II.4 Effect of Blood Processing on Infectivity

II.4.1 Production of Blood Components

Primary processing of donated whole blood involves segregation into the main components (red cells, plasma and sometimes platelets) by centrifugation but none of these procedures results in a product containing one component exclusively. The partitioning of infectivity can be estimated using the data from Section II.3.6. There is no evidence that any further reduction in infectivity occurs in the processing. It is possible that natural breakdown of white cells following donation leads to progressive increase of infectivity in platelets, hence reducing the infectivity in red blood cell preparations that include buffy coat. Recent studies have indicated that, at least with current methods of processing, such repartitioning of white cell fragments occurs. Even for blood donations processed before present methods were introduced, any such effect would be within the range of values obtained by the different estimates in Figure II.3.1.

II.4.2 Leucodepletion

II.4.2.1 Leucodepletion of Red Cells

Leucodepletion involves removal of white cells from the blood by simple filtering. This is at present applied to all donations collected since October 1999 but many of the incidents considered by the CJD Incidents Panel relate to donations made before the introduction of leucodepletion. The UK standard for white cell content of leucodepleted red cells is $<5 \times 10^6$ in 100% of units. It is believed to achieve a 3 log reduction in white cells compared to whole blood or red cell concentrate (Appendix I.4.5.5).

There is some evidence that infectivity is related to white cells (Section II.3.6.1), Hence a simple estimate of the effect of leucodepletion would be that it also achieved a 3 log reduction in infectivity compared to whole blood or red cell concentrate, as shown in Table II.3.9.

However, there are several reasons why infectivity may not reduce in proportion to white cell content:

- The experiments by Brown et al (1998 and 1999) also show considerable infectivity in the plasma, which is not consistent with the hypothesis that infectivity is proportional to white cell content.
- The possibility that fragments of white cell membranes might be formed has been considered. This is particularly likely if filtering is done at a late stage (immediately prior to transfusion rather than soon after donation), since white cells progressively break down during this time. However, filtration is currently carried out within 48 hours of donation, which should greatly reduce the occurrence of membrane fragments. Preliminary results (Krailadsiri et al 2002) indicate that whole blood and plasma filtration is very effective at removing any fragments present.

- It is possible in theory that filtration could release attached prion from the surface of the white cells into the leucodepleted component, although there is no evidence that this occurs and studies have indicated that the normal form of the prion protein is reduced by between 60% and 95% by whole blood and plasma filters (Krailadsiri et al 2002).
- The experiments by Brown et al (1998) show some infectivity in the buffy coat, which could be removed without leucodepletion. However, the filters used for leucodepletion would remove the majority of platelets.

In the absence of any experiments to show the effect of leucodepletion on infectivity, it is appropriate to assume that leucodepletion may reduce the infectivity but by less than the reduction in white cell content. The infectivity in leucodepleted red cells is assumed to be 2 logs lower than red cells with buffy coat removed. Combining with the selected infectivity breakdown by blood component from Section II.3.6.6, this gives a 2.6 log reduction compared to whole blood. This is shown in Table II.3.12, and used in the present study as the base case.

II.4.2.2 Filtration of Plasma

There is evidence that plasma contains infectivity that is unrelated to white blood cells or fragments thereof. Brown et al (2001) suggests that WBC reduction does not consistently reduce the infectivity of already separated plasma. No correction has been made to the infectivity of plasma for the effects of leucodepletion.

II.4.2.3 Filtration of Platelets

After 3-component segregation, most of the white cells from a blood donation remain in the buffy coat. In order to meet the specification for filtrated platelet units (Appendix I.4.6.2), some degree of filtration is required, amounting to approximately 3 log reduction of white cells. Based on the similar arguments to those for leucodepletion of red cells above, the reduction in infectivity is assumed to be 2 logs.

For unfiltered platelet units, reduction in WBC content is of the order of 2 logs; the associated reduction in infectivity is assumed to be 1 log.

II.4.3 Production of Plasma Derivatives

II.4.3.1 General Approach

Production of the various plasma derivatives (see Figure II.3.2) involves various stages of fractionation, precipitation, centrifugation, filtration, virus inactivation, formulation and heat treatment. Several of these steps are intended to achieve a major reduction in viruses, and it is possible that they also have a significant effect on CJD infectivity. A number of studies to assess the removal of the TSE agent in plasma fractionation processes have been carried out by "spiking" the starting material with extracts from the brains of animals with a TSE (Foster et al 1998, 2000). These studies suggest that a significant reduction in infectivity is achieved. However, concern has been expressed that the characteristics of any infectivity that may be present in plasma may not be the

same as those in the spiked material. However, the endogenous infectivity in blood is too low to assay, using currently available techniques, through the purification process.

Three possible approaches for estimating the infectivity in plasma derivatives have been considered. The second approach is a worst case scenario.

Approach 1: Largest single clearance factor. Infectivity based on the value for plasma in the Brown et al experiments (Table II.3.12), combined with an estimate of the TSE reduction capability of the process steps, so called TSE *clearance factors (CF).* An estimate of the potential for plasma fractionation processes to remove TSE infectivity has been made by Foster et al (1998, 1999 and 2000) on samples spiked with hamster adapted scrapie 263K and based on Scottish National Blood Transfusion Process (SNBTS) production methods, and the results of studies on the behaviour of brain-derived infectivity. Studies by Vey et al (2001) indicate that hamster prions partition very similarly to human prion strains. Foster estimated both cumulative CF for a combined process and also individual CF for single process steps. Further work by Reichl et al (2002) has since indicated that clearance is unlikely to be cumulative but may be complementary. In most processes, sequential steps reduced the infectivity to below the limit of detection. *This document considers the highest single clearance step only.* In this approach, the reduction in product volume is included in the CF, which refers to total infectivity, not infectivity concentration. Table II.4.1 summarises the highest single CF as estimated by Foster's experiments for the SNBTS processing methods. It should be noted that the CF are derived from experiments based on SNBTS processes and it is necessary to assume that the yields of product are the same under laboratory conditions as in the manufacturing process. Table II.4.1 includes estimates of the highest single CF that *may* result from BPL's processing techniques, as in some cases there are differences between the two processes. Where this is the case the reasoning behind the choice of highest single CF is given below Table II.4.1. It should be noted that studies by Kang et al (2002) indicate that solvent conditions (solvent pH, salt content and the presence of ethanol) can significantly affect the behaviour of the PrP in solution.

Approach 2: No additional clearance. Infectivity concentrations based on values in the Brown et al experiments (Table II.3.14) for the cryoprecipitate or other appropriate fraction, and it is assumed that there is *no further reduction* in infectivity. Hence all the load in infectivity present in the fraction is assumed to be present also in the appropriate derivative. This is a worst case scenario. Where a specific fraction is used to produce more than one derivative, it is possible that all the infectivity present in the fraction could potentially end up in any one of the derivatives. Since we do not know with which of the derivatives the infectivity might associate, it must be assumed that all the infectivity is in each. This approach also relies on values for the yields of product in the manufacturing process.

Approach 3: Protein content. Infectivity concentrations based on values in the Brown et al experiments on endogenous infectivity in the blood of animal models (Table II.3.14) for the cryoprecipitate or other appropriate fraction, combined with the *protein contents* in the plasma derivatives. This in effect assumes that infectivity partitions between the finished product and the waste material in proportion to the protein contents of the two. It assumes no further reduction in infectivity from further filtration steps after the fractionation (apart from that resulting from the

reduction in the protein content). The experts consulted indicated that, since there was no evidence that prion protein would partition with bulk protein, as the methods used are designed to separate different proteins largely on the basis of solubility characteristics (Foster 1994) this approach was not justified and these calculations have been removed from the report.

It should be noted that the 2 selected approaches estimate infectivity based mainly on SNBTS product yields (BPL yield for Anti-thrombin). Infectivity of specific products in individual incidents will require assessment of the particular batch details, particularly if the product was prepared by BPL.

Table II.4.1 Estimated Clearance Factors in Plasma Products (Foster 2000)

Product	SNBTS		BPL	
	Process Step resulting in Highest Single CF	CF (log_{10})	Process Step resulting in Highest Single CF	CF (log_{10})
Albumin	Fraction V depth filtration	4.5	Fraction V Depth filtration (1)	4
Immunoglobulins	Fraction I/III precipitation	3.5	Fraction I/III precipitation (2)	3.5
High purity Factor IX	*HIPFIX*- DEAE Sepharose chromatography	3	*Replenine* – DEAE Sepharose chromatography	3
Factor IX (intermediate II,IX,X)	*DEFIX*- DEAE Cellulose adsorption	3	*IXA*- DEAE cellulose adsorption	3
Thrombin (BPL Antithrombin)	Sepharose chromatography	3	DEAE cellulose adsorption (3)	2
Factor VIII (intermediate) (4)	*(Z8)* – $AlOH_3$ Adsorption	1.5	*(8Y)*- Cryoprecipitation	1
Factor VIII (high purity)	*Liberate* - SD+DEAE chromatography	3	*Replenate*- Ion exchange chromatography (5)	5

CF have been adapted to allow for differences between BPL & SNBTS Processes as follows:

(1) Depth filtration at BPL (Cuno filter) is different to SNBTS (Seitz KS80) and although it is understood to be an effective filtration method, a more prudent 4 log CF has been assumed for BPL.

(2) Although BPL use a different Fraction I/III precipitation method (Kistler & Nichmann) to SNBTS (cold ethanol), both fractionation conditions were examined (Foster 2000) with similar results.

(3) BPL produce anti-thrombin, not thrombin. Experts have advised that based on the fact that salt content/ionic strength during elution of ATIII from the heparin-sepharose adsorbent is higher than 250mM NaCl; then the expected log reduction would be about 2 for Anti thrombin.

(4) Factor VIII intermediates production are simpler processes; Experiment shows a CF of 1.7 for SNBTS Factor VIII Z8 (1.5 is selected for the purpose of these calculations) while for BPL's 8Y process a minimum of 1 log clearance is indicated to represent cryoprecipitation.

(5) BPL's high purity Factor VIII Replenate includes immunoaffinity chromatography (no data available) and an ion exchange chromatography steps, which literature (Foster et al 2000) suggests may have a higher single CF (up to 6 logs) than SNBTS processing for Factor VIII Liberate. A more conservative increased CF of 5 logs is selected.

The application of the first two approaches to the plasma derivatives produced by SNBTS (and BPL's anti-thrombin) is provided below. The calculations refer to derivatives made from a batch of plasma pooled from 20,000 donations (or 350 donations in the case of specific immunoglobulins, and 3,500 donations for Anti-thrombin) including one from a donor who went onto develop vCJD (in the case of specific immunoglobulins, the calculations assume that a pool of 350 includes 7 donations from a donor who went onto develop vCJD).

Infectivities calculated below are based on SNBTS process yields as follows:

Factor VIII Z8	– 200iu/l plasma
Factor VIII High purity	- 150iu/l plasma
Factor IX (intermediate)	- 200iu/l plasma
Factor IX (high purity)	- 150iu/l plasma
IgG	- 4g/l plasma
Albumin	- 25g/l plasma
Anti-thrombin	- 180iu/kg plasma (BPL)

II.4.3.2 Factor IX (Intermediate, Factor II, IX,X)

Factor IX (intermediate) is produced from cryosupernatant.

Using the highest single *clearance factor approach*, the infectivity in one batch of plasma of 20,000 donations (including one from a donor who subsequently developed vCJD) is estimated as 480 ID_{50} (see Table II.3.12). Allowing for a thousand-fold reduction in infectivity as a result of processing (see Table II.4.1), the potential infectivity is therefore estimated to be 0.48 ID_{50} in Factor IX (intermediate) produced by the batch. Now there are 200 iu of Factor IX (intermediate) produced per litre of plasma and there are 20,000 x 0.225 litres of plasma per batch, consequently there are 200 x 20,000 x 0.225 = 900,000 iu of Factor IX (intermediate) produced per batch. Therefore the infectivity is estimated as $0.48/900{,}000 = 5 \times 10^{-7}$ ID_{50}/iu.

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for cryosupernatant. Infectivity in Cryosupernatant is 50.6 ID_{50}/unit whole blood (Table II.3.14), hence 50.6 ID_{50} is the infectivity present in the cryosupernatant resulting from one plasma batch of 20,000 donations (including one from a donor who subsequently developed vCJD). If there is no further reduction in infectivity, there will therefore be 50.6 ID_{50} present in the 900,000 iu of Factor IX (intermediate) produced per batch (see above) which is equivalent to 5.6×10^{-5} ID_{50}/iu Factor IX (intermediate).

II.4.3.3 Factor IX (High Purity)

High purity Factor IX is produced from cryosupernatant (see Appendix I.5.4).

Using the highest single *clearance factor approach*, the infectivity in one batch of plasma of 20,000 donations (including one from a donor who subsequently developed vCJD) is estimated as 480 ID_{50} (see Table II.3.12). Allowing for a thousand-fold reduction in infectivity as a result of processing (see Table II.4.1), the potential infectivity is therefore estimated to be 0.48 ID_{50} in high purity Factor IX produced by the batch. Now there are 150 iu of high purity Factor IX produced per litre of plasma and there are 20,000 x 0.225 litres of plasma per batch, consequently there are 150 x 20,000 x 0.225 = 675,000 iu of high purity Factor IX produced per batch. Therefore the infectivity is estimated as $0.48/675{,}000 = 7 \times 10^{-7}$ ID_{50}/iu.

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for cryosupernatant. Infectivity in Cryosupernatant is 50.6 ID_{50}/unit whole blood, hence 50.6 ID_{50} is the infectivity present in the cryosupernatant resulting from

one plasma batch of 20,000 donations (including one from a donor who subsequently developed vCJD). If there is no further reduction in infectivity, there will therefore be 50.6 ID_{50} present in the 675,000 iu of high purity Factor IX produced per batch (see above) which is equivalent to 7.5×10^{-5} ID_{50}/iu.

II.4.3.4 Anti-thrombin

Anti-thrombin (BPL only) is produced from cryosupernatant.

Using the highest single *clearance factor approach*, the infectivity in one batch of plasma of 3,500 donations (including one from a donor who subsequently developed vCJD) is estimated as 480 ID_{50} (see Table II.3.12). Allowing for a hundred-fold reduction in infectivity as a result of processing (see Table II.4.1), the potential infectivity is therefore estimated to be 4.8 ID_{50} in Anti-thrombin produced by the batch. Now there are 180 iu of Anti-thrombin produced per litre of plasma and there are 3,500 x 0.225 litres of plasma per batch, consequently there are 180 x 3,500 x 0.225 = 141,750 iu of Anti-thrombin produced per batch. Therefore the infectivity is estimated as $4.8/141{,}750 = 3.4 \times 10^{-5}$ ID_{50}/iu.

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for cryosupernatant. Infectivity in Cryosupernatant is 50.6 ID_{50}/unit whole blood, hence 50.6 ID_{50} is the infectivity present in the cryosupernatant resulting from one plasma batch of 3,500 donations (including one from a donor who subsequently developed vCJD). If there is no further reduction in infectivity, there will therefore be 50.6 ID_{50} present in the 141,750 iu of Anti-thrombin produced per batch (see above) which is equivalent to 36×10^{-4} ID_{50}/iu.

II.4.3.5 Factor VIII (Z8)

Factor VIII (Z8) is produced from cryoprecipitate (see Appendix I.5.3).

Using the highest single *clearance factor approach*, the infectivity in one batch of plasma of 20,000 donations (including one from a donor who subsequently developed vCJD) is estimated as 480 ID_{50} (see Table II.3.12). Allowing for a 1.5 log reduction in infectivity as a result of processing (see Table II.4.1), the potential infectivity is therefore estimated to be 15 ID_{50} in Factor VIII (Z8) produced by the batch. Now there are 200 iu of Factor VIII (Z8) produced per litre of plasma and there are 20,000 x 0.225 litres of plasma per batch, consequently there are 200 x 20,000 x 0.225 = 900,000 iu of Factor VIII (Z8) produced per batch. Therefore the infectivity is estimated as $15/900{,}000 = 1.7 \times 10^{-5}$ ID_{50}/iu.

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for cryoprecipitate. Infectivity in Cryoprecipitate is 60 ID_{50}/unit whole blood (Table II.3.14), hence 60 ID_{50} is the infectivity present in the cryoprecipitate resulting from one plasma batch of 20,000 donations (including one from a donor who subsequently developed vCJD). If there is no further reduction in infectivity, there will therefore be 60 ID_{50} present in the 900,000 iu of Factor VIII (Z8) produced per batch (see above) which is equivalent to 6.7×10^{-5} ID_{50}/iu.

II.4.3.6 Factor VIII- High Purity

High purity Factor VIII is produced from both cryoprecipitate and albumin (see Appendix I.5.3).

Using the highest single *clearance factor approach*, the infectivity in one batch of plasma of 20,000 donations (including one from a donor who subsequently developed vCJD) is estimated as 480 ID_{50} (see Table II.3.12). Allowing for a thousand-fold reduction in infectivity as a result of processing (see Table II.4.1), the potential infectivity is therefore estimated to be 0.48 ID_{50} in high purity Factor VIII produced by the batch. Now there are 150 iu of high purity Factor VIII produced per litre of plasma and there are 20,000 x 0.225 litres of plasma per batch, consequently there are 150 x 20,000 x 0.225 = 675,000 iu of high purity Factor VIII produced per batch. Therefore the infectivity is estimated as 0.48/675,000 = 7 $\times 10^{-7}$ ID_{50}/iu. This does not include the contribution of infectivity due to added albumin (BPL process only), where there is 0.4 g of albumin added per 2000 iu of high purity Factor VIII (Appendix I.5.3); the extra relative contribution from albumin is negligible.

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for Cryoprecipitate. Infectivity in Cryoprecipitate is 60 ID_{50}/unit whole blood (Table II.3.14), hence 60 ID_{50} is the infectivity present in the Cryoprecipitate resulting from one plasma batch of 20,000 donations (including one from a donor who subsequently developed vCJD). If there is no further reduction in infectivity, there will therefore be 60 ID_{50} present in the 675,000 iu of high purity Factor VIII produced per batch (see above) which is equivalent to 8.9×10^{-5} ID_{50}/g. Again, this does not include for any contribution of infectivity due to added albumin (BPL process only) which will be negligible in comparison.

II.4.3.7 Albumin

Albumin is made from the Fraction V.

Using the highest single *clearance factor approach*, the infectivity in one batch of plasma of 20,000 donations (including one from a donor who subsequently developed vCJD) is estimated as 480 ID_{50} (see Table II.3.12). Allowing for a 4.5 log reduction in infectivity as a result of processing (see Table II.4.1), the potential infectivity is therefore estimated to be 0.015 ID_{50} in Albumin produced by the batch. Now there are 25g of Albumin produced per litre of plasma and there are 20,000 x 0.225 litres of plasma per batch, consequently there are 25 x 20,000 x 0.225 = 112,500 g of Albumin produced per batch. Therefore the infectivity is estimated as 0.015/112,500 = 1.4 $\times 10^{-7}$ ID_{50}/iu.

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for Fraction V. Infectivity in Fraction V is 3.4 ID_{50}/unit whole blood (Table II.3.14), hence 3.4 ID_{50} is the infectivity present in the Fraction V resulting from one plasma batch of 20,000 donations (including one from a donor who subsequently developed vCJD). If there is no further reduction in infectivity, there will therefore be 3.4 ID_{50} present in the 112,500 g of Albumin produced per batch (see above) which is equivalent to 3.0×10^{-5} ID_{50}/g.

II.4.3.8 Normal Immunoglobulins

IgG (iv) is produced by ethanol fractionation from Fraction II (and in the BPL process it also contains albumin - see Appendix I.5.6).

Using the highest single *clearance factor approach*, the infectivity in one batch of plasma of 20,000 donations (including one from a donor who subsequently developed vCJD) is estimated as 480 ID_{50} (see Table II.3.12). Allowing for a 3.5 log reduction in infectivity as a result of processing (see Table II.4.1), the potential infectivity is therefore estimated to be 0.152 ID_{50} in IgG produced by the batch. Now there are 4g of IgG produced per litre of plasma and there are 20,000 x 0.225 litres of plasma per batch, consequently there are 4 x 20,000 x 0.225 = 18,000 g of IgG produced per batch. Therefore the infectivity is estimated as $0.152/18{,}000 = 8.4 \times 10^{-6}$ ID_{50}/g. This does not include for the contribution of infectivity due to added albumin (BPL process only), which was derived from a separate pool. The contribution from albumin will be lower, due to its lower infectivity and also because for every 2 g of added albumin, there is 5 g of IgG.

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for Fraction II. Infectivity in Fraction II is 1.6 ID_{50}/unit whole blood (Table II.3.14), hence 1.6 ID_{50} is the infectivity present in the Fraction II resulting from one plasma batch of 20,000 donations (including one from a donor who subsequently developed vCJD). If there is no further reduction in infectivity, there will therefore be 1.6 ID_{50} present in the 18,000g of IgG produced per batch (see above) which is equivalent to 9.1×10^{-5} ID_{50}/g. Again, this does not include for any contribution of infectivity due to added albumin (BPL process only), which was derived from a separate pool. The contribution from albumin will be lower due to its lower infectivity and also because for every 2g of added albumin, there is 5g of IgG.

II.4.3.9 Immunoglobulins (im) - specific

IgG (im) is produced from Fraction II and contains no added albumin.

Using the highest single *clearance factor approach*, the infectivity in one batch of plasma of 350 donations (including seven donations from a donor who subsequently developed vCJD) is estimated as 7 x 480 = 3360 ID_{50} (see Table II.3.12). Allowing for a 3.5 log reduction in infectivity as a result of processing (see Table II.4.1), the potential infectivity is therefore estimated to be 1.06 ID_{50} in IgG produced by the batch. Now there are 4g of IgG produced per litre of plasma and there are 350 x 0.225 litres of plasma per batch, consequently there are 4 x 350 x 0.225 = 315 g of IgG produced per batch. Therefore the infectivity is estimated as $1.06/315 = 3.4 \times 10^{-3}$ ID_{50}/g.

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for Fraction II. Infectivity in Fraction II is 1.6 ID_{50}/unit whole blood (Table II.3.14), hence 11.2 ID_{50} is the infectivity present in the Fraction II resulting from one plasma batch of 350 donations (including seven donations from a donor who subsequently developed vCJD). If there is no further reduction in infectivity, there will therefore be 11.2 ID_{50} present in the 315g of IgG produced per batch (see above) which is equivalent to 3.7×10^{-2} ID_{50}/g.

II.4.3.10 Estimate of Infectivity in Plasma Derivatives

Table II.4.2 and Figure II.4.1 give the results from applying the above approaches to several key plasma derivatives. The results are expressed in the form of infectivity per iu or g dose given to patients.

Table II.4.2 Comparison of Estimates of Infectivity in Plasma Derivatives

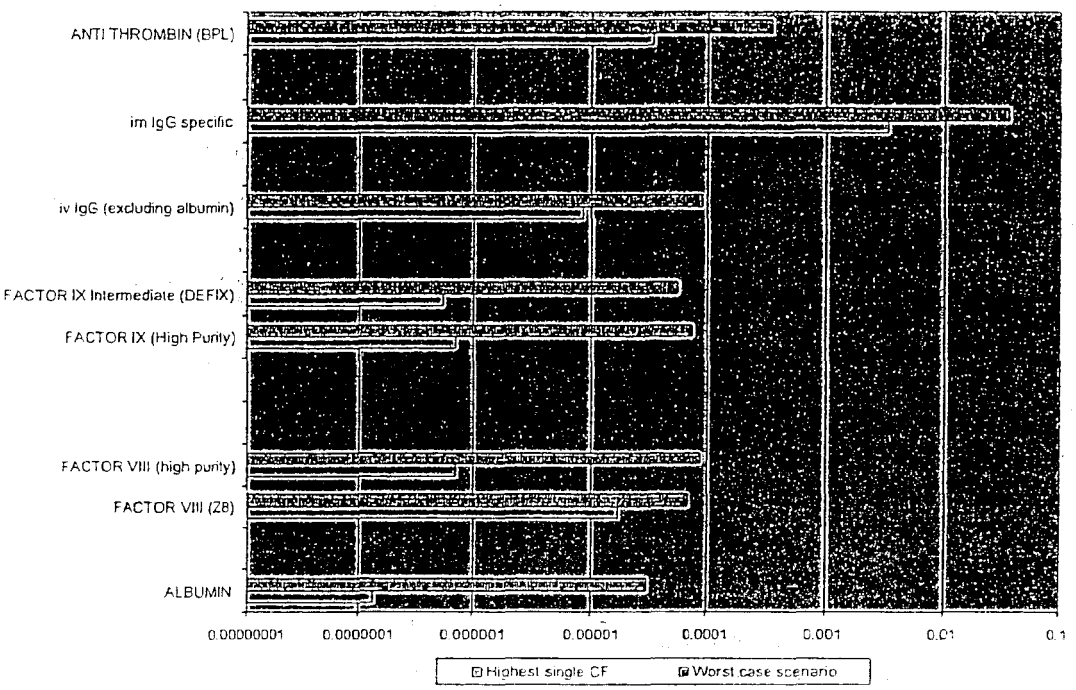
Plasma Derivative	Source	Based On largest single Clearance Factors	Based on no Clearance beyond initial Fractionation
Factor VIII Z8	Cryoprecipitate	1.7×10^{-5} ID_{50}/iu	6.6×10^{-05} ID_{50}/iu
Factor VIII high purity	Cryoprecipitate & Fraction V	7.1×10^{-7} ID_{50}/iu	8.9×10^{-05} ID_{50}/iu
High purity Factor IX	Cryosupernatant	7.1×10^{-7} ID_{50}/iu	7.5×10^{-05} ID_{50}/iu
Factor IX (intermediate)	Cryosupernatant	5.3×10^{-7} ID_{50}/iu	5.6×10^{-05} ID_{50}/iu
Normal IgG – excl albumin	Fraction II	8.4×10^{-6} ID_{50}/g	9.1×10^{-05} ID_{50}/g
I/m IgG* specific	Fraction II	3.4×10^{-3} ID_{50}/g*	3.7×10^{-02} ID_{50}/g*
Albumin 100%	Fraction V	1.4×10^{-7} ID_{50}/g	3.1×10^{-05} ID_{50}/g
Anti- Thrombin†	Cryosupernatant	3.4×10^{-5} ID_{50}/iu†	3.6×10^{-04} ID_{50}/iu†

Except for specific i/m IgG and anti-thrombin, the results are based on infectivity resulting from one plasma batch of 20,000 donations, including one from a donor who subsequently developed vCJD.

* For i/m IgG , the results are based on infectivity resulting from a plasma batch of 350 donations (50 donors), including 7 donations by a donor who subsequently developed vCJD

† For anti-thrombin, the plasma batch size is 3,500 donations, including 1 donation from a donor who subsequently developed vCJD.

Figure II.4.1 Comparison of Estimates of Infectivity in Plasma Derivatives (ID_{50}/iu or g)



II.4.3.11 Discussion

It should be noted that at present the 2 approaches estimate infectivity based mainly on SNBTS product yields (BPL for Anti-thrombin). Infectivity of specific products in individual incidents will require assessment of the particular batch details, particularly if the product was prepared by BPL.

It can be seen that for Factor VIII (Z8) there is little difference in the estimated infectivity between the two approaches, whereas for most of the others the differences are more substantial (e.g. about 100-fold for high purity Factor VIII).

In deciding which of the estimates to use in a particular context, factors other than scientific reasoning will need to be applied. However, the estimates may need to be revised on the basis of new scientific evidence and given the wide disparity between the current estimates, DNV considers that it is important that the assessment is updated accordingly.

II.5 Incubation Periods

II.5.1 vCJD Cases

Very little is known about the incubation period (from original infection to development of clinical signs) of vCJD. It is not known for certain when the 130 UK cases of definite and probable vCJD were infected. However, it is likely that they were infected by beef products between the first recorded case of BSE in 1986 and the offal ban in 1989. The first vCJD cases appeared in 1994, suggesting that the shortest incubation period is between 5 and 8 years for transmission across the species barrier. For example, one of the cases that appeared in 1997 had eaten no meat for 11 years, suggesting that in this case infection was in 1986 or earlier, with an incubation period of at least 11 years. It is not possible to obtain a median incubation period from this data, because it is not known how many other people are still incubating the disease.

The incubation period might be inferred from other TSEs. Incubation periods for sporadic and familial CJD cannot be determined, as there is no identifiable point of infection. However, incubation periods can be estimated for other acquired TSEs as follows.

II.5.2 Kuru

The incubation period for Kuru, a human TSE that occurred in cannibal tribes of Papua New Guinea, ranged from 5 to over 40 years (SEAC 1994 p25). No sources have been identified for a more detailed distribution. There are still some cases of kuru appearing, and there are some people exposed to kuru who are still alive and therefore potentially incubating the disease. Hence the upper limit of the incubation period is greater than 40 years.

II.5.3 Iatrogenic CJD

The incubation period in iatrogenic cases of CJD has ranged from 15 months to 30 years, and varies according to the route of exposure (see Table II.5.1, from Ricketts 1997).

The incubation period is in general longer for more peripheral routes of exposure, as illustrated in the table. Hence the values for growth hormone, which was injected via intra-muscular or subcutaneous routes, are the most appropriate for infection from blood transfusion. These have a mean of 12 years and a range of 5 to 30 years.

More detailed data has been supplied by the Institute of Child Health on the 27 cases of CJD due to human growth hormone (HGH) that have occurred in the UK up to March 1998. Incubation periods are difficult to estimate because the treatment with HGH lasted several years (for these 27 cases, the treatment duration had a mean of 6.4 years and standard deviation 2.7 years). Also, the onset of clinical symptoms is not well defined, and the time when they are detected is influenced by the patient's knowledge of the risks. For the present study, incubation periods have been estimated from the mid-point of treatment to the reported onset of symptoms.

Table II.5.1 Incubation Periods for Iatrogenic CJD

Mode Of Infection	No Of Cases	Route Of Entry	Mean (+ Range) Of Incubation Period
Instrumentation			
Neurosurgery	4	Intracerebral	20 months (15 - 28)
Stereotactic EEG	2	Intracerebral	18 months (16 - 20)
Tissue transfer			
Corneal transplant	2	Optic nerve	17 months (16 - 18)
Dura mater transplant	25	Cerebral surface	5.5 years (1.5 - 12)
Tissue extract transfer			
Growth hormone	76	Haematogenous	12 years (5 - 30)
Gonadotrophin	4	Haematogenous	13 years (12 - 16)

The resulting incubation periods of these 27 cases have a mean of 14.3 years, a standard deviation of 3.3 years, and a range of 8 to 19 years.

Figure II.5.1 shows the incubation periods in relation to the time of onset of symptoms. The trend line shows that these are tending to increase, as cases with longer incubation periods continue to appear. Any further cases would be expected to have incubation periods longer than 13 years, since treatment with HGH stopped in the UK in 1985. The final mean value may be in excess of 15 years, but it is difficult at present to predict what it might be.

Figure II.5.1 Incubation Period Trend for UK HGH Cases

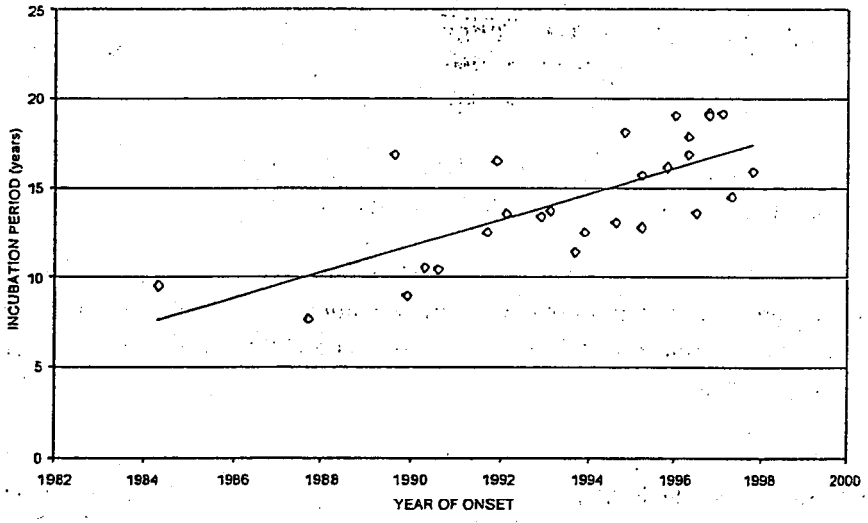
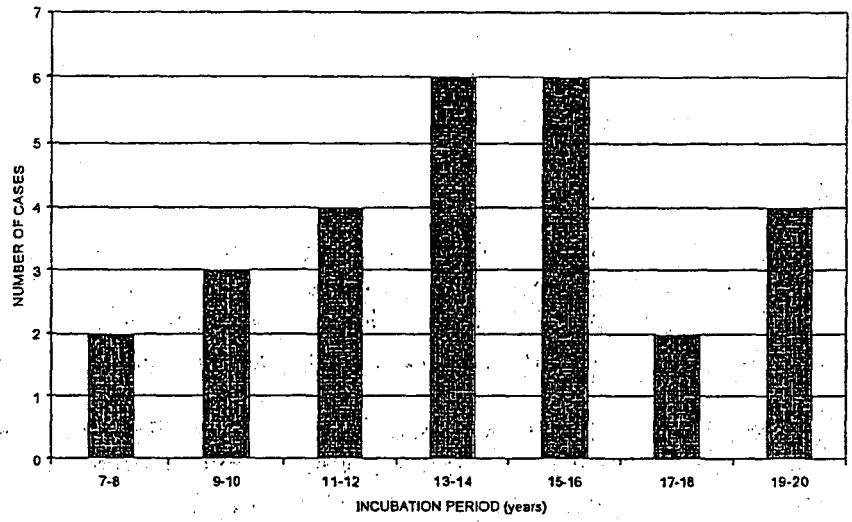


Figure II.5.2 shows the distribution of the incubation periods in the 27 cases. This has a peak around 15 years, but the higher incubation period end of the distribution, where cases are still appearing, is not well defined. Hence this does not show the shape of the final distribution.

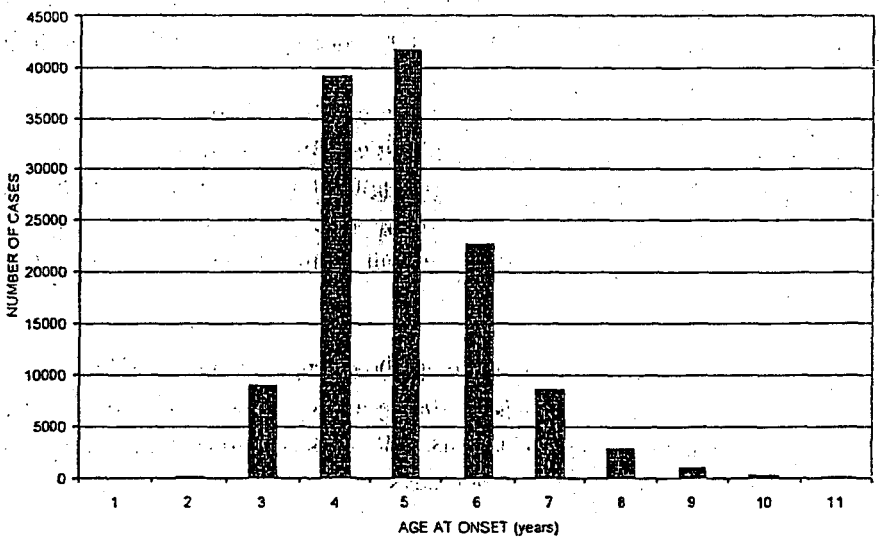
Figure II.5.2 Incubation Period Distribution for UK HGH Cases



II.5.4 BSE

The ages at onset of BSE in cattle have been supplied by MAFF for the 125963 cases of BSE reported up to January 1998. The mean is approximately 5 years with a 90% range of 3.5 to 7.5 years, i.e. 0.7 to 1.5x mean. The overall range is 20 months to 18 years (SEAC 1994 p35). Figure II.5.3 shows the distribution of the ages at onset, which has a peak around 4-5 years.

Figure II.5.3 Distribution of Age at Onset for BSE Cases



Most exposure to infected MBM was in calves, so the modal value may be appropriate for the incubation period. However, some adult cattle were also exposed, so the upper limit of the incubation period may be lower. The mean incubation period is usually assumed to be 5 years.

The distribution of the incubation period in cattle has been estimated from retrospective modelling of the BSE epidemic to be a gamma function with a standard deviation of 1.3 years, based on an assumed mean of 5 years (Anderson et al 1996). This gives a modal value of approximately 4.7 years, a 90% range of approximately 3 to 7.2 years (0.6 to 1.4x mean) and a 99% range of 2 to 9 years. This is consistent with the above data.

II.5.5 Scrapie

The peak age of onset of scrapie in sheep is 3½ years (SEAC 1994 p27) although this is understood to vary with the sheep type. The incubation period in experimental intra-ocular inoculations was 14-22 months (SEAC 1994 p 33). However, maternal transmission is thought to be more likely, so a typical incubation period for natural scrapie would be about 3½ years.

The natural life expectancy of sheep is about 12 years if fed on a farm, although this reduces to 6-7 years if the animal is left to feed itself (according to the Meat & Livestock Commission). The typical incubation period for scrapie of about 3½ years is 30% of the former figure.

II.5.6 Application of BSE and Scrapie Data to Humans

The above estimates of incubation periods within different species may be used as an alternative approach to estimating human CJD incubation periods. However, since human life expectancy is much greater than cattle and sheep, it is desirable to investigate whether this might have any effect on the incubation period.

At one extreme, it is possible that the incubation period is an inherent property of each strain of TSE and route of infection, and that life expectancy has no effect. Hence the incubation period of vCJD in humans would be 5 years, as for BSE in cattle. This is consistent with the time between peak exposure to BSE infectivity through food (1992) and the occurrence of vCJD cases during 1996-7.

At the other extreme, it is possible that the incubation period is proportional to the natural life expectancy of the species. The mean incubation period for BSE in cattle of about 5 years is 17% of their natural life expectancy of about 30 years. Applying to humans, with a current life expectancy of about 77 years, this would indicate a mean incubation period of about 13 years. This agrees reasonably well with the data from CJD due to growth hormone, but is considered a less reliable approach.

More realistically, each combination of TSE strain and host genotype may yield distinctive incubation periods that cannot at present be predicted. For example, some TSE strain/mouse genotype combinations give incubation periods as low as 100 days, whereas other strains in the same genotype give incubation periods as high as 1000 days.

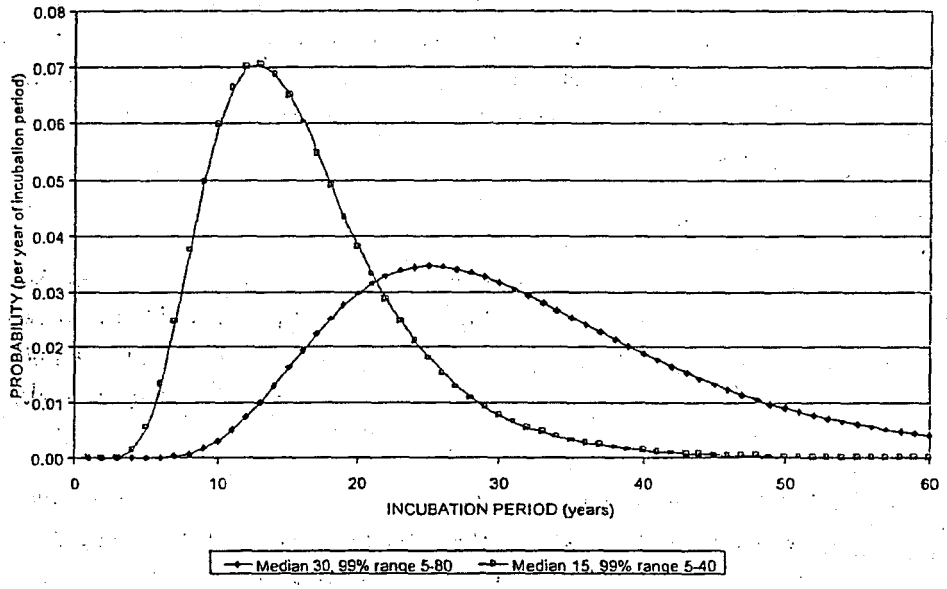
II.5.7 Previous Assumptions

Cousens et al (1997) assumed lognormal or gamma distributions with means in the range 10 to 25 years and 90%iles of 1.5x or 2x the mean.

II.5.8 Selection of Estimate for Infection from Blood

The HGH data is the best quality data on incubation periods of CJD, and being a peripheral route of exposure, it is most relevant to CJD from blood. However, the present data may under-estimate the incubation periods, because cases are still appearing. The UK data shows a mean incubation period of 14.3 years, which is increasing as cases continue to appear. Data on iatrogenic CJD from other countries and Kuru indicates a range of 5 to 40 years. These include at least 100 cases, and hence can be taken as a 99% range. This can be represented by a lognormal distribution with a median of 15 years and a 99% range 5 to 40 years, as shown in Figure II.5.4. This distribution has a mean of 16.3 years, a standard deviation of 7 years, and a 90% range of 7.5 to 30 years, or approximately 0.5 to 2x the mean.

Figure II.5.4 Assumed Incubation Period Distributions



II.5.9 Selection of Estimate for Infection from Food

Most of the above data involves infection within a species. For example, iatrogenic CJD involves infection with a human-passaged agent. This may be appropriate for vCJD from blood transfusion, but could be too short for vCJD obtained direct from BSE in food. When crossing a species barrier, the mean incubation period could be doubled, and the spread would be likely to increase. In order to model this for infections from BSE in food, a median of 30 years could be assumed, and a 99% range of 5 to 80 years. This distribution has a mean of 32.5 years, a standard deviation of 14 years, and a 90% range of 15 to 60 years, or approximately of 0.5 to 2x the mean. This is included in Figure II.5.4.

This distribution is consistent with the little available data on vCJD cases to date. However, it is highly speculative.

In addition, the incubation period might increase for the oral route, but available data on oral exposure of cattle to BSE does not support any further increase.

II.5.10 Effect of Dose

The incubation period for scrapie is inversely related to the dose received. Regression analysis of scrapie in hamsters has established this relationship (Prusiner et al 1982). Additionally, analysis of titration experiments in the murine scrapie model reveal that mean incubation periods rise linearly with logarithmic decreases in dose (McLean and Bostock 2000). Hence it may be assumed that variations in dose account for much of the observed variation in incubation periods.

If there was a large influence of dose on incubation period, it would be expected that the first cases of vCJD from food would have been people who had eaten bovine brain as a delicacy. Since this does not appear to be the case, it is consistent with the attack rate experiments in suggesting only a minor effect.

No information is known on how the average dose of infectivity from HGH might compare to the average dose from food or blood. However, it is likely that doses from blood transfusions could be very large, whereas doses from plasma derivatives could be very small. However, no model of these effects is adopted at present. It is assumed that the range of incubation periods accounts for variation in the doses received.

II.5.11 Effect of Genetic Factors

There is evidence that the nature of TSEs is affected by genetic factors, particularly in CJD in humans by the amino acid permutations (methionine/valine polymorphism) encoded by codon 129 on the PrP gene. In the UK population as a whole, 37% are homozygous for methionine at this locus (met/met); 11% are homozygous for valine (val/val) and 52% are heterozygotes (met/val) (Preece 1993).

So far, all cases of vCJD are homozygous for methionine at codon 129. This may indicate that only 37% of people are vulnerable to the disease. Alternatively, it may be that these people are more likely to have a short incubation period, and that others may be infected with a longer incubation period.

In sporadic CJD cases, 83% are homozygous for methionine. In iatrogenic CJD cases due to human growth hormone, 43% are homozygous for methionine (met/met); 47% are homozygous for valine (val/val) and 10% are heterozygotes (met/val) (Preece 1993). This suggests that the met/val genotype exerts some protective effect, but it is not complete. Such people may be vulnerable to the disease with a reduced probability of infection or a longer incubation period.

In the present study, it is assumed that the range of incubation periods accounts for variations in genetic factors, and all people are assumed to be capable of being infected with vCJD.