC Infect. Disease.* 3, 4-11.

¹⁰ Boelle *et al.* (2004) Epidemiological evidence of higher susceptibility to VCJD in the young. *BMC Infect. Disease.* 4, 26-32.

most pessimistic of three scenarios developed in the DNV risk assessment (described in paragraph 5 and provided at Annex 4).

16. The three scenarios (described in detail on pages 9-12 of the DNV report and pages 28-36 of Annex II of the report) are:

(i) the largest single clearance factor approach. This uses experimental data from studies that use plasma spiked with transmissible spongiform encephalopathy (TSE) infectivity to estimate the clearance from the various steps involved in plasma fractionation. As data suggest that clearance of infectivity from the fractionation steps is unlikely to be additive, the fractionation step with the largest clearance of infectivity is considered representative of the entire process in this scenario.

(ii) The no additional clearance approach. This assumes that other than the reduction in infectivity from separation of blood into blood components and plasma into fractions, there is no additional clearance of infectivity from processing into derivatives. Levels of infectivity on plasma fractions are derived from experiments measuring the infectivity in plasma fractions produced from blood from mice infected with mouse adapted CJD.

(iii) The protein content approach. This considered the infectivity level to correlate to the protein content of plasma derivatives but this approach was rejected as it was considered scientifically invalid.

17. There are uncertainties around the clearance values derived from studies using plasma spiked with TSE infectivity. Spiked plasma is used principally because the low levels of infectivity in plasma and the lower levels following plasma processing make it very difficult to demonstrate the effect of the various processing steps on the levels of endogenous infectivity in plasma. Plasma can be spiked with, for example, brain homogenate from infected animals to levels sufficient to allow clearance of infectivity or abnormal prion to be measured by bioassay or by biochemical tests, respectively. However, there are concerns that the physico-chemical nature of the infectious agent in spiked plasma is not representative of the form in plasma from infected hosts.

18. Approach (i) gives larger reductions in infectivity than approach (ii) with between approximately 10 to 100-fold differences in vCJD infectivity level depending on the plasma derivative (see figure 3.4 on page 11 of the DNV report). Currently approach (ii) is used,

which gives higher infectivity levels for plasma derivatives than approach (i).

19. DH has asked SEAC to consider whether it would be appropriate to re-assess the clearance of vCJD infectivity during plasma processing utilising data from spiking experiments.
20. To support this consideration, a recently published review is provided at Annex 4 of plasma derivative production and experimental studies examining the removal of TSE infectivity from plasma products during processing. Also, Annex A of Annex 2 discusses the possible physico-chemical differences between the form of abnormal prion protein in brain homogenate (the most commonly used spiking material) and the form of abnormal prion protein as it may appear in blood and how these differences may influence the nature of the results obtained from spiking experiments. In addition, SEAC will receive a presentation at SEAC 98 from a representative of Bio Products Laboratory (the main centre for plasma fraction in the UK) on plasma processing and on the possible reductions in vCJD infectivity during plasma fractionation.
21. DH would also welcome the committee's views on the potential infectivity of cryoprecipitate that is formed on thawing of fresh frozen plasma and collected by centrifugation in the initial plasma fractionation step.

ADVICE SOUGHT FROM THE COMMITTEE

22. The committee is requested to:
 - consider whether it is reasonable to assume that the time window of greatest risk of infections from UK sourced plasma products is shorter than the period from 1980 to 1998/99, and if so, suggest what limits might be appropriate for the period of greatest risk (see paragraphs 7-13).
 - consider whether the likely clearance of vCJD infectivity during the production process for plasma derivatives should be re-assessed to utilise data from experimental studies using plasma spiked with TSE infectivity (see paragraphs 14-19).
 - consider what research could be conducted to make more reliable assessments of the likely clearance of vCJD infectivity during plasma processing.



SEAC position statement on TSE infectivity in blood



POSITION STATEMENT TSE INFECTIVITY IN BLOOD

Issue

1. The UK blood services and Department of Health (DH) asked SEAC to consider data on the nature of transmissible spongiform encephalopathy (TSE) infectivity in blood and the implications for transmission of variant Creutzfeldt-Jakob disease (vCJD) via transfusion of blood products. The committee considered four specific issues:
 - (i) the level of TSE infectivity in whole blood and the distribution of infectivity amongst individual components of blood,
 - (ii) the change in the level of TSE infectivity in blood over the course of the incubation period of disease,
 - (iii) the relative efficiencies of the intracranial (ic) and intravenous (iv) routes of inoculation,
 - (iv) the dose-response relationship for TSE infection.

Background

2. Blood has been shown to carry TSE infectivity in a number of different animal models¹⁻⁴. Three cases of probable vCJD transmission via transfusion of non-leucodepleted red blood cells provide strong evidence that blood from humans infected with vCJD can carry the infectious agent during the pre-clinical stage of the disease⁵⁻⁷.
3. Precautionary measures, including leucodepletion and importation of fresh frozen plasma for children, have been implemented by the UK blood services to reduce the risk of vCJD transmission via blood transfusion. Additional blood processing technologies that may further reduce transmission risks are under consideration. Assessment of the potential effectiveness of new technologies relies on assumptions about the nature of the infectivity in blood, particularly the level and distribution of vCJD infectivity in blood components. However, there is much uncertainty about the nature, level and distribution of infectivity in blood. An assessment produced by Det Norske Veritas Consulting (DNV) and reviewed by SEAC

provides a working model for the level of vCJD infectivity in blood and the distribution of infectivity between blood components⁸.

4. At SEAC 92, SEAC reassessed some of the assumptions in the DNV risk assessment by consideration of recent published literature and unpublished data presented by a number of researchers⁹⁻¹³. Many of the available data were derived from animal studies that have used prion strains, inocula and routes of administration that may not be directly applicable to the human blood transfusion situation. Most of the data are from studies of infectivity in hamster blood infected with hamster scrapie^{3,10,13,14} and mice infected with mouse adapted vCJD². Many of the hamster studies have not yet been published and therefore, have not been subject to the usual peer review process. Extrapolation of data from studies of hamster scrapie to vCJD is complicated by differences in the pathogenesis of these diseases, particularly the low level of lymphoreticular system (LRS) involvement in the pathogenesis of hamster scrapie in contrast to vCJD. Limited data, that may be more relevant, are available from ongoing studies of the infectivity in the blood of sheep experimentally infected with BSE or scrapie as the pathogenesis of these diseases involve the LRS in this model¹¹.

Level of TSE infectivity in whole blood

5. The levels of infectivity reported in rodent studies to examine the infectivity in blood of animals with TSEs vary widely, ranging from about one to 300 infectious doses*(ID)/mL of blood. One large unpublished study¹⁰ involving a series of experiments to measure the infectivity in samples of pooled blood from large groups of hamsters with hamster scrapie suggests a mean level of infectivity of around 10 ID/mL of blood (range of two to 24 ID/mL of blood). In a published study, levels of mouse adapted vCJD infectivity within this lower range were found in blood components from mice at late pre-clinical or clinical stages of infection². There are no data on the infectivity in the blood of humans with vCJD to assess the relevance of these data to humans.

Origin of blood infectivity

6. The source of infectivity in blood is not understood. Unpublished comparisons of the infectivity in blood from intact and splenectomised hamsters suggest that the spleen is not the source of infectivity in blood¹⁰. Unpublished comparisons¹⁰ of the rate of increase of infectivity in pooled blood and brain from infected hamsters during the incubation period of

* An infectious dose is the minimum single dose expected to cause one infection in a given population.

hamster scrapie suggest that it is not the result of leakage from the central nervous system (CNS) into the blood supply¹⁰. However, a single published study that measured abnormal prion protein (PrP^{Sc}) in the buffy coat (white blood cells and platelets) from single hamsters infected with hamster scrapie suggests that PrP^{Sc} concentrations in blood are bimodal with a peak in the pre-clinical phase from peripheral replication in the spleen and other lymphoid tissues, followed by a larger rise in PrP^{Sc} concentrations leading into the clinical stage of the disease from leakage from the CNS¹³.

Distribution of infectivity in blood components

7. Published and unpublished data from studies of the infectivity in components of blood from hamsters with hamster scrapie show that around one half of the infectivity in blood can be removed by depleting blood of white blood cells³, and that the infectivity associated with the white blood cells can be substantially depleted by extensive washing¹⁰. In addition, infectivity is not, or is minimally, associated with platelets¹⁴ or red blood cells¹⁰. These data suggest, at least in this model of TSE infection, that infectivity may be distributed equally between plasma and white blood cells but is weakly bound to white blood cells. Data from published experiments to measure mouse adapted vCJD infectivity in components of blood taken at the late pre-clinical or clinical stages of disease also suggest that infectivity is principally associated with plasma and white blood cells, minimally associated with red blood cells but that there may be some association with platelets². The buffy coat from sheep with scrapie or BSE has also been shown to transmit infection by transfusion to healthy recipient sheep^{1,11}. It is possible that there are inter-species and inter-strain differences in the distribution of TSE infectivity in blood components. Therefore, additional research to examine the infectivity in blood components, particularly from models using TSE strains closely related to vCJD, will allow assessment of the relevance of these data to humans infected with vCJD.

Change in infectivity during the incubation period

8. A number of studies in animals have examined the level of infectivity in blood during both the pre-clinical and clinical stages of TSE infection^{2,9,11}. An unpublished study¹⁰ examined the infectivity in the blood of hamsters after ic inoculation with hamster scrapie at a number of time points during the preclinical stage of infection. Infectivity was first detected at the mid-point of the incubation period with the level of infectivity increasing linearly towards the clinical stage of infection. Extrapolation of these data suggests infectivity may first appear in blood at around a third of the way into the

incubation period. Similar findings were obtained when the experiment was repeated using oral inoculation. Although the relationship between PrP^{Sc} and infectivity is unclear, PrP^{Sc} concentrations in the blood of hamsters infected with hamster scrapie show a bimodal profile (as described in paragraph 7)¹³. Studies of mouse adapted vCJD^{2,9} and sheep infected with scrapie or BSE^{1,11} only examined the level of infectivity at one point during the preclinical stage of infection but show that blood is infectious during the second half of the incubation period. Two cases of probable blood transfusion associated transmission of vCJD from blood donors 20 months⁵ and 3.5 years⁷ prior to the onset of disease have been identified, indicating that human blood can be infectious in the preclinical phase. More extensive data, particularly from models using TSE strains closely related to vCJD, will inform on the relevance of the findings in the hamster scrapie model to changes in infectivity in the incubation period of vCJD in humans.

Relative efficiency of the ic and iv routes of transmission

9. The efficiency of transmission varies depending on the route of administration, host, TSE strain, source of inoculum and how it is prepared. Most measurements of TSE infectivity are derived from bioassays using the ic route of administration. Since the efficiencies of the ic and iv routes of transmission may not be equivalent, the infectivity of an inoculum measured by the ic route may not reflect the infectivity of the same inoculum administered by the iv route, this latter route being the most relevant to blood transfusion. A small number of studies using different animal models have compared the infectivity of brain homogenate or purified blood components administered by the ic or iv routes. These studies suggest that the efficiency of transmission by the iv route is between 10% and 100% of the efficiency of ic route^{2,4,9,14,15}. One unpublished study¹⁰, comparing the efficiency of iv transfusion of intact whole blood and ic inoculation of sonicated whole blood from hamsters with hamster scrapie showed the iv route to be considerably less efficient than suggested by this range. In addition, a published study² showed that mouse adapted vCJD could be transmitted equally efficiently from inoculation of buffy coat from infected animals by the ic or iv routes but the transmission efficiency from inoculation of plasma was lower by the iv compared with the ic route. These studies suggest that the form of inoculum may strongly influence the relative transmission efficiencies of inoculation by different routes.

Dose-response relationship

10. Evidence from animal studies¹⁰ suggests that TSE infectivity is quantal in nature. An infectious dose diluted by distribution to a number of individuals reduces the risk of transmission to an individual. However, at the population level, one of the exposed individuals would be still be expected to become infected. Higher doses split between individuals would lead to more than one infection. The implication of this relationship between dose and probability of infection for strategies to reduce the public health risks in relation to blood transfusion is that pooling blood to dilute infectivity does not decrease the risks to public health. Indeed, depending on the dose, pooling is likely to increase the risks to public health. Strategies to remove or inactivate infectivity in blood would reduce the risks of transmission to both the individual and at the population level.

Conclusions

11. The available data show that blood is infectious during the preclinical stage of vCJD. Although the precise time in the incubation period of vCJD at which blood becomes infectious is unclear, data from animal models suggests it may be infectious from at least, if not before, the middle of the incubation period. The source of infectivity in blood is not understood. Data from rodent studies suggests that infectivity in whole blood is around 10 ID/mL and that it mostly resides in the plasma and white blood cell components with infectivity associated with white blood cells substantially depleted by extensive washing. However, additional information from other animal models is required to assess whether these findings may be closely representative of vCJD infectivity in human blood. It is clear that an infectious dose in blood can be disseminated but not diluted by distribution to a large number of recipients. Consequently, pooling of potentially infectious material, or in other ways disseminating infectious material between a number of recipients, will not reduce the number of people infected, and is likely to increase the number of people infected.

*SEAC
July 2006*

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**Risk of vCJD transmission from UK-derived plasma products:
reassessment of infectivity assumptions**

Dr Peter Bennett (DH)

Risk of vCJD Transmission from UK-derived Plasma Derivatives: the case for a reassessment of infectivity assumptions

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27th April 2007

The following note for SEAC 97 sets out the case for a reassessment of the vCJD infectivity likely to be contained in plasma derivatives sourced from an infected donor. If this case is accepted, it is requested that SEAC carry out such a reassessment at its next meeting (SEAC 98 on 20.07.2007)

Issue

DH Risk Assessments for vCJD transmission via blood and blood products (plasma derivatives) have considered a wide range of assumptions, but have until recently made use of scenarios for infectivity suggested in work commissioned from DNV (1999, 2003) which summarised existing research on animal models.

For blood *components* (red cells, platelets, fresh frozen plasma), revised infectivity scenarios are now available, based on new evidence discussed by SEAC in 2006. It is *not* suggested that these be re-examined at this stage.

However, assumptions for plasma derivatives - e.g. Factor XIII or albumin - were *not* formally reconsidered in 2006. Furthermore, the UK risk assessment is based on more pessimistic scenarios than those used in other countries. This has had a significant impact on risk management, via highly “precautionary” recommendations of CJDIP. (By contrast, here is much less international variation with regard to the potential risks from receipt of infected blood components.)

The *time* at which infectivity would appear in blood, within an infected donor’s incubation period is relevant, as well as the *level* of infectivity present. To date, risk management has been based on the precautionary assumption of infectivity being present throughout the incubation period, though there is some evidence to suggest a delay. A change in this assumption could have implications both for recipients of plasma derivatives and for other “at risk” groups.

Background

Prior to 1999, pooled plasma products such as clotting agents were manufactured from UK-sourced plasma. Given the size of the pools used (of the order of 20,000), risk assessment has been based on the assumption that each pool would have contained one donation from an individual infected with vCJD. The risk to any individual recipient then depends on how great an infective dose they would have received – bearing in mind that many recipients have repeated and long-term need for the products. This dose is calculated by estimating how much of any product would have been received (exposure), then multiplying this by the assumed infectivity (infective doses per ml) of the product(s) in question. The figures involved are subject

to large and multiple uncertainties. CJDIP advice classifies recipients as “at risk for public health purposes” unless their estimated additional risk of infection is clearly less than 1%, even using pessimistic assumptions.

The calculations for these recipients are carried out using a spreadsheet held by HPA, using an infectivity scenario suggested by DNV. (DNV noted various ways of estimating the residual infectivity in plasma products: the calculations use the most pessimistic of these.) This approach has led to around 5,000 individuals being placed in the “at risk” category, the large majority being haemophiliacs exposed to UK-sourced products, who were categorised *as a group* rather than individually. This reflects their generally high exposure to clotting agents.

Coincidentally, we understand that new data on historical exposure to plasma products may become available from Bio Products Laboratory (BPL), the main centre for plasma fractionation in the UK. Revised risk calculations for the Incidents Panel will need to make use of this. This strengthens the case for ensuring that any calculations are based on the most up-to-date assumptions regarding both exposure *and* infectivity.

The historical nature of this transmission route is significant. Any vCJD risk from products manufactured by BPL should have been avoided from 1999 onward, when plasma imported from the US replaced UK-sourced plasma for fractionation.

Discussion

(1) Levels of infectivity in plasma products

A starting point for any calculation is the infectivity assumed to be present in plasma from an infected donor. In the DNV scenarios, about half the infectivity per unit of whole blood prior to leucodepletion is associated with the plasma component, and this is also true in the scenarios discussed in 2006. Preliminary calculations suggest that adopting the working assumptions endorsed by SEAC in 2006 *for components* would not dramatically change the calculations for derivatives.

Rather, **the key issue is the clearance of infectivity during manufacturing processes such as fractionation and filtration.** It has been shown that these processes can remove large proportions of infectivity experimentally spiked into blood. However, these high “clearance factors” have not been demonstrated for endogenous infectivity. Indeed, the relatively low levels of endogenous infectivity in blood means that high clearance factors are probably impossible to demonstrate experimentally *even if they occur*. Given this, the question is one of how much weight should be attached to the “spiking” results. Discussion in international fora – e.g. provided by WHO - has not led to any consensus. Some of the issues are summarised in Annex A, kindly provided by Dr John Stephenson.

This problem is similar to that of evaluating the efficacy of prion removal from blood components. In that context, SEAC recommended that the efficacy of new methods could be sufficiently validated experimentally by demonstrating large reductions in spiked infectivity combined with a (necessarily) much smaller removal of endogenous infectivity. This raises the question of why no consensus exists over the removal of

infectivity from plasma derivatives – specifically, whether the standards of proof demanded are consistent with each other.¹

The approach adopted for prion filter evaluation combines use of spiked blood to evaluate the efficacy of filters quantitatively, with experiments to confirm that endogenously infected blood when processed through a filter is not infectious in bioassays. This acknowledges that low initial infectivity prevents bioassay being used to quantify any large reductions in infectivity. This suggests that the weight attached to spiking experiments by SEAC may depend on the availability of data from “confirmatory” endogenous experiments.

Current UK risk assessments *ignore results from spiking experiments altogether* when calculating infectivity in plasma derivatives. An initial key question is therefore whether this is still judged to be appropriate.

If some weight were to be given to spiking experiments, a supplementary question is that of what differential to apply as against removal of endogenous infectivity. For example, one might decide that a process demonstrably reducing spiked infectivity by, say, a factor of 4 logs should be assumed to reduce endogenous infectivity by 2 logs – still a very different proposition from assuming no reduction.

A further consideration is that the clearance factors achieved by a succession of steps would not necessarily be additive. That is, combining two steps with clearance factors of 2 logs and 3 logs would not necessarily produce a reduction of 5 logs. This is illustrated in an alternative approach considered by DNV: to count only the *highest single clearance factor* demonstrated (from spiking experiments) for any of the manufacturing steps. In the DNV scenario, this leads to estimated levels of infectivity for individual derivatives typically 10 to 100-fold lower than those currently used, though these differentials vary considerably. (For example, the reduction for Factor VIII Z8 is only roughly 4-fold, though that for High Purity Factor VIII is roughly 100-fold). As a further comparison, the FDA risk assessment used in the US considers clearance factors ranging from 100 to 100 million (8 logs).

Note that in estimating risks to existing recipients of UK-sourced plasma products, the relevant questions concern processes that were standard in the UK prior to 1999. Any more recent evidence would need to be interpreted in that light.

(2) Timing of infectivity

A further consideration is the *timing* of possible donations from infected donors. In contrast to the basic DNV scenario, that adopted by SEAC recognises that blood-borne infectivity is likely to rise during a donor’s incubation period. Specifically, it assumes that infectivity would be low during the first third of the incubation period.

¹ Our understanding is that the UK blood services are currently planning for the efficacy of prion filters to be evaluated without any *independent* (non-manufacturer’s) test for removal of endogenous infectivity, which arguably widens the difference in approach.

If we assume that most primary infections will have taken place in the late 1980s / early 1990s, this suggests that **the blood of most infected donors would not have become infective until the early to mid 1990s**. Any risk to recipients would therefore have been largely confined to donations made during a relatively short period between (say) 1992 and 1998/9, especially toward the end of that period. (This would fit with the timing of the *component*-associated transmissions seen so far.) If this argument is accepted, its implications for exposure to “implicated” products would bear examination.

Suggested way forward

Pursuing these questions would require a scientific re-examination of assumptions on plasma derivatives, for which SEAC provides the authoritative forum. To ensure effective discussion, the committee may wish to invite additional contributors to this session. Preparatory scientific work on the part of the secretariat may also be needed. For these reasons, it is suggested that a full discussion should be scheduled for **SEAC 97 on 20.07.2007**. Even if the end result reaffirms the current highly-precautionary view, there would be merit in having prompted a more up-to-date discussion.

Annex A: On using spiked blood samples to evaluate screening tests

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The following points are drawn from a scan of the published literature

PrP Structure

The native form of the PrP protein is a member of a class of proteins called GPI-anchored membrane proteins. A unique feature of this class of membrane proteins is that they sit on the external surface of the cell's plasma membrane, unlike classical membrane proteins which are firmly anchored to the cell membrane by part of their amino acid chain which either penetrates the membrane's lipid layer or passes through it. GPI-anchored proteins are however fixed to the lipids of the cell membrane through a glycosylphosphatidylinositol (GPI) bond (see the figure for details of these structures).

Unlike conventional membrane-bound proteins, GPI-anchored proteins can be readily released into the external environment by the action of membrane-associated phospholipases. As GPI-anchored proteins typically contain lipophylic domains (and PrP is no exception), the released form of these proteins are normally dimers or small oligomers. Thus, the molecular size of PrP aggregates in plasma or preparations of blood products is likely to be only twice that of the monomer or at the most 4-6 times its size.

Most materials used for spiking studies are derived from infected brain homogenates and therefore contain mainly membrane fragments from disrupted cells. The PrP molecules in these preparations are likely to be mainly membrane bound and present in large membrane fragments, typically 100-1,000 times the size of a PrP molecule, or even larger.

Procedures for fractionating blood and blood products

It is my understanding that most of the processes used to separate plasma and to fractionate blood products employ centrifugation and/or chromatography. Both these techniques rely on the relative size of molecules or molecular complexes for their effect and therefore native PrP and PrP in spiked samples will behave differently because of their size difference. Moreover, spiked samples may give a falsely optimistic estimate of purification as the large membrane aggregates will sediment faster in a centrifuge or are more easily separated by chromatography than the smaller, soluble native oligomers of PrP.

Other technologies used by the blood services employ differential precipitation. Again, the use of spiked samples derived brain homogenates will give a falsely optimistic estimate of purification as their very high lipid content will result in them behaving differently from native soluble PrP oligomers.

I have only discussed the behaviour of PrP here as we have accurate structural information on only the native molecule, but it would be reasonable to assume that PrP^{sc} will behave in an analogous manner.

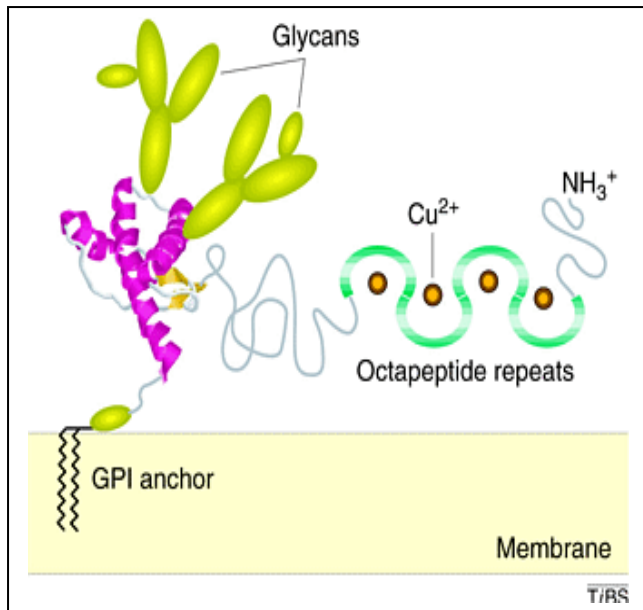


Figure 1. Schematic of the normal cellular form of the prion protein (PrP^c) showing its GPI membrane anchor, the two N-linked glycans and the octapeptide repeats that bind metal ions.

◀ Cleavage site



Donnelly *et al.* (2002) Implications of BSE infection screening data for the scale of the British BSE epidemic and current European infection levels. *Proc. R. Soc. Lond. B.* 269, 2179-2190.

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Provided to SEAC members with the hard copy of the paper