II.3 Infectivity in Blood

II.3.1 Experimental Evidence for Infectivity

II.3.1.1 Tests on Blood from Human CJD and vCJD Cases

Available experimental evidence on the infectivity of human blood from CJD and vCJD patients is summarised in Table II.3.1 (from a review by Brown 1995). All these experiments involved inoculating the blood into animals. If the animal died, its brain was then examined for signs of TSE.

Table II.3.1 Attempts to detect Infectivity in the Blood of Humans with CJD

Study	Donor Diagnosis	Material Inoculated	Assay Animal	Route	Transmissions/ Total Inocul'd*
Manuelidis et al (1985)	Sporadic CJD	Buffy coat	Guinea pig Hamster	i/c	2/2
Tateishi (1985)	Sporadic CJD	Whole blood	Mouse	i/c	1/3
Tamai et al (1992)	Sporadic CJD	Concentrated plasma	Mouse	i/c	1/1
Brown et al (1994)	Sporadic CJD Sporadic CJD Sporadic CJD Sporadic CJD Sporadic CJD Kuru	Whole blood Whole blood Whole blood Buffy coat Whole blood Serum	Chimpanzee Spider monkey Squirrel monkey Squirrel monkey Guinea pig Mouse	i/v i/c, i/v, i/p i/c, i/p, i/m i/c, i/p i/c, i/p i/c	0/3 0/1 0/1 0/4 0/1 0/3
Deslys et al (1994)	hGH CJD	Whole blood	Hamster	i/c	1/1
Bruce et al (2001)	vCJD	Buffy Coat Plasma	Mouse Mouse	i/c i/c	0/18 0/23

* Fraction of blood samples found to transmit disease. Number of animals infected is larger.

The table shows that bioassays on mice failed to detect infectivity in blood components (buffy coat and plasma) from vCJD cases. In 4 experiments infectivity was detected in the recipient animal (mouse, hamster or guinea pig). Three used blood from sporadic CJD cases, and one used the blood of an iatrogenic CJD case. However, several other studies have failed to show infectivity in the blood of sporadic CJD cases when inoculated into monkeys, chimpanzees and guinea pigs. These conclusions are surprising because it would be expected that primates would be relatively easy to infect with human CJD, and rodents would be relatively difficult. A possible explanation is that blood is infective, but only at a level near the detectability threshold in the tests, at least when crossing the species barrier. Another possible explanation is that all the experiments that showed infectivity were flawed. Although this seems unlikely for 4 independent experiments, they all involved very few animals and all had some puzzling features (Brown 1995).

Hence these studies are taken to indicate the *possibility* of infectivity in the blood of humans with sporadic and iatrogenic CJD, and they do not amount to proof. In the absence of better data, it would be appropriate to assume that the blood of sporadic and iatrogenic CJD cases is infective at a low level.

II.3.1.2 Tests on Blood from Animals with TSEs

Available experimental evidence on the infectivity of blood from animals with TSEs is summarised in Table II.3.2 (based on a review by Brown 1995). These tests have the advantage that inoculation does not have to be across a species barrier, but they have the disadvantage that human CJD may differ from experimental (e.g. mouse-adapted) CJD and scrapie, and hence the results may be invalid for humans.

Table II.3.2 Attempts to detect Infectivity in the Blood of Animals with TSEs

Study	Host Animal	Material Inoculated	Assay Animal	Route	Transmissions/ Total Inocul'd*
Natural scrapie					
Hadlow et al (1980)	Goat	Clot/serum	Mouse	i/c	0/3
Hadlow et al (1982)	Sheep	Clot/serum	Mouse	i/c	0/18
Hunter et al (2002)	Sheep	whole blood buffy coat	sheep	i/v	4/21**
Experimental scrapie					
Pattison & Millson (1962)	Goat	Whole blood	Goat	i/c	0/14
Gibbs et al (1965)	Sheep	Serum	Mouse	i/c	1/1
Clarke & Haig (1967)	Rat	Serum	Rat	i/c	1/1
Clarke & Haig (1967)	Mouse	Serum	Mouse	i/c	1/1
Eklund et al (1967)	Mouse	Whole blood	Mouse	i/c	0/39
Dickinson et al (1969)	Mouse	Whole blood	Mouse	i/c	3/13
Hadlow (1974)	Goat	Clot	Mouse	i/c	0/3
Diringer (1984)	Hamster	Blood	Hamster	i/c	5/5 (see also further positive transmissions in Table II.3.4)
Casaccia et al (1989)	Hamster	Blood	Hamster	i/c	10/11 (see also further positive transmissions in Table II.3.5)
Experimental CJD					
Manuelidis (1978)	Guinea pig	Buffy coat	Guinea pig	All	10/28
Kuroda (1983)	Mouse	Buffy coat	Mouse	i/p	4/7 (see also further positive transmissions in Table II.3.3)
Brown et al (1998)	Mouse	All components	Mouse	i/c	
Brown et al (1999)	Mouse	All components	Mouse	i/c	
Experimental BSE					
Taylor et al (2000)	Mouse	Plasma	Mouse	i/c	4/48
Hunter et al (2002)	Sheep	Whole blood	Sheep	i/v	2/17**
Bons *et al* (2002)	Microcebus murinus	buffy coat	Microcebus murinus	i/c	1/1

Fraction of blood samples found to transmit disease. Number of animals infected is larger.
** Only preliminary data available

The table shows that infectivity has been found in the blood of laboratory mice, sheep, guinea pigs and primates infected with CJD or BSE and natural scrapie in sheep when inoculated back into the same species. Infectivity has also been found in the blood of laboratory sheep, mice, rats and hamsters infected with scrapie. This again suggests that the level of infectivity in blood is near the detectability threshold in the tests.

The studies that showed infectivity mainly involved direct intracerebral inoculation of infected blood. Some peripheral inoculations of infected blood have caused infections, but intravenous transfusion of blood from human CJD cases into chimpanzees did not lead to infection. This would be expected if the intracerebral infectivity was near the detectability threshold, as peripheral inoculation is less efficient in most experimental models. However, transfers within the same species are more efficient, and preliminary reporting (Houston et al 2000, Hunter et al 2002) indicates that transfusion of whole blood (not leucodepleted) or buffy coat from a BSE infected but asymptomatic sheep to an uninfected sheep has resulted in transmission of the BSE infection. The Hunter study also showed transmission of natural scrapie in sheep by blood transfusion.

The studies of experimental TSE demonstrate that blood is infective, at least in some strains of TSEs. They provide further evidence for the *possibility* of infectivity in the blood of human CJD patients, particularly vCJD. It should be noted that infectivity in patients with vCJD is found in lymphoid tissue, similar to the distribution of scrapie infectivity in sheep (see Section II.3.3). In the absence of better data, it would be appropriate to assume that the blood of vCJD cases is infective at the same level as animals with experimental CJD.

II.3.2 Epidemiological Evidence for Infectivity

Several epidemiological studies have reviewed CJD cases to investigate whether they may have resulted from blood transfusion. Epidemiological studies in the UK, the USA and Japan (reviewed by Ricketts et al 1997 and by Riggs 2001) have shown that CJD patients have no more than average exposure to blood transfusions. This suggests that blood transfusion is not a major cause of infection for sporadic CJD cases.

Four Australian CJD cases were identified who had received blood transfusions (Klein & Dumble 1993). Their symptoms were consistent with those of iatrogenic CJD, but there is no clear evidence that blood transfusions were the cause.

A Canadian CJD case had received albumin from a pool that included the blood of a CJD patient (reported by Ricketts et al 1997). However, the recipient died only 8 months after receiving the albumin, which short incubation period suggests this was not the cause of infection.

A similar French CJD case received albumin during a liver transplant from a pool that included the blood of a probable CJD patient (Creange et al 1996). The donor developed probable CJD 3 years after the donation. The recipient died only 2 years after receiving the transfusions, which is an unusually low incubation period, and suggests that the albumin was not the cause of infection.

Other studies have reviewed recipients of blood products to investigate whether they may be at increased risk of developing CJD. A review of haemophilia centres in the USA revealed no CJD cases in haemophiliacs (reported by Ricketts et al 1997).

It is understood (letter from National Blood Authority to CJD Incidents Panel 2001) that at present there are no reported cases of vCJD among the haemophiliacs and immunodeficient patients who have been exposed during regular replacement therapy to potentially contaminated UK plasma derivatives (factor VIII and IV/IgG) by vCJD. This may be explained however by a long incubation period.

Investigation of a CJD patient who had donated 35 units of blood in 20 years identified 27 people who had definitely received the patient's blood, and 8 probable recipients (Schlesselmann 1982). For 20 units the recipients could not be identified. Of the 35 identified recipients, 18 had died (51%). None of the identified recipients had neurological disease.

A similar study of a German CJD patient who had donated 55 units of blood (Heye et al 1994) identified 35 recipients, of whom 21 (60%) had died from non-CJD illnesses up to 22 years later, and 14 were alive an average of 12 years after receiving the blood.

In an ongoing US study of patients who received blood from CJD patients (reported by Ricketts et al 1997), 147 recipients have been identified, of whom 80 have died (54%). There were no CJD cases among the 65 for whom the cause of death is known.

A retrospective surveillance study for CJD among persons with haemophilia has been reported by Evatt et al (1998). Samples from 24 haemophilia decedents were obtained and examined for signs of spongiform encephalopathy by a panel of expert neuropathologists. The panel found no evidence of CJD in any of the specimens.

It is possible that cases of transmission of CJD by blood transfusion might be masked by the much larger numbers of genuinely sporadic cases. However, unless the incubation period is very long, this would only apply to older recipients, since there are significant numbers of transfusions to neonates, and cases of sporadic CJD in young people are rare.

It is concluded from the above that there is no evidence that sporadic CJD has ever been transmitted by blood transfusion. Although such transmissions may have occurred, the numbers would have to have been very small to escape detection. However, it is not certain that this also applies to vCJD. It has been argued (Brown, personal communication) that the risks from blood transfusions in humans may be similar for vCJD and sporadic CJD because the infectivity in animal models of variant CJD is similar to that found in non-variant animal models of the disease. However, the extent to which this similarity in experimental animals can be extrapolated to humans is unknown.

For variant CJD, less epidemiological evidence is available. The Annual Report 2001 from the National CJD Surveillance Unit reports a case control study of vCJD risk factors among 51 cases and 116 community controls. Five of the cases were reported by relatives to have had a history of blood transfusion compared with 6 of the 116 community controls (O.R. 2.3 (0.7, 7.8) p = 0.2). Although this suggests that previous blood transfusion may have been the cause in one or two of the 51 vCJD cases, there is a 20% likelihood that this result arose by chance due to the relatively small numbers involved. This information leaves open the possibility that blood transfusion may be a factor in vCJD transmission.

II.3.3 Indirect Evidence for Infectivity

Most of the evidence above applies to sporadic CJD in humans or to experimental scrapie, BSE and CJD in animals. There are some theoretical reasons why infectivity may be more likely or higher

in the blood of vCJD cases. In sporadic CJD, the abnormal form of PrP and infectivity are largely confined to the central nervous system and the eye and transmission has only been reported through procedures involving these tissues. In contrast, abnormal PrP (Wadsworth *et al* 2001) and infectivity (Bruce *et al* 2001) has been found in the lymphoid tissues of patients who died of vCJD. Furthermore, the abnormal protein has been found in lymphoid tissue prior to the onset of symptoms of disease (Hilton *et al* 1998, Hilton *et al* 2002). This pattern of tissue distribution of infectivity is similar to that observed in sheep infected orally with BSE (Foster *et al* 2001). This indicates that the distribution of infectivity in the body in vCJD patients differs from that in patients with the sporadic form of the disease and may include blood. The role of circulating white cells in the pathogenesis of the disease and the contribution of lymphocytes to the total infectivity in whole blood is far from clear (Raeber 2001) but the normal form of the prion protein has been shown to be present in human lymphocytes and monocytes (Dodelet & Cashman 1998) and in other blood cells and platelets (Barclay *et al* 2002) so these components could support prion replication.

Animal studies provide some evidence that the infectivity in blood in models of variant CJD behave in a similar fashion to models of other TSEs. It has been proposed (Brown, Personal Communication) that you might therefore expect the risks of transmission by blood would be similar for vCJD as other forms of the disease in humans. However, the consensus expert view was that there was currently insufficient evidence to support this argument.

It is concluded from the above that infectivity in the blood may be more likely or higher in vCJD cases than in sporadic CJD. Hence the epidemiological evidence against infection via blood may be valid for sporadic CJD but not for vCJD.

II.3.4 The Variation of Infectivity in Blood Through the Incubation Period

II.3.4.1 Tests by Manuelidis et al

Manuelidis et al (1978) investigated infectivity in blood during the incubation period in experimental CJD in hamster-adapted CJD from intracerebral inoculation. The results were expressed in terms of numbers of recipient hamsters with CJD, but were incomplete when reported and difficult to interpret. Nevertheless, they indicate the presence of infectivity throughout the incubation period.

II.3.4.2 Tests by Kuroda et al

Kuroda et al (1983) investigated the variation of infectivity through the incubation period in mouse-adapted CJD following intracerebral inoculation. Recipient mice were inoculated intracerebrally. The results were expressed in terms of numbers of recipient mice infected and their mean incubation period. The results for blood are given in Table II.3.3. They show significant infectivity in blood starting at about 30% of the incubation period and increasing towards the end of the incubation period.

Table II.3.3 Infectivity in Blood by Kuroda et al

Time After Infection Of Donor (Assay) Animal (Weeks)	Recipient Animals Infected	Mean Incubation Period Of Recipient Animal (Days)
1	0/4	-
2	0/4	-
3	0/2	-
6	5/5	281
9	3/3	213
14	3/3	156
18	5/5	142

II.3.4.3 Tests by Diringer

Diringer (1984) estimated the infectivity in the blood from donor hamsters with hamster-adapted 263K scrapie early in the incubation period following intraperitoneal inoculation. The blood was concentrated so that each 50 µl of inoculum contained 2 ml blood equivalent. This was inoculated intracerebrally into groups of recipient hamsters. Table II.3.4 gives the results. It indicates a relatively high level of infectivity up to 40 days, i.e. about the first third of the incubation period. It gives no information on the later period.

Table II.3.4 Infectivity in Blood by Diringer

Time After Infection Of Donor (Assay) Animal (Days)	Recipient Animals Infected	Mean Incubation Period Of Recipient Animal (Days)
5	1/6	214
10	8/8	151
20	3/3	186
30	4/4	191
40	2/2	150

II.3.4.4 Tests by Casaccia et al

Casaccia et al (1989) conducted similar tests to Diringer, but with lesser concentration of the blood and covering the whole incubation period. Blood from donor hamsters with hamster-adapted 263K scrapie following intraperitoneal inoculation was concentrated so that each 50 µl of inoculum contained 0.2 ml blood equivalent. This was inoculated intracerebrally into groups of recipient hamsters. The level of infectivity was estimated from the mean incubation period of the hamsters that died of scrapie. Table II.3.5 gives the results scaled from the plot in Casaccia et al (1989). This indicates a relatively high level of infectivity in the first half of the incubation period, and a subsequent decline, with negligible infectivity at the end of the incubation period.

Table II.3.5 Infectivity in Blood by Casaccia et al

Time After Infection Of Donor (Assay) Animal (Days)	Recipient Animals Infected	Mean Incubation Period Of Recipient Animal (Days)	Estimated i/c log ID_{50}/0.05 ml
1	6/6	107	2.45
8	6/6	116	1.8
16	6/6	115	1.9
27	6/6	114	2.0
41	6/6	129	1.2
51	6/6	124	1.4
60	?/6	132	1.0
70	6/6	120	1.6
80	1/6	132	1.0
90	1/6	138	0.8
100	0/6	>160	-

II.3.4.5 Tests by Brown et al

Brown et al (1999) looked at blood infectivity during the pre-clinical and clinical phases of disease and conducted numerous bioassay experiments with blood from mice inoculated intracerebrally by a mouse-adapted strain of human TSE. The blood was then processed and inoculated intracerebrally in infectivity assays. Brown concluded that infectivity was present in the preclinical phase in the buffy coat, but was either absent or present in only trace amounts in the plasma or plasma fractions. Infectivity rose sharply at the onset of clinical signs in both the buffy coat and in the plasma. Table II.3.6 below illustrates that the experiments show that the preclinical infectivity is less than 8% of the clinical infectivity levels for buffy coat and less than 3% for plasma. If this is averaged out proportionate to volumes, it gives a combined relative infectivity of preclinical to clinical infectivity for buffy coat/plasma of only 3%.

Table II.3.6 Comparison of Pre-clinical and Clinical Infectivity based on Brown et al 1999

	Average Preclinical Infectivity (IUs/ml)	Clinical Infectivity (IUs/ml)	% infectivity preclinical to clinical
Buffy Coat	8.2	106.0	7.7%
Plasma	0.6	21.9	2.7%
Cryo + I + II + III	0.7	-	-

II.3.4.6 Tests by Houston et al

Houston et al (2000) described preliminary reports of one case of transmission of BSE to a sheep by transfusion of whole blood taken from a sheep asymptomatic from experimental BSE. (This sheep was half way through the incubation period and was infected orally). Although this study on experimental BSE and natural scrapie in sheep is still incomplete, further results from the study have been reported (Hunter *et al* 2002). In total, 24 transfusions from BSE-inoculated sheep have been carried out. Of these 17 received whole blood and 7 received a fraction enriched in white cells (buffy coat). Both the positive results to date were in sheep that received whole blood. In both

cases, the blood was taken from the donor before clinical signs of illness were apparent and the donors were estimated to be about half way through the incubation period. Two additional animals who received whole blood are currently showing signs characteristic of early BSE. If these are confirmed as positive, this brings the rate of transmission to at least 17%. The donor animals in these transfusions were themselves in the clinical phase of BSE. Of the remaining 20 sheep, one died of unrelated causes and 19 animals remain apparently healthy. The transfusions for these healthy animals were carried out between 68 and 1243 days ago.

In total, 21 transfusions from sheep naturally infected with scrapie have been carried out. Of these, 4 have developed scrapie. One of the four received the buffy coat preparation from the blood of a sheep in the clinical phase of the disease. The remaining 3 received whole blood from donors who were not yet showing clinical signs. Of the remaining 17 transfused sheep, one died of unrelated causes and 16 remain apparently healthy although it is too early to be confident that they will not go on to develop scrapie. The transmission rate in this part of the study was at least 19%.

The volumes of blood transfused were comparable with those used in the treatment of humans.

The EC Scientific Steering Committee assessed the implications of Houston and Hunter papers detailed above and concluded that the BSE agent is now clearly identified in sheep transfused with blood from BSE infected Cheviot sheep, and that the papers reinforced the substance of previous opinions by the scientific committees regarding the safety of blood (European Commission 2002).

II.3.4.7 Interpretation

The available data suggests that the infectivity may remain stable, decrease or increase during the incubation period. However, the evidence for low infectivity at the beginning of the incubation period comes mainly from animals inoculated intracerebrally (Manuelidis, Kuroda and Brown). When considering vCJD infections from food or blood transfusions, the model used in the sheep transfusion studies are the most relevant. Preliminary results indicate that infectivity is present before clinical signs but a comparison of infectivity at different stages of the disease has not been provided.

The intraperitoneal inoculation used by Diringer and Casaccia may be more relevant than the intracerebral route. These both indicate a high level of infectivity early in the incubation period. Cassacia found that infectivity declines as the disease progresses. Such a pattern might explain why several tests on blood from clinical cases have not detected infectivity. Diringer's study indicated rising levels of infectivity up to 40 days but later times were not reported.

For the present study, it will be assumed that the level of infectivity remains constant through the incubation period. The levels estimated below are based on tests on blood from clinical cases, this may be either an optimistic or a pessimistic assumption.

II.3.5 The Level of Infectivity in Whole Blood

II.3.5.1 Tests by Brown et al (1998)

The most detailed available titration of infectivity in blood is from tests by Brown et al (1998). Three sets of experiments were performed:

- A high input ("spiking") experiment, using hamster-adapted 263K scrapie. Infected hamster brain was added to human blood, which was then separated into components and inoculated intracerebrally at various dilutions into hamsters. Although an infectivity was estimated for whole blood in this experiment, the approach produces an artificially high value which does not indicate the overall infectivity in normally infected blood. These tests can be used to indicate the relative level of infectivity in different blood components.
- A low input "endogenous" experiment, using mouse-adapted CJD. Blood from symptomatic mice inoculated intracerebrally was extracted, separated into components and inoculated intracerebrally at various dilutions into mice. Although no infections resulted from whole blood, these tests can be used to indicate the relative level of infectivity in most of the different blood components.
- Transfusion experiments, using hamster-adapted scrapie. Blood from symptomatic hamsters was inoculated intravenously and intracerebrally into other hamsters. Although the full results are not yet available, Rohwer (1997) has given some results. Rohwer has also reported in some presentations at conferences that he obtained one positive infection from blood inoculated intravenously. This was 1 out of 22 transmissions attempted. These results have not been published.

Available information on the test results is incomplete and unclear, and no single best-estimate of infectivity is available for whole blood. In the endogenous experiment, 45 ml of blood was obtained from mice with mouse-adapted CJD. Of this, 0.15% (i.e. 68 µl) was inoculated as whole blood into 11 mice, i.e. an average of 6 µl each. None of these 11 mice died. This suggests that the ID_{10} for whole blood is more than 6 µl. Assuming a linear dose-response function, this would indicate that the ID_{50} was more than 30µl, i.e. an infectivity less than 30 ID_{50}/ml. Interim results from Brown et al quoted a value of <76 ID/ml, but this does not appear in the final paper.

Rohwer (1997) stated that by adding the infectivity levels estimated in the individual blood components in the same tests "we get a titre for blood of about 10 infectious units (IU)/ml". Analysis of the infectivity of the plasma and buffy coat components from the low input experiment (Section II.3.6.2, Table II.3.11) indicates about 340 IU (680 ID_{50}) from a 45 ml blood sample (equivalent to 7.6 IU/ml), but the infectivity of the red cell component was not determined. Hence an infectivity for whole blood of 10 i/c IU/ml would be consistent with a 24% contribution from red cells.

For the transfusion experiments using hamster-adapted scrapie, Rohwer (1997) also stated "the titre in the blood by these experiments is about 2-10 infectious units/ml". This is assumed to refer to the intracerebral route, although this is not clear from the source. No complete results are available.

This source has the advantage that it included investigation of the infectivity in different blood components (see Section II.3.6.2). However, it has the disadvantage that reporting is incomplete and unclear, with no single infectivity result for whole blood. It only considers infectivity at the end of the incubation following intracerebral inoculation.

II.3.5.2 Tests by Brown et al (1999)

Brown et al (1999) conducted further experiments to assess the infectivity of buffy coat, plasma and plasma fractions, at both preclinical and symptomatic stages, as summarised in Table II.3.7 below, which compares infectivity with the levels determined in his previous experiment (II.3.5.1). Unfortunately no infectivity experiments would appear to have been conducted on red blood cells or whole blood since 1998. However, infectivity of whole blood can be estimated from its components.

Table II.3.7 Comparison of Infectivity in Brown et al Experiments 1999 with 1998

	Brown et al 1999 Asymptomatic	Brown et al 1999 Symptomatic	Brown et al 1998 Symptomatic
Buffy Coat	16.4	212	88.8
Plasma	1.2	56.2	20.6

(All units in ID_{50}/ml component, where $2ID_{50}$/ml = 1 IU/ml

Both buffy coat and plasma contained higher infectivity levels during the symptomatic stage than those found in the earlier Brown et al (1998) experiments (by a factor of approximately 2.5). Brown et al considered that the higher infectivity levels in the buffy coat in the 1999 paper were expected, because in the earlier 1998 paper some mice receiving buffy coat had died with typical clinical signs but were not included in the infectivity calculations because their brains were not examined for PrP, hence death was not proven to have resulted from disease transmission. However, Brown (2001) considers that the higher symptomatic infectivity value found in the 1999 experiments for the buffy coat is "clearly an overestimate, or we would already have seen many cases of CJD in blood recipients". The experiments indicated that preclinical infectivity is less than 10% of the clinical infectivity levels.

II.3.5.3 Tests by Diringer

Diringer (1984) estimated the infectivity in the blood from donor hamsters with hamster-adapted 263K scrapie from intraperitoneal inoculation, early in the incubation period (Section II.3.4.3). Based on an incubation period range of 130-200 days, Diringer estimated an infectivity level of 5-50 i/c IU/ml.

This source has the advantage of considering infectivity during the incubation period following intraperitoneal inoculation. However, it has the disadvantage that it was based on scrapie, and that titration was not performed.

II.3.5.4 Tests by Casaccia et al

Casaccia et al (1989) conducted similar tests to Diringer, but with lesser concentration of the blood and covering the whole incubation period. Blood from donor hamsters with hamster-adapted 263K scrapie from intraperitoneal inoculation was concentrated so that each 50 µl of inoculum contained 0.2 ml blood equivalent. This was inoculated intracerebrally into groups of recipient hamsters. The level of infectivity was estimated from the mean incubation period of the hamsters that died of scrapie. Table II.3.3 gives the results.

The average infectivity during the first half of the incubation period was $10^{1.8}$ ID_{50}/0.05 ml inoculum (= 63 ID_{50}/0.05 ml inoculum). Since each inoculum contained 0.2 ml blood equivalent, this can be expressed as 310 ID_{50}/ml. The average over the whole incubation period would be approximately half of this.

This source has the advantage of considering infectivity during the whole incubation period following intraperitoneal inoculation. However, it has the disadvantage that it was based on scrapie, and that titration was not performed.

II.3.5.5 Tests by Bruce et al

Bruce et al (2001) conducted bioassay experiments on mice with plasma and buffy coat from vCJD patients. The experiments failed to detect infectivity in either source but could be used as an upper limit for infectivity, as follows. For plasma, each animal received an undiluted 0.02 mls inoculum. The absence of infections indicates that 0.02 mls of plasma must contain much less than 1 ID_{50}. In total 39 animals were inoculated with plasma from 4 vCJD patients. This indicates a probability of infection less than 1/39 = 0.026. Assuming a linear dose-response model (Section II.6), this is equivalent to 0.026/ 0.5 ID_{50} units (i.e. the dose with 0.5 probability of infection) in the dose volume of 0.02 ml. Hence the limit of detection is estimated as 0.026/ (0.5 x 0.02) = 2.6 mouse ic ID_{50}/ml. Since the buffy coat was diluted two-fold before inoculation into a similar number of animals, the limit of detection for it would be approximately a factor of 2 higher.

These experiments were conducted across the human-mouse species barrier. Bruce et al assume that this barrier is approximately 500, based on the cattle-mouse species barrier. Following this assumption, the limit of detection for infectivity within a species would be 2.6 x 500 = 1,320 human ic ID_{50}/ml for plasma. This is significantly more than the infectivity assumed in the present report for plasma and buffy coat, hence it is not surprising that the experiment did not detect any infectivity, and the upper limit is of very limited use.

II.3.5.6 Wadsworth et al

Wadsworth et al (2001) indicates that although highly sensitive immuno-blotting analysis methods for detection of PrP have improved, they are not yet sensitive enough to be used for estimation of blood infectivity unless spiking has taken place to raise infectivity levels. Additionally, the quantitative relationship between PrP and infectivity is uncertain.

II.3.5.7 Selection of Estimate

The above approaches are compared in Table II.3.8. In order to put them into comparable units, the values are converted using 1 IU = 2 ID_{50}. They give estimates of infectivity in blood ranging from 4 to 300 i/c ID_{50}/ml. The experiments by Brown et al (1998 and 1999) are at the lower end of this range.

Since these experiments all used species and disease strains that have been selected to achieve transmission, it is likely that they would provide an over-estimate of the infectivity of vCJD in humans. Infectivity in brains from patients dying of vCJD has been estimated to be in the region of 10^8 ic ID_{50}/g tissue. Infectivity levels were in the range of 10^6 - 10^7 icID_{50} in the two mice brain pools used in Brown et al (1999) experiments and the higher of the brain pools was associated with the higher blood infectivity levels. However, a value of 10 i/c ID_{50}/ml is used for this assessment, because the lower end of the range has been reported more consistently.

Table II.3.8 Estimates of Overall Infectivity in Blood

Source	Original Estimate	Estimate In Comparable Units
Rohwer (1997) hamster adapted scrapie	2-10 hamster i/c IU/ml hamster blood	4-20 hamster i/c ID_{50}/ml hamster blood
Brown et al (1998) mouse adapted CJD ("Endogenous experiment")	10-15 mouse i/c IU/ml mouse blood	20-30 mouse i/c ID_{50}/ml mouse blood
Brown et al (1999) mouse adapted CJD (range allows for pre-clinical and clinical infectivity)	0.5-15 mouse i/c IU/ml mouse blood (buffy coat/plasma only)	1-30 mouse i/c ID_{50}/ml mouse blood (buffy coat /plasma only)
Casaccia et al (1989) hamster scrapie tests (first half of incubation period)	1.8 log hamster ID_{50}/0.2 ml hamster blood equivalent	310 hamster i/c ID_{50}/ml hamster blood
Diringer (1984) hamster scrapie tests (first third of incubation period)	5-50 hamster i/c IU/ml hamster blood equivalent	10-100 hamster i/c ID_{50}/ml hamster blood

II.3.5.8 Conversion to Intravenous Route

All the above estimates are based on intracerebral (i/c) inoculation, which is in general the most efficient route for transmitting infection. Intravenous (i/v) inoculation is reported to be 10 times less efficient than the i/c route, based on other tests with mouse-adapted scrapie (Kimberlin 1996). Brown et al (1999) conducted a comparison between i/v and i/c administration routes of inoculation and found a 7-fold reduction in infectivity by the i/v route using plasma from a murine model and a 5 fold reduction using buffy coat from a murine model. The research was conducted with blood from mice that had earlier been infected with a mouse-adapted strain of human TSE.

DNV propose a reduction factor of 5 for conversion from i/c to i/v inoculation. This will be used for whole blood, blood components and plasma fractions.

Combined with the estimate of i/c infectivity of 10 i/c ID_{50}/ml blood above, this gives 2 i/v ID_{50} /ml blood, with a range of approximately 0.2 to 60 i/v ID_{50} /ml.

It should be noted that Brown et al (1998) suggested that the i/v route might be 100 times less efficient, based on earlier work by Kimberlin and this indicates the uncertainty in this parameter.

II.3.6 The Level of Infectivity in Blood Components

II.3.6.1 Based on White Cell Content

In most studies, the highest levels of infectivity are found in fractions containing white blood cells. The involvement of lymphoreticular tissue has already been discussed but the contribution of the cells to total blood infectivity is uncertain. Nevertheless, one possibility is that, when blood is segregated into components and processed into blood products, infectivity may remain in the products in proportion to the number of white cells present.

The mean white cell content in a 450 ml donation of whole blood is taken as 4×10^9 cells per unit (Appendix I.4.2), i.e. 8.9×10^9 cells per litre. If the infectivity is taken as 2 i/v ID_{50}/ml and assumed proportional to the number of white cells, it can be expressed as 2.2×10^{-7} ID_{50} per white cell. The infectivity in each blood component can then be estimated from the white cell content (Appendix I.4) as summarised in Table II.3.9.

Table II.3.9 Infectivity Based on White Cell Content

Blood Product	Volume (ml/unit)	Fraction Of Volume	Mean WBCs (per unit)	Infectivity (ID50/unit)
Whole blood	450	1.000	4×10^9	900
Plasma	225	0.500	4×10^6	0.9
Plasma (filtered)	225	0.500	1,000	0.0002
Red cells (with buffy coat)	225	0.500	4×10^9	900
Red cells (buffy coat removed)	212	0.470	5×10^8	110
Red cells (leucodepleted)	212	0.470	5×10^5	0.11
Buffy coat	14	0.030	3.5×10^9	800
Platelets (unfiltered)*	0.75	0.002	6×10^6	1.4
Platelets (filtered)*	0.75	0.002	1.2×10^5	0.03

* Figures are for platelets as derived from 1 unit of blood, while therapeutic platelet doses are typically derived from the pooling of 4 individual donations. Figures do not include the plasma present within a platelet dose. Also, it should be noted that a filtered platelets therapeutic dose has a UK specification that 100% of units have a WBC content of less than 5×10^6 per therapeutic dose (1.2×10^6 per unit of blood); routine testing indicates an average WBC count of 5×10^5. Unfiltered platelets refer to the removal of buffy coat.

This approach may under-estimate the level of infectivity in plasma and in leucodepleted red cells because it is possible that infectivity could be present in other cellular components, including fragments of white cells, not just white cells. Protein is also a component of plasma, so it is also possible that prion exists in soluble form. Hence this approach may be regarded as an extreme estimate, optimistic for plasma and leucodepleted components, and pessimistic for red cells with buffy coat attached.

II.3.6.2 Tests by Brown et al (1998)

Brown et al (1998) investigated the distribution of infectivity in different blood components. Two separate experimental methods were used - a high input ('spiking') experiment and a low input endogenous one.

In their high input ("spiking") experiment, artificially high levels of hamster-adapted scrapie infectivity from brain homogenate were added to normal human blood, which was separated by centrifugation into red cells, white cells/platelets and plasma components, and the plasma was subjected to Cohn fractionation, as used by the American Red Cross. Titrations in each component were then determined as shown in Table II.3.10.

These results show that the majority of the infectivity went into the red cell component, although the infectivity concentration was higher in the white cell/platelet component. Only 32% of the infectivity in whole blood was recovered in the blood components, and only 1.5% of the infectivity in plasma was recovered in the plasma fractions. While the latter effect could be a genuine effect of fractionation, a more cautious interpretation might be that this method under-estimates the infectivity in the components. As a sensitivity test, the percentages could be adjusted in proportion so as to sum to 100%.

This experiment is questionable because the partitioning of the infectivity may reflect the properties of the brain homogenate, which would be very different from blood. However, the results of the experiment are broadly consistent with the more realistic endogenous experiment described below.

Table II.3.10 Infectivity Based on Spiked Blood Experiments

Component	Specimen Quantity	Infectivity Concentration (log ID_{50}/ml) or log ID_{50}/g)	Total Infectivity (log ID_{50})	% Of Infectivity In Whole Blood
Whole blood	46.8 ml	8.3	9.3×10^9	100 %
Red cells	20.0 ml	8.0	2.0×10^9	22 %
White cells/platelets	2.0 ml	8.5	6.3×10^8	7 %
Plasma	24.0 ml	7.1	3.0×10^8	3 %
Cryoprecipitate	0.26 g*	6.6	1.0×10^6	0.71 %#
Fraction I+II+III	0.93 g*	6.1	1.2×10^6	0.86 %#
Fraction $IV_1 + IV_4$	0.87 g*	4.0	8.7×10^3	0.006 %#
Fraction V	1.66 g*	2.5	0.5×10^3	0.0004 %#

* Based on fractionation of an 11 ml plasma sample.
# Percentage of infectivity in plasma.

In the endogenous experiment, blood from mice with mouse-adapted CJD was separated into components and fractionated as above. Titrations were not carried out, but the infectivity in the blood components can be estimated from results provided by Rohwer as shown in Table II.3.11.

Table II.3.11 Infectivity Based on Low Input Experiments

Component	Vol (ml)	Positive/ Inoculated Animals	Total Infectivity (ID_{50})**	% Of Total Infectivity	Infectivity Concentration (ID_{50}/ml or ID_{50}/g)
Whole blood	45	0/11	-	-	-
Red cells	18	0/7	-	-	-
White cells/platelets	3.5	2/12	200.6	29.4	57.4
Plasma pellet*	0.2	4/23	14.6	2	73
Plasma	22.6	8/124	466	68.4	20.6
Cryoprecipitate	0.15	5/11	46	9.6 #	308
Fraction I+II+III	0.4	6/43	36	7.4 #	90
Fraction IV_1 + IV_4	0.86	0/86	-	-	
Fraction V	1.22	0/94	-	-	-

* White cell pellet recovered by centrifugation of plasma.
* * Based on Poisson Titre approach
Percentage of infectivity in plasma + white cell pellet

These results show that a large proportion of infectivity was in the pellet extracted from the plasma component by centrifugation. It is assumed that this infectivity would normally remain in the plasma component. The small number of animals inoculated with red cells mean that no infectivity can be determined for this component, but the results are consistent with a high level of infectivity in the red cells, as in the spiking experiment.

Only 17% of the infectivity in plasma (including the plasma pellet) was recovered in the plasma fractions. As a sensitivity test, the percentages could be adjusted in proportion so as to sum to 100%, as for the spiking experiment.

The results show a low level of infectivity in Fractions IV and V, but do not quantify it. However, they are consistent with the results from the spiking experiment, and could be combined with it to quantify the infectivity in these fractions.

II.3.6.3 Tests by Brown et al (1999)

Brown et al (1999) conducted further experiments to determine infectivity of buffy coat and plasma as summarised previously in Table II.3.7. Unfortunately, no experiments were conducted on red blood cells. The relative infectivity of the plasma and buffy coat during the symptomatic stage are very similar in both the 1998 and 1999 tests.

II.3.6.4 Tests by Taylor et al (2000)

Taylor et al (2000) developed a mouse-adapted BSE strain (301v) model which gave preliminary results of plasma infectivity of 5 ic ID_{50} /ml, which is consistent with the value of 10 i/c ID_{50}/ml for whole blood selected in Section II.3.5.7.

II.3.6.5 Other Experiments

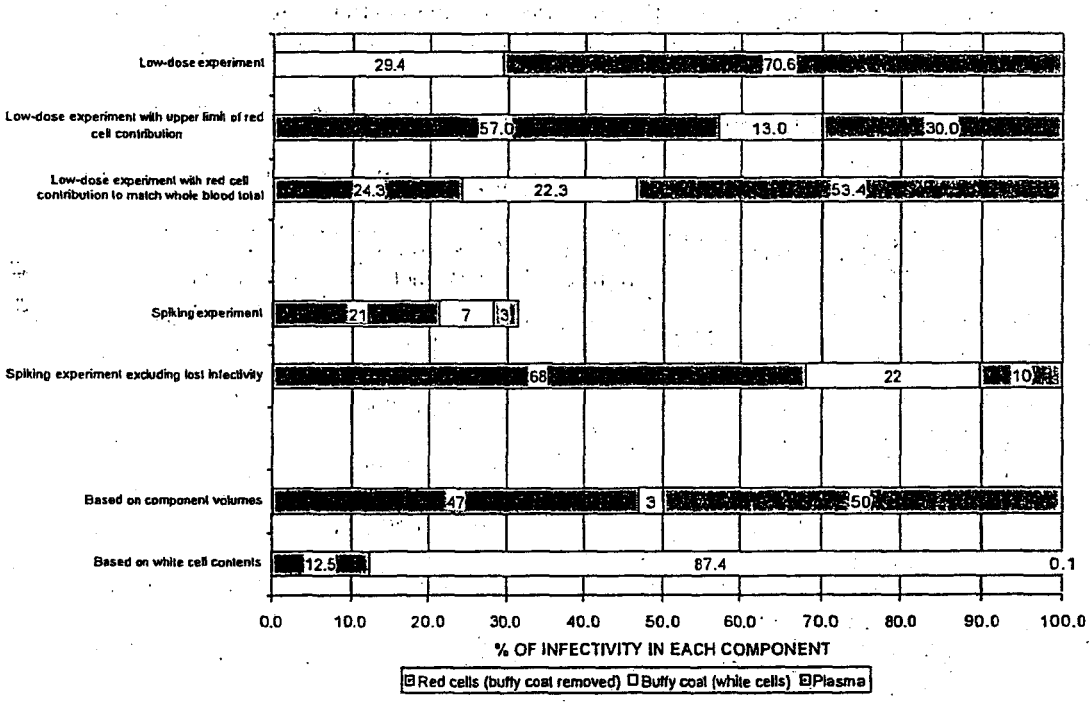
Several experiments that indicated infectivity in blood used buffy coat (platelets and white cells) (see Tables II.3.1 and II.3.2). The fact that they demonstrated infectivity in the buffy coat does not prove that there is none in the other components.

Other tests indicated infectivity in plasma from a sporadic CJD case (Tamai et al 1992) and serum (i.e. plasma with fibrinogen removed) from experimental scrapie in sheep and rats (reviewed by Brown 1995). It is possible that white cells were incompletely removed from these components, so this does not prove that infectivity is present outside white cells.

II.3.6.6 Comparison of Estimates

Figure II.3.1 compares various ways of estimating the breakdown of infectivity in whole blood donations between the different components.

Figure II.3.1 Comparison of Estimates of Infectivity in Blood Components



The methods are:

1. Direct analysis of low input experiment (Brown et al 1998) with zero for red cell component. Note that Brown et al experiments in 1999 gave similar proportions for infectivity breakdown, hence they are not shown on Figure II.3.1.

2. Analysis of low input experiment but with 1 out of 7 inoculated animals assumed infected, giving a pessimistic estimate of the red cell contribution.

3. Analysis of low input experiment (Brown et al 1998) with a red cell contribution of 24% calculated from another study (see Section II.3.5.1). This is considered to be the best approach, and the infectivity proportions are then combined with the whole blood infectivity of 2 i/v ID_{50}/ml (as derived in Section II.3.5.9) to give infectivity values for each blood component.

4. Direct analysis of spiking experiment with no correction for lost infectivity.

5. Analysis of spiking experiment with percentages increased to eliminate lost infectivity.

6. Infectivity concentrations assumed to be the same for all components, giving percentages proportional to the component volumes.

7. Infectivity in each component assumed proportional to white cell content (Section II.3.6.1).

The wide variation between the possible approaches illustrates the substantial uncertainties involved.

Using the Brown et al data leads to the conclusion that, although the highest infectivity *concentration* is in the buffy coat, much of the *total* infectivity remains in the red cell and plasma components. Clearly it would be desirable to have better quality data on these aspects.

The selected infectivity breakdown based on Approach 3 above is summarised in Table II.3.12.

Table II.3.12 Selected Infectivity of Blood Components Based on Brown et al (1998)

Blood Product	Volume (ml/unit)	Infectivity (ID50/unit)*	Infectivity Concentration (ID50/ml)*
Whole blood	450	900	2.0
Plasma	225	480 (=53.4% of 900)**	2.1
Red cells	212	219 (=24.3% of 900)**	1.0
Buffy coat	14	201 (=22.3% of 900)**	14.9
Plasma (filtered)	225	480***	2.1
Red cells (leucodepleted)	212	2***	0.01
Platelets (unfiltered) (4 donations)	3	20****	7
Platelets (filtered) (4 donations)	3	2****	0.7

* Note that infectivities given are for Intravenous administration

** See Section II 3.6.6, Approach 3

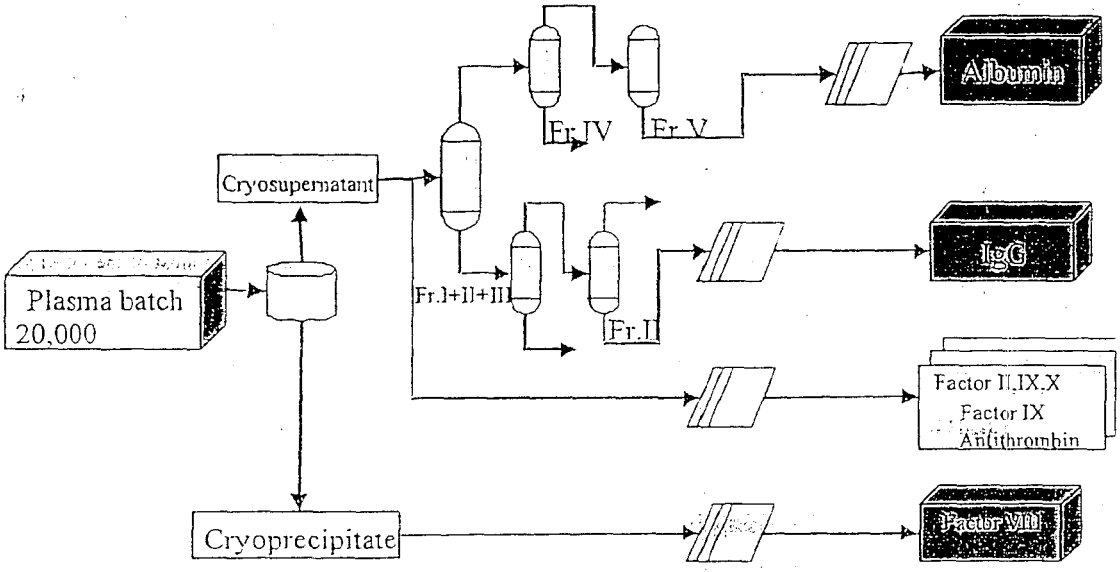
*** The basis for the filtered components is described in Section II.4.2.

**** Figures for Platelets are for a therapeutic dose, which originates from the pooling of 4 separate donations (infectivities shown assume only 1 of the donations was from a patient with vCJD). Platelet figures do not include data for the plasma present in a therapeutic platelet dose (typical platelet therapeutic dose is 300ml, comprising 297 ml plasma and 3 ml platelets)

II.3.7 The Level of Infectivity in Plasma Fractions

Figure II.3.2 provides an outline of the plasma fractionation process, illustrating the intermediate fractions that are used in manufacturing the final plasma derivatives.

Figure II.3.2 Outline of Plasma Fractionation



The low dose experiments by Brown et al (1998) estimate the infectivity in plasma fractions as detailed previously in Table II.3.11. Further experiments carried out by Brown et al (1999) estimated infectivity of the various fractions during both the pre-clinical and symptomatic stages of disease. It was concluded that plasma and its fractions contained only trace amounts during the pre-clinical stage (at the limit of assay recognition, and were detectable only by the inoculation of larger numbers of animals than usual). Cryoprecipitate levels at the symptomatic stage of disease contained low infectivity levels (but approximately twice the infectivity levels identified in the 1998 experiments). Also, the experiments detected trace infectivity levels in fractions IV and V at the symptomatic stage, unlike the 1998 endogenous experiment. Table II.3.13 summarises the experimental data and compares it with data from Brown's 1998 experiments.

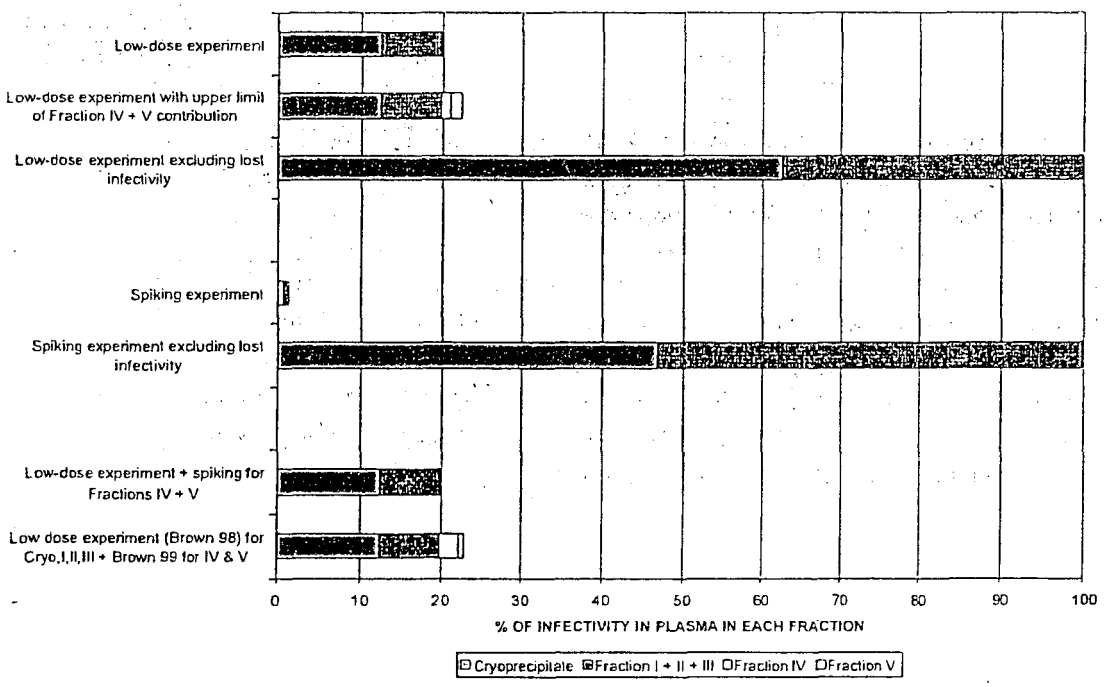
Table II.3.13 Comparison of Plasma Derivatives' Infectivity in Brown et al Experiments in 1999 with 1998

	Brown et al 1999 Asymptomatic	Brown et al 1999 Symptomatic	Brown et al 1998 Symptomatic
Plasma	1.2	56.2	20.6
Cryoprecipitate	1.4 (combined with I,II,III)	5.2	2.4
Fraction I+II+III *	1.4 (combined with cryo)	NT	1.6
IV	NT	1.0	< 0.22
V	NT	0.3	< 0.30
V supernatant	NT	ND	NT

Note that some infectivity levels for the 1999 experiments have been combined from the 2 pools of experiments to enable simple comparison of relative infectivities to be made. All units in ID_{50}/ml plasma.
NT = not tested
ND = not detected
* The Brown et al (1999) experiment also separated Fraction II from Fractions I and III, and most of the infectivity (95.6%) was found to remain with Fractions I and III.

There are several possible ways of analysing the available data, as shown in Figure II.3.3.

Figure II.3.3 Comparison of Estimates of Infectivity in Plasma Fractions



The methods are:

1. Direct analysis of low input experiment (Brown 1998) with zero for Fractions IV and V.

2. Analysis of low input experiment (Brown 1998) but with 1 of the inoculated animals for Fractions IV and V assumed infected, giving a pessimistic estimate of their contributions.

3. Analysis of low input experiment (Brown 1998) with percentages increased to eliminate lost infectivity.

4. Direct analysis of spiking experiment (Brown 1998) with no correction for lost infectivity.

5. Analysis of spiking experiment (Brown 1998) with percentages increased to eliminate lost infectivity.

6. Analysis of low input experiment (Brown 1998) with contributions for Fraction IV (0.006% of plasma infectivity – see Table II.3.10) and Fraction V (0.0004%) from the spiking experiment.

7. Analysis of low input experiment (Brown 1998) provides data for Cryoprecipitate and Fractions I, II and III with contributions provided by the low dose Brown et al experiment (1999) for Fractions IV (from Table II.3.13, the infectivity of Fraction IV is 19.2% of Cryoprecipitate = 2.39% of plasma infectivity) and Fraction V (0.72% of plasma). The infectivity of Fraction II can also be estimated from Brown et al's experiment (1999) as 4.6% of the infectivity of Fractions I, II, III.

Approach number 7 is the favoured approach.

The wide variation between the possible approaches illustrates the substantial uncertainties involved, particularly regarding the reduction in infectivity due to fractionation processes. However, all approaches indicate that most infectivity remaining after fractionation is in the cryoprecipitate and Fractions I and III, and that infectivity in Fractions II and V is relatively low.

The selected infectivity breakdown based on Approach 7 above is summarised in Table II.3.14.

Table II.3.14 Selected Infectivity in Plasma Fractions Based on Brown et al (1998 &1999)

Plasma Fraction	Volume (ml/450 ml whole blood)	Infectivity (ID_{50}/unit whole blood)	Infectivity Concentration (ID_{50}/ml fraction)
Plasma	230	480	2.1
Cryoprecipitate	3	60	19.6
Fraction I + II + III	8	36	4.4
Fraction II	3.8	1.6	0.43
Fraction IV	17.5	11.5	0.66
Fraction V	24.8	3.4	0.14
Cryosupernatant	227	50.6	0.22

II.3.8 Summary of Derivation of Infectivity Levels

Figure II.3.4 overleaf summarises the key steps involved in the derivation of infectivity levels.

Figure II.3.4 Summary of Derivation of Infectivity Levels

		Summary	Relevant Section	Selection of Infectivity
STEP 1	*Whole Blood Infectivity*	Different experiments gave a range of infectivity from 4-300 ic ID_{50}/ml. Lower end of range selected (see Section II.3.5.7).	Section II.3.5.1 to II.3.5.7	10 i/c ID_{50}/ml whole blood
STEP 2	*Conversion Intracerebral to Intravenous*	Five fold reduction factor chosen for conversion from i/c to i/v inoculation, based on experiments.	Section II.3.5.8	2 i/v ID_{50}/ml whole blood (= 900 i/v ID_{50}/unit whole blood)
STEP 3	*Blood Components Infectivity*	A 24% infection contribution from RBC is derived as detailed in section II.3.5.1. For plasma and buffy coat, the Brown et al experiments give their proportional infectivity and combining this with 24% RBC, these are then related to the 900 iv ID_{50}/ unit whole blood infectivity derived in Step 2.	Section II.3.5.1 Section II.3.6.2 to II.3.6.6	Whole Blood: 900 i/v ID_{50}/unit Plasma: 480 i/v ID_{50}/unit whole blood RBC: 219 i/v ID_{50}/unit whole blood Buffy: 201 i/v ID_{50}/unit whole blood
STEP 4	*Plasma Fractions Infectivity*	Brown's 1998 and 1999 experimental data is used to determine the proportions of the plasma fractions infectivity and this is then related to the plasma infectivity derived in Step 3.	Section II.3.7	Cryoprecip: 46 i/v ID_{50}/unit whole blood Fract 1&III: 34.4 i/v ID_{50}/unit whole blood Fraction II: 1.6 i/v ID_{50}/unit whole blood Fraction IV: 8 i/v ID_{50}/unit whole blood FractionV: 2.6 i/v ID_{50}/unit whole blood Cryosuper: 48 i/v ID_{50}/unit whole blood

The above is for non-leucodepleted blood. Any necessary adjustments for leucodepleted blood products should be made with reference to Appendix II.4.2.