



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL
Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

**SCIENTIFIC COMMITTEE ON EMERGING AND NEWLY
IDENTIFIED HEALTH RISKS
(SCENIHR)**

Opinion on

**The Safety of Human-derived Products with regard to Variant
Creutzfeldt-Jakob Disease**

Adopted by the SCENIHR
during the 7th plenary meeting of 28-29 September 2005

TABLE OF CONTENTS

EXECUTIVE SUMMARY	4
1. BACKGROUND	7
2. TERMS OF REFERENCE	8
3. SCIENTIFIC RATIONALE	9
3.1 Introduction	9
3.2 Current status of knowledge on vCJD – overview	10
3.2.1 The infectious agent	10
3.2.2 vCJD pathogenesis and the immune system	11
3.2.3 Distribution of infectivity and PrP ^{Sc} in CJD	13
3.2.4 Evidence for the presence of vCJD infectivity in peripheral blood	15
3.2.5 Conclusions	15
3.3 Review of the TSE detection methods	15
3.3.1 Biochemical assay methods for the detection of PrP ^{Sc}	16
3.3.2 Infectivity methods for the detection of TSE agents	17
3.3.3 Surrogate markers of TSE disease	19
3.3.4 Other methods for the detection of TSE agents	19
3.3.5 Tests in development for blood	19
3.3.6 Conclusions	20
3.4 Evaluation of current epidemiological data	21
3.4.1 Surveillance systems and methodologies	21
3.4.2 Current variant CJD figures	23
3.4.3 Modelling and predictions of vCJD figures	25
3.4.4 Epidemiological approaches to secondary transmission	28
3.4.5 Problems & possible deficiencies in current systems	33
3.4.6 Conclusions	33
3.5 Evaluation of prion decontamination procedures for surgical instruments	34
3.5.1 Cleaning	34
3.5.2 Decontamination and sterilisation	35
3.5.3 Conclusions	37
3.6 Risk assessment on transmission of vCJD	37
3.6.1 Risk assessment for transmission of vCJD by blood or blood components	37
3.6.2 Risk assessment on transmission by surgical instruments or invasive procedures	45
3.6.3 Risk assessment on vertical transmission	50
3.6.4 Risk assessment on transmission by transplantation of Umbilical Cord Stem Cells	51

3.6.5	Conclusions.....	52
3.7	Evaluation of previous opinions of the SCMPMD on human product safety for vCJD transmission with regard to current scientific knowledge	53
3.7.1	Opinion on Quality and Safety of Blood (Adopted by SCMPMD on 16 February 2000).....	53
3.7.2	Opinion on update of the opinion given by SCMPMD on the risk quantification for CJD transmission via substances of human origin (Adopted by SCMPMD on 16 February 2000).....	53
3.7.3	Opinion on The Safety Of Human-Derived Products With Regard To TSE's (Adopted by SCMPMD on 18 January 2002).....	54
4	COMMITTEE OPINION.....	58
5	MINORITY OPINION.....	62
6	REFERENCES.....	63
7	ACKNOWLEDGEMENTS	77
	GLOSSARY	78

EXECUTIVE SUMMARY

In 2004 two cases were reported indicating the possible transmission of variant Creutzfeldt-Jakob disease (vCJD) by blood transfusion. This prompted a review of the current state of knowledge and practice of vCJD infection in relation to the safety of blood and blood components, including the evaluation of previous scientific Opinions of Scientific Committee for Medicinal Products and Medical Devices (SCMPMD). The general conclusions and recommendations of the previous Opinions on the safety of human derived products including blood and blood components are still valid. However, two aspects do need our attention: 1) the possibility of transmission of vCJD by blood and blood components, and 2), the presence of asymptomatic vCJD infected individuals in the population who may be responsible for secondary transmission of the disease by blood/blood components or surgery.

While neither of the two transfusion-related cases reported is definitively proven to be caused by the preceding blood transfusion this is the most likely explanation. These two cases raise serious concern over the possibility of infection by blood transfusion (or through surgery) from asymptomatic preclinically or subclinically infected donors. Therefore, it is assumed that vCJD infectivity is likely to be present in human peripheral blood which is in accord with the results of recent work in experimental animals on transmission by transfusion. The present risk assessment of exposure to vCJD infectivity in whole blood and in blood components allows a rationale to define precautionary measures to reduce vCJD transmission within the human species by the intravenous or other routes.

While all the clinical cases of vCJD so far have been homozygous for methionine, (MM) at codon 129 of the prion protein (*PRNP*) gene, one of the two reported transfusion-related cases was heterozygous (MV). In addition, the results of the UK study evaluating anonymised appendix surgical specimens, showed that vCJD infection might be more common than is suggested by the numbers of actual cases of vCJD to date. It is therefore possible that following exposure to BSE, vCJD infectivity is present in a considerable number of individuals in the UK in an asymptomatic phase of the disease, including individuals with MV genotypes. This poses an additional threat to the use of blood and other products of human origin as a potential source of secondary transmission. The potential transmission by blood raises concern, especially in view of the fact that routine screening with respect to vCJD is not (yet) possible. A possible iatrogenic transmission through surgical instruments used in invasive procedures also has important implications.

Considerable advances in test methodologies for prion diseases have been made in recent years. However, no diagnostic system has yet emerged with the level of sensitivity and specificity required for routine screening of blood or urine. It is essential that confirmatory assays are available for any assay proposed for large scale screening of donated blood. In addition, prior to introduction into routine practice, such assays should be independently assessed and validated for their analytical performance. Validation of any new methodology should be mandatory prior to introduction, and it is recommended that the EU adopts a procedure similar to that used for the BSE testing. For validation, carefully controlled vCJD reference materials should be used. The issue of false positives needs especially careful consideration. Even minute percentages of false positives may

actually involve a large number of individuals if the tests are performed on a large scale in the EU population, with a varying "prevalence" of asymptomatic carriers. The ethical implications of testing and informing an individual of a positive test result, without providing any certainty as to the likelihood of progression to clinical disease, should not be considered lightly.

Based on conservative assumptions made for the purposes of this risk assessment, there is a considerable risk that an asymptomatic donor infected with vCJD could cause infective material to be passed on to one or more recipients of blood or blood components. In the worst case scenario each therapeutic unit of blood from an asymptomatic infected donor could contain as much as 4500iv ID50. This amount of infectivity is deemed sufficient to cause transmission of the infection, with or without development of the disease. Because the donor population is much younger than the recipient population, with only a small overlap in age, modelling studies strongly indicate that blood transfusion alone will not be sufficient to maintain vCJD in the human population at large.

Taking into account the eligible blood donor population, and using the data of the UK appendix study, the number of donations and the percentage of the population actually donating blood, up to 1250 infected donations may occur per year. As donations are typically split between 3 recipients, 3750 new infections would occur each year in the UK as a result of these infected donations in the worst case scenario. Transfusion statistics show that in general only about 50 % of blood recipients survive more than 3 years. Accordingly half of the blood recipients will not live long enough after transfusion to develop vCJD. If all the surviving recipients do develop disease, 1875 new individuals per year could develop vCJD in the UK population

The current decline in the onset of clinical vCJD in the UK and the general low number of cases in the older age groups who comprise the majority of blood recipients, strongly indicate that this worst case scenario considerably overestimates transfusion-related vCJD disease development.

There are several possible explanations for this. It is possible that most infections have a very long incubation period so that the individual dies before disease develops, or that infections in some groups such as the MV heterozygotes are not associated with blood infectivity, or that different genotypes do not transmit efficiently to each other even if the unit is infectious. There are analogies in animal models for these scenarios, and they reflect the difficulty in making realistic estimates of the number of cases expected from blood transfusion. Taking the lower limit of the confidence interval of the prevalence from the UK appendix study and assuming that only ten percent of infectious donations actually transmit the infectious agent, the number of infections resulting would be 9 per year in contrast to the 1250 predicted by the worst case scenario. Independent of the method of calculation transmission by blood transfusion may occur. Based on current data, the frequency cannot be reliably estimated, but even in the UK it is probably low. The frequency is largely dependent on the number of asymptomatic vCJD infected individuals in the general population which is likely to differ from one Member State to another. Epidemiological studies, similar to the UK appendix study, are needed to collect data on the presence of infection (PrP^{Res}) in the general population before estimations can be made on the possible frequency of contaminated blood donations in countries other than the UK.

There is no evidence that individuals working in hospital settings have developed vCJD by virtue of their profession. Transmission of TSEs during surgical procedures remains a concern, but to date there is no evidence that it has actually occurred in relation to vCJD. To minimize the risk of transmission of vCJD by surgical instruments procedures are recommended based on the probability of the patient under investigation/treatment being infected with vCJD (or any other TSE).

There are no proven instances of vertical transmission of any human prion disease. The available animal data are inadequate to allow firm conclusions concerning vertical transmission to be drawn. It is recommended that there is a follow-up for children that are born to mothers who had or developed clinical vCJD. There are no data indicating that breast milk transmits human prion disease.

In the absence of evidence on vertical transmission in man, the risk posed by the use of cord blood can be considered to be negligible.

In conclusion, as long as there is a risk that infectious prion protein is present in blood and blood components, there will be a risk of transmission of vCJD disease by transfusion. Blood transfusion appears the most likely route for inter-human transmission of vCJD. The Committee does not consider that specific measures are needed to reduce the risk from vCJD infectivity in blood. However, it recognises that there are good practices to reduce any risk for transmission of infectious diseases such as optimal use of the transfusion to reduce the number of patients exposed, and optimal blood donation techniques and blood transfusion practices which minimize the number of blood donors to which an individual patient is exposed. The Committee recognises that it is important that Member States maintain the principle of regional blood supply structures, national surveillance systems and international information exchange at the EU level.

1. BACKGROUND

In July 2004, the United Kingdom announced that a second case of probable vCJD transmission via blood transfusion had been identified. The patient received the blood donated by an individual who was confirmed in 2001 as a definitive vCJD case. Although, the receiver died of unrelated causes, and showed no clinical signs of vCJD at the time of death, abnormal prion protein was found in spleen tissue and a cervical lymph node by autopsy. This patient was found to be heterozygous for codon 129 of the *PRNP* gene and differed thus from the genotype found so far in patients who have developed the disease.

As a safeguard against the possible transmission of vCJD and in order to protect the blood supply the British Government had previously introduced a number of precautionary measures. This second case has led to the introduction of further precautionary measures ‘because of a small but unquantifiable risk’, according to the Minister of Health, tightening the donor exclusion criteria.

The significance of the second case of vCJD infection transmission by blood is that while all previous clinical cases of vCJD were genotyped as homozygous (MM) at codon 129 of the prion protein gene, this new case was heterozygous (MET-VAL, MV) at codon 129. It is known, from studies of other prion diseases, that *PRNP* codon 129 genotype affects the susceptibility of individuals to disease and longer incubation periods are seen in acquired prion disease in non-MM genotypes.

The following considerations should be taken into account:

- A second human case with evidence for vCJD infection gives further confirmation that vCJD can be transmitted by blood transfusion. The finding was consistent with the existence of a substantial risk associated with the receipt of non-leucodepleted blood from a donor incubating vCJD. The extent to which leucodepletion reduces that risk is not known. Published studies confirm that intra-species infection by blood transfusion is possible in sheep and non-human primates.
- This case provides evidence that vCJD infection can be transmitted to heterozygotes, and is not just restricted to homozygotes. It is unknown whether vCJD disease develops in only MM homozygous individuals or if cases will emerge in the other genotypes at a later time because of a longer incubation period.
- Although it cannot be known whether this individual would have eventually developed the disease, the finding of PrP^{Res} is significant as it indicates that iatrogenic transmission by blood in the incubation phase (or in genuinely subclinical disease), through surgical instruments or by instruments used in invasive procedures can occur.

According to the UK Spongiform Encephalopathy Advisory Committee (SEAC) Statement of 7 August 2004, the Committee agreed inter alia that careful post-mortem examination of all recipients of blood (leucodepleted or not) from donors incubating vCJD would help to quantify the nature and magnitude of the risks of transmission of the vCJD agent through blood donated by preclinical cases of vCJD.

The Scientific Committee for Medicinal Products and Medical Devices (SCMPMD) issued its last Opinion on ‘the Risk quantification of CJD transmission via substances of human origin’ in February 2000 (SANCO/SCMPMD/2000/0005) and on the ‘Safety of Human-derived Products with regards to TSEs’ in January 2002 (SANCO/SCMPMD/2002/0001). The latter did not cover exclusion criteria for donors (covered in the opinion adopted 21.10.98) and 16.02.2000) nor the substitution with alternative products and is not up to date with regards to recent developments in the science e.g. in the infectivity in blood and transmissibility via blood.

In the light of the new findings, a request for an updated Opinion on the risk quantification of vCJD transmission by blood including an update with regard to recent scientific developments has been sought.

It should also be noticed that all previous opinions issued were related to the use of certain products and did not address other possible paths of transmission such as vertical transmission or the infectivity of umbilical cord cells stored in cell banks. Considering that there is a relatively high possibility that the onset of clinical signs of vCJD occur during the fertile period of humans, it is desirable to extend the scope of the request to include the two items.

The Committee for Medicinal Products for Human Use (CHMP) of the EMEA has recently issued a review of its ‘Position Statement on Creutzfeldt-Jakob disease and Plasma-derived and Urine-derived Medicinal Products’ (EMA/CPMP/BWP/2879/02/rev1). Several recommendations and proposals related to vCJD were issued.

2. TERMS OF REFERENCE

In light of the recent evidence mentioned above, the Scientific Committee on Emerging and Newly Identified Health Risk (SCENIHR) is requested:

- to review the previous scientific SCMPMD Opinion on the ‘Safety of Human-Derived Products with regard to TSEs’ adopted on 18 January 2002, and the SCMPMD Opinion on ‘Quality and Safety of Blood’ adopted on 16 February 2000 in order to quantify, if possible, the nature and magnitude of the risks of transmission of the vCJD agent through blood donated by preclinical cases of vCJD, through surgical instruments or by instruments used in invasive procedures,
- to evaluate the risk of vertical transmission of vCJD in pregnant women and
- to evaluate the risk of vCJD transmission by the tissues stored in umbilical cord cell banks.

3. SCIENTIFIC RATIONALE

3.1 Introduction

The Directorate General Health and Consumer Protection of the European Commission has published several Opinions of the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) dealing with the safety of human-derived products with regard to variant Creutzfeldt - Jakob disease (vCJD) (SCMPMD 1998, 2000a, b, 2002). Concern over the possible transmission of variant Creutzfeldt - Jakob disease (vCJD) by blood products was raised by publications indicating the transmission of Transmissible Spongiform Encephalopathy (TSE) in animal models. While exposure to other tissues is possible, the largest exposure to products of human origin involves blood, either through transfusion of whole blood or components, through proteins derived from blood such as immunoglobulins, albumin or clotting factors, or by contamination of surgical instruments. This opinion relates to blood and blood components and not to plasma derived products.

The Opinions stated there was at that time no proof or disproof for vCJD infectivity in human blood. Also in animal studies TSE infectivity could not be reproducibly detected in blood. Thus the presence of vCJD infectivity in human blood could neither be excluded nor confirmed (SCMPMD 2002). Bioassays in laboratory animals are currently the only way to investigate the presence of TSE infectivity.

The risk was considered minimal, so no additional measures were proposed. On an individual basis, however, several Member States instigated donor deferral for people who had stayed for some time in the UK, while others did not.

Whereas the previous Opinions solely concerned themselves with blood, the current Opinion covers a wider area including possible transmission of vCJD by tissue and cells stored in cell banks and by the use of surgical instruments.

In 2004 two reports were published indicating the possible transmission of vCJD by blood transfusion (Llewelyn et al 2004, Peden et al 2004).

Llewelyn et al (2004) reported on a survey including forty-eight recipients who were identified as having received blood from a total of 15 donors who developed vCJD. One of the recipients developed symptoms of vCJD 6.5 years after transfusion, although infection due to past dietary exposure could not be excluded.

Peden et al (2004) reported an individual who died from a non-neurological disorder 5 years after transfusion from a donor who subsequently developed vCJD. Protease resistant protein (PrP^{Res}) was detected in this patient by immunohistochemistry in the spleen and in a cervical lymph node. Interestingly, this patient was heterozygous (MV) at the prion protein (*PRNP*) gene codon 129. All tested clinical cases of vCJD to date have been homozygous for methionine at codon 129 (MM).

Although both cases could not be definitively proven to be caused by the preceding blood transfusion, these two cases raise serious concern about the possibility of infection by blood transfusion from preclinically or subclinically infected donors especially in view of the fact that routine screening for vCJD is not (yet) possible. This concern was increased by the report of transmission of BSE in sheep blood transfusion experiments (Hunter et al 2002, Houston et al 2000).

This may have important implications for possible iatrogenic transmission by blood and/or cells and organs from donors in the incubation phase, and transmission through surgical instruments used in invasive procedures.

The evidence that potential vCJD infectivity may be present in heterozygotes at codon 129 poses an additional serious threat to the use of blood and other products of human origin, especially in view of the fact that routine screening for vCJD is not (yet) possible. This may have important implications for possible iatrogenic transmission by blood and/or cells and organs from donors in the incubation phase, and transmission through surgical instruments used in invasive procedures.

These observations resulted in the formulation of several questions (section 2) for the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) regarding the safety of blood and other human tissues in relation to the possible transmission of vCJD.

3.2 Current status of knowledge on vCJD – overview

Variant Creutzfeldt-Jakob disease (vCJD) is a neurodegenerative disorder and belongs to the group of TSEs. The histological features of TSEs include vacuoles in the brain of the infected species, neuronal loss and gliosis, leading to dementing disease and death. The distinctive feature of TSEs is the accumulation of a particular abnormal (prion) protein in the brain. vCJD was first described as an independent disease entity, different from other forms of Creutzfeldt-Jakob disease (CJD) in 1996 (Will et al 1996). The great majority of yearly notified CJD cases arise without identifiable cause (sporadic CJD, sCJD). Another form of CJD (genetic CJD, gCJD, or familial CJD, fCJD) is associated with mutations of the prion protein gene (*PRNP*). In addition, CJD can result from medical exposure to infectious material (iatrogenic CJD, iCJD). Different forms of CJD occur at different frequencies in different countries (especially vCJD) but sCJD affects approximately 1.5 to 2 persons per million/ population per year in all studied countries.

Current evidence indicates that vCJD is caused by the agent responsible for bovine spongiform encephalopathy (BSE). The ingestion of BSE contaminated food is the most likely transmission route and disease-induction mechanism of human vCJD (Hill et al 1997a, Bruce et al 1997).

As of September 1st, 2005, 182 cases of vCJD have been reported, there of 157 cases in the United Kingdom, 3 cases in Republic of Ireland, 14 cases in France, 1 case in Italy, 1 case in Saudi-Arabia, 1 case in Portugal, 1 case in the Netherlands, 1 case in Spain, 1 case in the United States of America, 1 case in Canada and 1 case in Japan. The understanding of the nature of the infectious agent of vCJD and of the pathogenesis of vCJD is critical to the question of vCJD transmission *within* the human species by the intravenous or other routes.

3.2.1 The infectious agent

The nature of the infectious agent causing TSEs has been discussed controversially for a long time (Soto and Castilla 2004). There is substantial scientific evidence in favour of the “protein only” hypothesis. In 1967, Alper and colleagues proposed for the first time that TSEs might be caused by particles replicating without nucleic acid (Alper et al 1967). In 1982, Prusiner introduced the “protein only” hypothesis (Prusiner 1982): A protein that purifies with the scrapie infectivity was characterized in 1982 (Bolton et al 1982). The gene encoding the prion protein (PrP) was cloned in 1985. In 1988, it was shown that infectivity of the prion can be

neutralized by anti-PrP antibodies. The first mutation in the *PRNP* gene associated with familial TSE was identified in 1989. In 1990, transgenic animals overexpressing mutant PrP were shown spontaneously to develop clinical and pathological signs of TSE. In 1993, PrP knockout mice were observed to be resistant to scrapie. Conformational differences between the physiological prion protein, PrP^C, and its pathological form, PrP^{Sc}, were reported in 1993 (for detailed references on the historical overview, see (Soto and Castilla (2004))). The *in vitro* conversion of the physiological prion protein, PrP^C, into its pathological form, PrP^{Sc}, was reported in 2001 (Saborio et al 2001). The generation of *de novo* infectious prions from protein *in vitro* was reported in 2004 (Legname et al 2004), and in 2005 (Castilla et al 2005), although in these cases alternative explanations of the origin of infectivity can not be excluded. In addition, the current protein only hypothesis fails to explain adequately all features of prion diseases, for example strain variation.

Different models currently exist for the conversion of PrP^C to PrP^{Sc} (Soto 2004). The infectious PrP^{Sc} may occur as a small oligomer acting as a seed for recruiting, converting and stabilizing the misfolding of the physiological prion protein PrP^C (nucleation-polymerization model). According to the template-associated conversion model, the main step of formation of the pathological prion protein is the formation of an intermediate state on binding to a molecular chaperone. The intermediate is proposed to interact with monomeric or oligomeric PrP^{Sc}, which acts as a template for its conversion. Consistent with both models, conformational transition of the prion protein from an alpha-helical isoform to a beta-sheet-rich isoform is now widely considered to underlie the pathogenesis of prion-related diseases at the structural level, making TSEs similar to other protein conformational disorders such as Alzheimer's disease (Baskakov et al 2004, Bucciantini et al 2002, Lundmark et al 2002, Soto 2001).

Barriers for prion transmission between different species exist, and different prion strains characterized by different biological properties exist (Bruce et al 1997, Tanaka et al 2005, Jones and Surewicz 2005). For example, prion proteins from BSE case can be distinguished from those from scrapie cases (Scott et al 2005).

In summary, the pathogenesis of prion-related diseases is currently widely considered to be related to abnormal protein folding. Normal cellular protein is converted into an insoluble, aggregated, beta-sheet rich form which is deposited in the brain. The mechanism of prion-related diseases is believed to involve disease transmission by replication of protein conformation (Soto and Saborio 2001). The accumulation of protease resistant prion protein in tissues is a characteristic feature of prion disease. vCJD is a typical prion disease in this respect.

3.2.2 vCJD pathogenesis and the immune system

PRNP polymorphism is a determinant of vCJD in humans. All tested clinical cases of vCJD to date have been homozygous for methionine at codon 129 of the prion gene. Wadsworth et al (2004) demonstrated that the generation of the vCJD phenotype in a particular transgenic mouse model required expression of human prion protein with methionine in position 129. Expression of human PrP with valine 129 resulted in a distinct phenotype that was associated with the persistence of a barrier to transmission of BSE-derived prions. In this mouse model, the polymorphic residue 129 of human PrP dictated propagation of distinct prion strains after BSE prion infection, suggesting that human infection with BSE-derived prions could result in a variety of phenotypes of vCJD, depending on the genotype of the prion source and the recipient (Wadsworth et al 2004). Such complexity of phenotypic determination in human prion diseases has recently been confirmed by Pan and colleagues (Pan et al 2005). Whether

certain MHC genotypes are associated with vCJD remains controversial (Jackson et al 2001, Pepys et al 2003, Laplanche et al 2003).

Besides a genetic influence on vCJD susceptibility, the cellular environment seems to be an important modifier of vCJD: vCJD prions typically accumulate in nervous and lymphoid tissues (Hill et al 1999, Hilton et al 2004a). In animal models of scrapie, proinflammatory cytokines and immune cells are required for lymphoid prion replication, and chronic lymphocyte inflammation enables prion accumulation in otherwise prion-free organs such as kidney, pancreas, or liver. Thus, chronic inflammatory conditions may act as modifiers of natural and iatrogenic prion transmission (Aguzzi and Heikenwalder 2005, Heikenwalder et al 2005). Urinary prion excretion was recently demonstrated in presymptomatic and in sick animals in a murine model for scrapie (Seeger et al 2005). It was dependent on specific intrarenal inflammatory conditions (i.e. presence of lymphofollicular foci). Prion excretion due to renal inflammation may be an explanation for the horizontal transmission of TSEs among various animal species like sheep, deer and elk (Seeger et al 2005).

Acquired disease pathogenesis is a dynamic process including infection, peripheral prion replication, and prion transmigration from the periphery to the central nervous system. Although the mechanisms of prion lymphoinvasion and neuroinvasion are not yet completely understood, many molecular details have been elucidated (Aguzzi and Heikenwalder 2005). The cellular basis for prion transmigration from the gut into the lymphoid system seems to depend on membranous epithelial cells (M cells) that may represent a site of prion entry. Evidence for such mechanisms, so far, comes from coculture models (Heppner et al 2001).

Within the lymphoid system, B cells are important for peripheral prion spread and neuroinvasion (Klein et al 1997). B cell effects on prion replication seem to derive primarily from the expression of cytokines such as the proinflammatory cytokine lymphotoxin α (= TNF beta). Within lymphoid organs, B cells contribute to the maturation and maintenance of pre-existing mature follicular dendritic cells (FDCs). FDCs accumulate PrP^{Sc} after scrapie infection, and prion replication in spleen depends on PrP^C expressing FDCs (for detailed literature, see (Aguzzi and Heikenwalder (2005)). Defects in haematopoietic compartments, such as impaired B-cell maturation, or in stromal compartments, such as abrogation of FDCs, can delay or prevent lymphoreticular prion accumulation. Kaeser and colleagues studied the distribution of infectivity in splenic fractions of mice expressing the gene that encodes PrP^C solely on haematopoietic or on stromal cells following intraperitoneal challenge with prions, and observed that optimal prion replication requires PrP^C expression by both stromal and haematopoietic compartments (Kaeser et al 2001).

Under physiological conditions, FDCs contribute essentially to antigen presentation by antigen trapping via capturing of immune complexes by Fc γ receptors, and via the binding of opsonized antigens to the CD21/CD35 complement receptors, suggesting a role for complement components in prion-related diseases. Indeed, mice genetically engineered to lack complement factors (Klein et al 2001) exhibited enhanced resistance to peripheral prion inoculation. Consistent with this observation, Mabbott and colleagues observed that temporary depletion of the complement component C3 or genetic deficiency of C1q significantly delayed the onset of scrapie (Mabbott et al 2001). It is still unclear how prions reach FDCs, how they disseminate throughout the lymphoid system, and how they travel from FDCs to nerve endings, resulting in neuroinvasion.

Peripheral blood may be involved in propagation and transport of vCJD prions, although PrP^{Sc} has not yet been detected in peripheral blood of vCJD patients (Wadsworth et al 2001). Currently, evidence of blood-borne infectivity in other forms of TSE exists and implies that mobile cells contribute to the spread of infectivity (Brown et al 1998, Houston et al 2000,

Hunter et al 2002). Thus, lymphocytes were shown to acquire prion infectivity when residing in lymphoid organs (Raeber et al 1999), and may contribute to the prion load in blood. Consistent with this observation, blood infectivity in experimental systems could be related to the white cell content of blood components (Brown et al 1999, Brown et al 1998), and leucodepletion of cellular blood components for haemotherapeutic purposes is considered to reduce peripheral blood infectivity (Turner 1998, Turner 2000). However, it has been reported that 50% of infectivity in blood is not cell associated but is transmitted by the plasma (Gregori et al 2004). Dendritic cells may represent another type of immune cells that facilitate transport of prions from the periphery to sites of prion replication (Aucouturier et al 2001, Huang et al 2002). Direct penetration into the brain across the blood-brain barrier may occur (Soto 2004).

A completely different mechanism of prion transport in the peripheral circulation has been proposed by Fevrier and colleagues (Fevrier et al, 2004): they observed that both PrP^C and PrP^{Sc} were actively released into the extra cellular environment by PrP expressing cells before and after infection with sheep prions, respectively. PrP^C and PrP^{Sc} in the medium were associated with exosomes, membranous vesicles that are secreted upon fusion of multivesicular endosomes with the plasma membrane. Exosomes bearing PrP^{Sc} were found to be infectious, suggesting that exosomes may contribute to intercellular membrane exchange and to the spread of prions throughout the organism.

Vaccination against the abnormal prion protein is challenging. Strategies for immunization against prion diseases represent a field of sincere efforts (Aguzzi and Sigurdson 2004, Nikles et al 2005). PrP^C is a normal protein with yet unknown function and is present at the surface of a variety of cells including immune cells under physiological conditions (Cashman 2001). In addition, prion-specific antibodies are not generated during the course of prion infections. However, passive immunization with antibodies directed against the normal PrP^C can prevent prion disease, and induction of humoral immune responses to PrP^C or PrP^{Sc} might be protective. Future research in the prion immunology field is likely to focus on the establishment of efficient diagnostic, prophylactic and therapeutic anti-prion concepts (Aguzzi et al 2004, Aguzzi and Sigurdson 2004, Heppner and Aguzzi 2004, Soto 2004, Weissmann and Aguzzi 2005). A successful vaccination should result in an immune response to either the normal or abnormal prion protein, protecting against disease without adversely affecting the functionality of normal PrP^C.

Taken together, current evidence on the pathogenesis of vCJD indicates that orally ingested prions enter mainly via the gut lymphoid tissue, and are transported to the other lymphoid organs. Prions are replicated in the periphery in lymphoid tissues such as the spleen, the appendix and tonsils, and are then transported to the brain mainly by peripheral nerves. Direct penetration into the brain across the blood-brain barrier may occur. Thus, peripheral blood may be involved in propagation and transport of vCJD prions, although PrP^{Sc} has not yet been detected in peripheral blood of vCJD patients.

3.2.3 *Distribution of infectivity and PrP^{Sc} in CJD*

The broad picture of the distribution of infectivity and PrP^{Sc} in cases of TSEs is that in general the highest titres in clinical cases are found in the brain and other nervous tissues while the major other types of tissue of concern are the lymphoid organs, such as the spleen. However the details vary depending on the species, the strain of agent, the individual case and its clinical status and possibly pathophysiological parameters (see above on the effect of inflammatory cytokines). Specifically large amounts of PrP^{Sc} may be found in organs such as the spleen, appendix and tonsils in cases of variant CJD but not in sporadic CJD. It has been shown by assays of infectivity in a mouse model that spleen from vCJD cases was infectious at a level about one thousand fold less than in

brain (Bruce et al 1997). While it is possible that some infectivity or PrP^{Sc} can be found in lymphoid tissues from cases of sporadic CJD, the scale is very different. It is also suspected that lymphoid tissue involvement in sCJD occurs relatively late in the illness, whereas it occurs preclinically in vCJD. Similarly in scrapie infected sheep more infectivity and PrP^{Sc} can be found in the peripheral non-neurological tissues of certain genotypes than of others. There may be specific combinations of circumstances in which a tissue is more or less infectious than others.

Table 1 presents the reported distribution of infectivity and/or PrP^{Sc} in vCJD and includes the other forms of CJD for comparison. It is consistent with data for other TSEs from both natural and model animal systems. Tissues are classified into high, medium and low infectivity, but it should be born in mind that detection is mostly based on the detection of PrP^{Sc}, and the quantitative relationship between PrP^{Sc} and infectivity is not precisely established. A tissue with very little PrP^{Sc} could therefore in theory be infectious.

Table 1. Presence of abnormal Prion Protein and infectivity in human tissues

Tissue	Presence of abnormal Prion Protein and level of infectivity			
	CJD other than vCJD		vCJD	
	PrP ^{Res} detected	Assumed level of Infectivity	PrP ^{Res} detected	Assumed level of Infectivity
Brain	+	High *	+	High *
Spinal cord	+	High *	+	High
Spinal ganglia	+	High	+	High
<i>Dura mater</i>	NT	High	NT	High
Cranial nerves	+	High	+	High
Cranial ganglia	+	High	+	High
Posterior eye	+	High *	+	High
Anterior eye and cornea	-	Medium	-	Medium
Olfactory epithelium	+	Medium	NT	Medium
Tonsil	-	Low	+	Medium *
Appendix	-	Low	+	Medium
Spleen and thymus	+	Low *	+	Medium *
Other lymphoid tissues	-	Low *	+	Medium *
Peripheral nerve	-	Low	-	Low
Dental Pulp	-	Low	-	Low
Gingival Tissue	-	Low	-	Low
Blood and bone marrow	NT	Low	NT	Low
CSF	-	Low *	-	Low
Placenta	NT	Low	NT	Low
Urine	NT	Low	NT	Low
Other tissues	NT	Low *	NT	Low

Key: + = tested positive - = tested negative NT = not tested
* = infectivity proven in experimental transmission studies

3.2.4 Evidence for the presence of vCJD infectivity in peripheral blood

In February 2004, Llewelyn et al reported on the first case of possible transmission of vCJD by blood transfusion in humans (Llewelyn et al 2004). The case had been identified by the efforts of the UK National Blood Authorities and the National CJD Surveillance Unit (NCJDSU). Forty-eight individuals were identified by the NCJDSU as having received a labile blood component from a total of 15 donors who later became vCJD cases and appeared on the surveillance unit's register. One of these recipients was identified as developing symptoms of vCJD 6.5 years after receiving a transfusion of red cells donated by an individual 3.5 years before the donor developed symptoms of vCJD. A second case of possible transmission of vCJD infection by blood transfusion in humans was reported in August 2004 (Peden et al 2004). Pre- or sub-clinical vCJD was reported in a patient who died from a non-neurological disorder 5 years after receiving a blood transfusion from a donor who had subsequently developed vCJD. Protease-resistant prion protein, the typical feature of prion disease, could be detected in the spleen and in a cervical lymph node, but not in the brain. The patient was heterozygous at codon 129 of the *PRNP* gene providing the first evidence in humans that susceptibility to vCJD infection is not confined to the methionine homozygous *PRNP* genotype (MM).

These indications of the presence of vCJD infectivity in human peripheral blood are in line with experimental data proving blood borne transmission of TSEs in animals. In 2000, preliminary evidence indicated the sheep-to-sheep transfusion transmission of BSE (Houston et al 2000). Such evidence was confirmed in preclinically infected and clinically affected animals in 2002 (Hunter et al 2002). In 2004, BSE transmission in primates was demonstrated to be at least as efficient by the intravenous route as by the intracerebral route (Herzog et al 2004).

3.2.5 Conclusions

The current evidence indicates that infectivity is likely to be present in the peripheral blood of humans infected with vCJD. The risk assessment of exposure to vCJD infectivity in whole blood and in blood products allows a rationale to define precautionary measures to reduce the risk of vCJD transmission within the human species by the intravenous or other routes.

3.3 Review of the TSE Detection Methods

A detailed review of potential screening methods for the detection of vCJD or other TSE diseases was not included in previous opinions of the EU Scientific Committee on Medicinal Products and Medical Devices (SCMPMD). This opinion will therefore provide a summary of the current state of the art as it relates to TSE detection.

Given the continuing debate around the nature of TSE agents, for the purposes of this review it will be assumed that the Prion Protein (PrP) is essential for infectivity, and that the infectious aggregated form of this protein (PrP^{Sc} for PrP^{Scrapie}) is closely associated with the principal agent responsible for transmissibility, and therefore serves as a suitable marker for infectivity. It should be noted that methods for PrP^{Sc} detection usually involve detection of PrP^{Res}, the protease resistant core. For the purposes of this review, PrP^{Res} and PrP^{Sc} are often used as equivalent terms. To date PrP^{Sc} remains the only disease specific marker, and this assumption underpins most of the development efforts aimed at developing diagnostic tests for the detection of TSE agents.

A distinction should be made between detection methods amenable for rapid large scale screening, and tests that are useful in confirming initial diagnoses. A considerable effort has been expended in attempts to develop ante-mortem tests for prion agents as such tests could provide a possible risk reduction measure for screening purposes. Other less rapid detection methodologies remain important, and still play an important role in confirming diagnosis, characterising strain differences and estimating levels of infectivity.

Rapid and effective diagnostic procedures for large-scale screening rely on the availability of easily collectable samples for analysis (i.e. blood or urine) as well as a screening assay with the required level of sensitivity combined with a high level of specificity. In the UK, examination of routinely removed lymphoid tissues has found PrP^{Res} positivity typical of that found in vCJD in three out of 12,674 appendices that could be evaluated (Hilton et al 2004a), but such a survey was performed at some considerable effort, and using samples not suitable for general routine screening (since it requires appendicectomy). Low levels of sensitivity will lead to false negative results, whereas a low specificity will result in large numbers of false positive results, with ensuing ethical difficulties associated with how such results are handled. Ethical issues are apparent even if a specific and sensitive assay would be available as there is no cure for the disease.

The levels of TSE infectivity likely to be present in the blood of individuals with vCJD are likely to be very low (section 3.2.3). Ante-mortem screening tests, to be useful as a risk reduction measure, must be capable of identifying individuals prior to the onset of clinical disease, at a time where potential TSE infectivity will be lower than during the clinical phase of disease. Where infected brain contains levels of infectivity during the clinical phase of disease in the order of 10^6 - 10^7 IU/ml (an infectious unit is defined as the lowest concentration of agent required to initiate infection when inoculated using the optimal bioassay system; i.e. intra-cerebral inoculation in the same species), sensitivities in the range of 1-10 infectious units per ml will be required for testing of blood. The low limit of detection required represents a formidable challenge, especially in a complex matrix such as blood.

3.3.1 Biochemical assay methods for the detection of PrP^{Sc}

All commercial test kits currently available for the detection of the PrP^{Sc} require proteolytic digestion of the sample prior to detection of any residual PrP^{Sc} (which has a partial resistance to proteolytic digestion). The digestion is required to remove any normal PrP, which might otherwise interfere with the subsequent immunological detection of PrP^{Sc}. Assays have also been developed which do not require proteolytic digestion (e.g. the Conformational Dependent Immunoassay, or CDI assay, Safar et al 2002). The CDI assay relies on substantial enhancement of immuno-reactivity of PrP^{Sc} following protein denaturation, mediated through the exposure of “buried” epitopes.

Assays, which do not rely on proteolytic digestion prior to detection of PrP^{Sc}, are likely to demonstrate a higher level of sensitivity, although exceptions to this rule have been reported (Bellon et al 2003). The PrP^{Sc} protein is not completely resistant to proteolytic digestion, and the degree of resistance is strain specific (Kuczius and Groschup 1999). There may also be PrP^{Sc} forms within the same sample with varying resistances to proteolytic digestion (Minor 2004, Yakovleva et al 2004). The relevance of these

various PrP^{Sc} forms to infectivity is not clear, and has only further complicated the development of any diagnostic test that relies on proteolytic digestion prior to detection. An additional complication is that assumptions derived from observations in animal systems, may not be applicable to disease in humans.

To circumvent the issue of potential loss of signal following proteolytic digestion, attempts have been made to generate monoclonal antibodies that detect conformational epitopes present only on PrP^{Sc} (Korth et al 1997, 1999) or antibodies that are targeted at epitopes expressed on the surface of PrP^{Sc} but not on the normal form of the PrP protein (Paramithiotis et al 2003). The applicability of such antibodies in routine diagnostic testing is still under investigation, and test systems have been reported utilising such approaches.

The sensitivity of most immunological assays for the detection of PrP^{Sc} is such that most would not be suitable for screening of blood. An improved CDI system has reported levels of sensitivity approaching that necessary for the detection of PrP^{Sc} in blood, and is comparable to the detection limit of vCJD and CJD in transgenic mice carrying the human prion gene (Bellon et al 2003, Safar et al 2002). The sensitivity of detection in transgenic mice, however, cannot be correlated to human infectious units, and thus this level of sensitivity may not be sufficient for routine screening. An evaluation of the limit of detection of this test using WHO vCJD reference materials is currently ongoing.

The concentration of PrP^{Sc} by techniques such as phosphotungstic acid precipitation (Wadsworth et al 2001) may be useful in any diagnostic test (Bellon et al 2003). The initial report that concentrations of PrP^{Sc} can be “amplified” through a process of repeated protein denaturation and subsequent refolding (Protein Misfolding Cyclic Amplification: PMCA) (Saborio et al 2001) has been repeated in other laboratories, and although initial attempts to show a corresponding increase in infectivity were unsuccessful, the *in vitro* generation of infectivity has recently been claimed (Castilla et al 2005).

Shaked et al (2001) reported the detection of PrP^{Sc} in the urine of hamsters, bovines and humans in the clinical stages of TSE infection although intra-cerebral inoculation of the urine associated PrP^{Sc} in hamsters did not result in disease transmission. The possibility that the PrP^{Sc} signal observed in urine may originate from cross-reacting protease resistant enterobacterial proteins has been raised (Furukawa et al 2004). A recent report provides evidence that this signal is in fact due to immunoglobulin light chain fragments rather than PrP^{Res} and the test proposed by Shaked et al (2001) did not discriminate between cases and controls in a blinded study (Head et al 2005). As indicated in section 3.2.2 it was reported very recently that urinary prion excretion could take place in a murine model of scrapie under conditions of lymphofollicular renal inflammation (Seeger et al 2005). However, the authors were unable to detect PrP^{Sc} by biochemical methods although the urinary proteins were able to induce scrapie in indicator mice.

3.3.2 Infectivity methods for the detection of TSE agents

Animal infectivity assays remain the definitive standard for the measurement of TSE agents, but the long incubation times, cost and use of large numbers of animals precludes their use in routine screening. Confirmation of TSE infection in the animal bioassay is by histopathology and the identification of characteristic spongiform changes

or amyloid deposition. In addition, confirmation by detection of PrP^{Sc} is commonly used. This may be done by immunohistochemistry or immunological assays such as immunoblotting. Recent data have indicated that using a sensitive immunoassay provides a higher level of sensitivity for disease confirmation than immunohistochemistry or standard histology (Safar et al 2005).

Most animal bioassay systems are only optimally sensitive for prions originating from the same species. The species barrier that exists when transmitting prion agents from another species (Hill and Collinge 2002) further limits the sensitivity of the test for screening human samples. Transgenic mice engineered to express the human prion gene have resulted in increased sensitivity for the detection of human prion agents (Korth et al 2003), but such systems are not amenable for use in routine screening, and have sometime yielded unexpected results (e.g. increased susceptibility to sCJD and decreased susceptibility to vCJD in the same model) (Taguchi et al 2003).

Animal infectivity assays remain an important tool in a number of areas of prion biology:

- In the characterisation of new strains of agent. Strain differences manifest themselves in different incubation times, different neuropathological lesion profiles (Bruce et al 1997) or different protein/glycosylation profiles (Collinge et al 1996). To date there is one particular protein/glycosylation type that has been uniquely associated with BSE and the associated infections of the animals and humans (Collinge et al 1996, Head et al 2004). Such assays can therefore provide important information when analysing unknown or newly emerging strains of TSE agents.
- In the characterisation of reference materials. Such reference materials are used in assessing new diagnostic technologies e.g. titres of vCJD reference materials in the human PrP transgenic mouse model will enable a determination of the limits of detection for biochemical assays (Safar et al 2002).
- In the confirmation of biochemical *in vitro* assays. For example when prion reduction studies are performed using only biochemical determinations of PrP^{Sc}.

The route of inoculation plays an important role in transmission in animal bio-assay systems, and in general intracerebral inoculation is the most efficient route followed by intraperitoneal, intravenous and oral routes respectively. Recent studies in primates have cast some doubt on the view that intravenous inoculation is less sensitive than intracerebral inoculation (Herzog et al 2004), but additional work is needed to determine if this effect is consistently observed in the animal system used in this study.

In vitro tissue culture systems for the detection of TSE infectivity have been described, and for levels of sensitivity comparable to that observed in the animal bioassay, the selection of specific sub-populations of cells sensitive to infection and replication appears necessary (Klohn et al 2003). Other publications have suggested that such selection may not be necessary (Vorberg et al 2004), and that indeed a wide selection of cell lines is susceptible to TSE infection, but a detailed analysis of the limit of detection was not performed in these studies. Tissue culture systems have the advantage of speed (~3 week assay time) and could be potentially automated. The systems to date have only

been described for the detection of rodent adapted scrapie strains, but hold out hope that comparable systems could also be developed for the detection of human TSE agents.

3.3.3 Surrogate markers of TSE disease

Efforts have been expended to identify markers other than PrP^{Sc} that are associated with progression to clinical disease or found during clinical disease. A number of candidate surrogate markers have been proposed, including blood levels of erythroid differentiation related factor (EDRF) or erythrocyte associated relaxing factor (EARF) (Miele et al 2001).

Elevated concentrations of 14-3-3, a cytosolic protein present in high concentrations in mammalian brains, are found in the cerebrospinal fluid (CSF) of patients with sporadic Creutzfeldt-Jakob Disease (sCJD) (Baxter et al 2002, Hsich et al 1996). In the appropriate clinical setting, the detection of elevated CFS 14-3-3 has a high degree of accuracy for the diagnosis of sCJD (Zerr et al 1998). Other proteins such as tau protein, a microtubular protein, are also elevated in sCJD and may also be a useful surrogate marker (Otto et al 1997). CSF 14-3-3 is less useful in vCJD, where only half the patients have elevated levels, however elevated concentrations of tau protein have been found to have a high sensitivity and specificity for this condition (Green et al 2001). Phosphorylated tau protein is also raised in both vCJD and sCJD, but the diagnostic accuracy of phosphorylated tau is no better than that of total tau protein for either condition (Goodall et al 2005).

Continued research is needed with such surrogate markers, using demographically defined population groups, to determine the predictive diagnostic value in the general population. Care also needs to be exercised when translating results observed in animals to humans, as pathophysiological responses may differ between species.

3.3.4 Other methods for the detection of TSE agents

TSE diseases result in spongiform changes in the brain, with the subsequent alteration of brain function, brain activity and visible signs that manifest during the clinical phase of disease. The assessment of clinical symptoms remains an extremely important tool in the diagnosis of vCJD, and in distinguishing vCJD from other forms of TSE. In support of clinical assessments, Magnetic Resonance Imaging (MRI) of the brain is now the principal test in the diagnosis of vCJD (Zeidler et al 2000, Zeidler et al 2001), and different MRI appearances usually distinguish between sCJD and vCJD. The characteristic MRI changes may occur early in disease (Shiga et al 2004). Such methods currently provide strong support for the diagnosis but are not in themselves diagnostic tests. Tonsil biopsy is a helpful test in suspected cases of vCJD where diagnostic doubt exists. It rests on the involvement of the lymphoreticular system and the detection of disease-associated PrP, however it is an invasive procedure and useful only as a supportive clinical diagnostic test (Hilton et al 2002, 2004b, Hill et al 1997b)

3.3.5 Tests in Development for Blood

The development of assays for TSEs based on blood samples has been a focus of activity for commercial and other groups for some time and is apparently making real progress (Brown 2005). Currently the most promising approaches aim to detect PrP^{Sc}.

Few of the relevant studies have appeared in the peer reviewed literature because of commercial interests although some of the enabling technology has been published. The applications of the technology have been presented at meetings and in general the level of detail available is less than would be required for a scientific publication. It seems probable that in the very near future detection of infected individuals in the preclinical and subclinical phases through testing blood samples will be possible. For the purpose of this opinion it was decided to review the methods, bearing in mind that the list may be incomplete and the supporting information inadequate.

Early approaches to assays of very low amounts of PrP^{Sc} included immunocapillary electrophoresis (ICE) (Schmerr et al 1999) in which samples were digested with proteinase and residual PrP was detected by competition with a synthetic peptide for binding to a specific antibody. The key features were the concentration of the blood sample and resolution of the complexes by capillary electrophoresis. The method was technically complex and proved difficult to reproduce on human and chimpanzee samples (Cervenakova et al 2003b). A modified method has been published, but is not yet validated (Yang et al 2005). SIFT (Screening for Intensely Fluorescent Targets) is based on the fact that because PrP^{Sc} aggregates, it presents a higher number of antibody binding sites than the unaggregated normal form, so that fluorescent intensity in immunoassays is more intense for PrP^{Sc} (Bieschke et al 2000). While this is a sensitive method in the detection of known infectious material such as brain samples, the current state of development is not known and it has not apparently been applied to blood.

As already discussed above, CDI under the right conditions is very sensitive (Safar et al 1998, Safar et al 2005, Bellon et al 2003), and its suitability for the assay of blood is currently being explored.

A palindromic ligand has been developed by Adlyfe which converts from the alpha helical to beta sheet form in response to interactions with PrP^{Sc}. The transition is detected by fluorescence, and the method is sensitive (Grosset et al 2005). It has been reported to distinguish samples from infected and uninfected laboratory animals, sheep and cows (Pan et al 2004). A ligand (Seprion) has been developed by Microsens which is reported to bind specifically to PrP^{Sc} of any species and to distinguish blood from infected and uninfected sheep (Lane et al 2003). It has also been reported to distinguish blood from an iatrogenic case of CJD from controls (Wilson 2004).

An antibody, 15B3, reported to be specific for PrP^{Sc} (Korth et al 1999) has been used in the development of methods for the detection of PrP^{Sc} in blood (Zwald et al 2004). Finally, the cyclic amplification of PrP^{Sc} *in vitro* (PMCA) has been reported to be sufficiently sensitive to detect prions in blood from the preclinical phase (Castilla et al 2005, Soto et al 2005).

3.3.6 Conclusions

Considerable advances in test methodologies for prion diseases have been made in recent years, and the application of these advances in the diagnosis of vCJD has, in particular, been fruitful. However, no diagnostic system has yet emerged with the high level of sensitivity and specificity required for routine screening of blood or urine. Care must be exercised in applying conclusions derived from animal experiments to the situation in humans.

As with screening tests approved within the EU for BSE, test validation of any new methodology should be mandatory prior to implementation. Given the scarcity of vCJD material, a stepwise approach should be followed, where only those tests which have attained a prescribed level of sensitivity and specificity for example in carefully controlled animal experiments, are allowed to proceed into testing with vCJD reference materials. This approach will ensure that any test implemented will generate meaningful and interpretable data suitable for risk management decisions in relation to vCJD. Options have been presented using a combinatorial approach, where individuals identified as positive using a low specificity test could be subsequently screened using other tests intended to either validate or invalidate the first positive result. The use of such approaches warrants extreme caution, and the ethical issues of informing an individual of such results, without providing any certainty as to the likelihood of progression to clinical disease, should not be considered lightly. Quite aside from the likely emotional impact on individuals with a positive test, decisions will be needed as to what level general public health protection measures should be taken (e.g. if such individuals require dental treatment or surgical operations).

It is essential that the assays are independently validated, confirmatory assays are available and all ethical implications are considered and carefully taken into account before implementing testing. It is recommended that assays are approved by EU similar to the situation for BSE testing.

3.4 Evaluation of current epidemiological data

The epidemiological data reviewed here include the incidence/prevalence of vCJD, the basic case characteristics, studies of risk factors and observational data related to possible instances of secondary transmission. This necessitates a review of disease surveillance systems as well as specific research approaches such as case-control studies.

3.4.1 Surveillance Systems & Methodologies

Epidemiological data on vCJD depend fundamentally on case ascertainment and, therefore, on the nature of CJD surveillance systems and their methodologies. Efficient and organised disease surveillance is vital to proper risk evaluation and risk management of CJD. Complete case ascertainment for vCJD requires surveillance of all forms of human prion disease, different forms of CJD may be sometimes clinically similar and require careful evaluation.

Background

The present UK surveillance system was established in 1990 and first described vCJD in 1996 (Will et al 1996). Aside from identifying CJD cases and collecting detailed relevant data, the NCJDSU (National CJD Surveillance Unit) undertakes a variety of research projects including a case-control study. Suspect cases of all forms of CJD, including vCJD, are referred to the NCJDSU. Wherever possible, cases are visited in life and all clinical and investigation data are collected. Cases are also identified through neuropathology and death certificates. There is a separate paediatric surveillance system, established to identify vCJD cases in children (Devereux et al 2004). Cases are classified according to the accepted diagnostic criteria (WHO 2002). A detailed face-to-face

interview of the family along with an examination of hospital and General Practice (Primary Care) notes allows recording of relevant data including surgical operations and blood donations/treatments. The relatives of all vCJD cases and appropriate controls are interviewed using a standard protocol.

Other EU member states have established CJD surveillance systems; while these vary in structure, there are good grounds for expecting that cases of vCJD would be promptly identified. There are established collaborations within the EU with harmonization of methodologies. In particular three collaborative groups have received EU-funding: EUROCCJD began in 1993, NEUROCCJD began in 1996, and SEEC in 2001. Collaborative research is now continuing under the umbrella of Neuroprion (a Framework VI, DG Research funded project). There is an established system of reporting of cases from countries of European Union to DG Health and Consumer Protection.

Clinical identification

The core of any system is the clinical identification of cases of vCJD. The absence of a specific, clinical, non-invasive test requires the involvement of clinical neurologists in case identification and classification. There are established clinical diagnostic criteria that are both sensitive and specific. (WHO 2002, Will et al 2000).

Neuropathological identification

A definite diagnosis of vCJD requires neuropathology, usually via autopsy, but sometimes via brain biopsy. Neuropathological expertise and a high post mortem examination rate are, therefore, important, especially in clinically doubtful cases. However, overall autopsy rates are low in many countries (in the UK, for hospital deaths, around 5%) and especially in neurodegenerative/dementing illnesses in the older age groups. In suspected prion disease, the rates have been substantially higher, but, in the UK, they have declined in the last few years. Some CJD autopsy data in the UK are given in Figure 1.

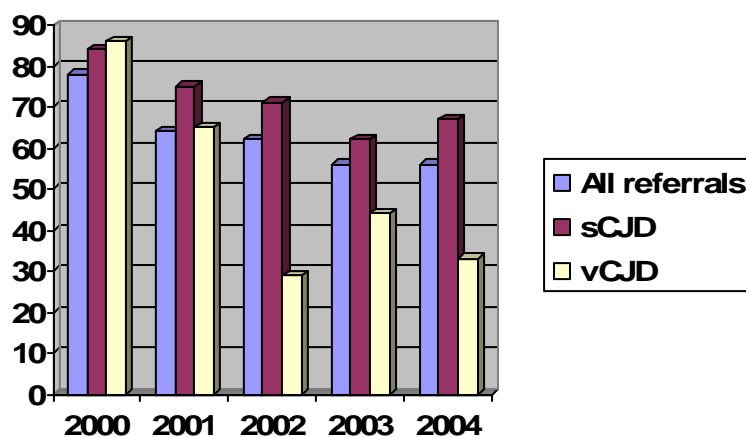


Figure 1. Autopsy rates (% of cases) in the UK 2000-2004 for all referrals of suspect CJD cases, definite & probable sCJD cases, definite & probable vCJD cases

Clearly, relatively low and declining autopsy rates are a concern in relation to complete case ascertainment, especially in relation to atypical cases, atypical age groups and the identification of any newly emerging disease phenotypes.

Lymphoreticular surveillance

There is involvement of lymphoreticular system (LRS) tissue in vCJD (Hill et al 1999, Hilton et al 2004b) and this can be (indeed, perhaps, must be) pre-clinical. There are two reported instances of definitive pre-clinical LRS involvement (Hilton et al 2002). In one, vCJD symptoms developed about 8 months (Hilton et al 1998), and in the other, 2 years after a routine appendectomy (Hilton et al 2004a). In both, the appendix specimen was later obtained and found to be positive on PrP immunohistochemistry. In a further case, an appendix removed 9-10 years prior to vCJD onset was negative for PrP^{Sc} (Hilton et al 2004a, Ironside 2000). Appendices and tonsils are fairly frequently surgically removed and their analysis for vCJD-PrP allows a form of lymphoreticular surveillance (Hill et al 1999, Ironside et al 2000, Sachse et al 2003, Hilton et al 2004a). There is one published study, reporting on anonymised routine appendectomy samples (details below) and results from a tonsillectomy study are pending (Hilton et al 2002, Hilton et al 2004a). In addition, there was a recently reported individual in whom PrP^{Res} was found in the spleen and a cervical lymph node (Peden et al 2004).

Case-control studies

Case-control studies allow an analysis of potential risk factors and there is one current study of vCJD (being undertaken in the UK). The study has analysed data from over 100 cases. To date (September 2005) there are provisional data to support the dietary theory of vCJD and no other risk factors have been identified in the case-control study (including surgery and blood transfusion). Analysis is on-going. (Knight, personal communication, NCJDSU 2005).

Retrospective Reviews

There have been retrospective clinical and pathological reviews aimed at determining whether cases of vCJD were missed prior to 1996. The UK National Retrospective Review undertook a pathology-based review from 1970 onwards without, to date, any reported previously missed cases of vCJD but the final report is awaited. A death-certificate based retrospective study covering England between 1979 and 1996 and a similar study covering Wales between 1985 and 1995, did not identify any previously missed vCJD cases (Majeed et al 2000, Hillier et al 2002).

3.4.2 Current Variant CJD Figures

UK data

157 cases of definite and probable vCJD have been identified in the UK (as of 1st September 2005). It is always possible that cases have been missed but it is very unlikely that large numbers have been overlooked. The UK has a comprehensive state-based health system, an established, active, well-funded surveillance system and there has been intense interest in vCJD with a high level of awareness of the condition in both medical services and the general population. The retrospective reviews discussed above

have not identified previously ‘missed’ cases. Figures from the UK are reviewed on a quarterly basis and the analyses placed on the NCJDSU’s website (www.cjd.ed.ac.uk). Since 2003 the analyses have shown a statistically significant decline in cases; the current best fit model to the data is a quadratic one with a peak in the number of deaths in 2000 (see Figure 2).

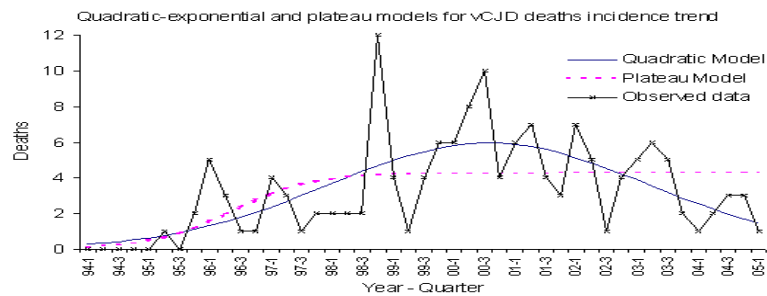


Figure 2. UK May 2005 Quarterly Analysis

The current incidence in the UK is estimated at 1.5 deaths/quarter and a total of 5 deaths is predicted in the twelve months from March 2005 (95% prediction interval of 1 to 11). (NJ Andrews, www.cjd.ed.ac.uk).

The age-distribution of the UK vCJD cases is notable for three reasons. Firstly, the relative youth of cases needs explanation. The UK mean age at death is 30 (median: 28, range: 14-74) and at onset is 28 (median: 26, range: 12-74). The details are given in Figure 3.

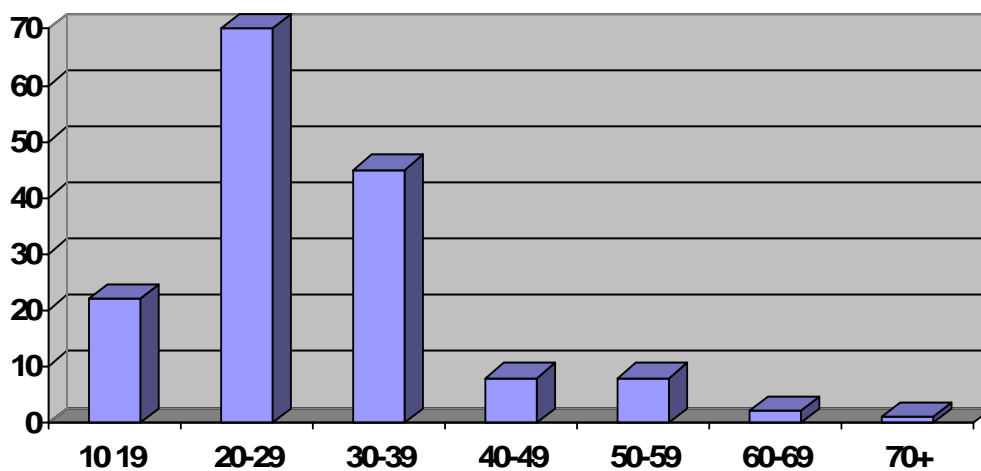


Figure 3. Age Distribution of deaths due to vCJD in the UK (first 156 cases)

Secondly, the mean age of cases has not changed significantly over the course of the epidemic and this also needs explanation.

Thirdly, vCJD occurs in an age group that is likely to contain most blood donors.

The age-data suggest the possibilities of age-related exposure to infection, age-related incubation periods and age-related susceptibility. There is no definitive explanation at present but age-related exposure is not thought likely to be the complete explanation. Age-related susceptibility is plausible; for example, gut-associated lymphoid tissue decreases with age and is a likely portal of infection entry.

Data from other countries

In other countries, the figures at 1 September 2005 are: France 14, Republic of Ireland 3, Italy 1, Netherlands 1, Portugal 1 and Spain 1. Outside Europe, one case has occurred in each of the following countries: USA, Canada, Japan and Saudi Arabia. It is important to understand that cases are attributed to countries on the basis of their country of residence at the time of their developing symptoms and this does not necessarily mean that the infection was acquired in these countries; on present evidence, one of the Irish cases, the American, Canadian and Japanese cases are thought to have acquired infection whilst in the UK. It is impossible to provide a definitive statement about the completeness of case ascertainment in all countries. It is highly probable that cases occurring in EU countries would be identified, if they presented to neurological services, given the long-standing collaborative systems described above.

Appropriate surveillance systems are dependent on the continued funding within Member States. The declining incidence in the UK and the lower number of cases (to date) in many EU countries might lead to reduced interest and support in some countries. This would be premature particularly considering the increasing numbers of vCJD cases outside the UK and would clearly undermine confidence in future epidemiological data. Autopsy rates are also of importance as discussed above.

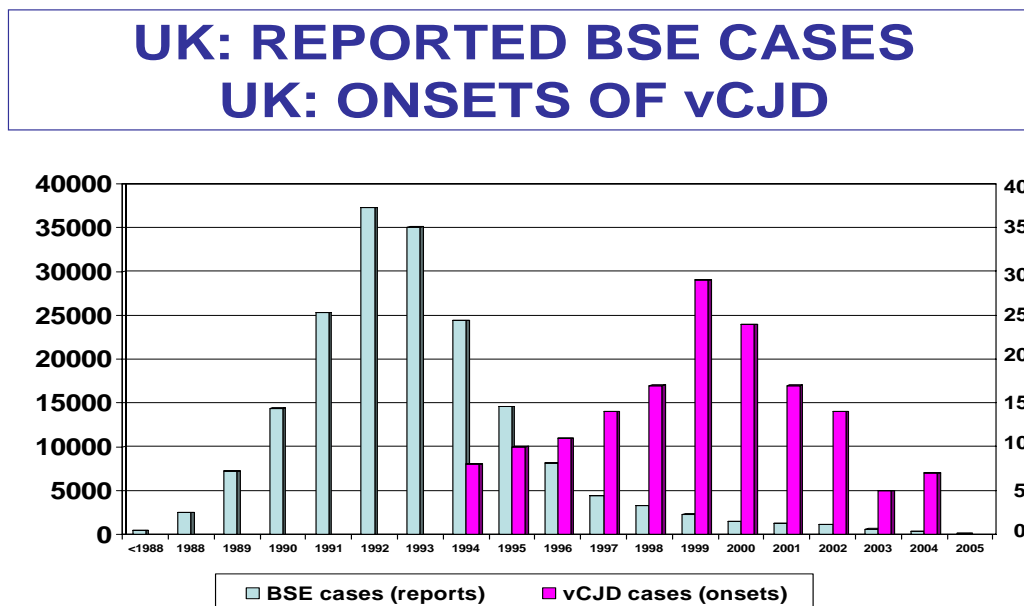
3.4.3 Modelling & Predictions of vCJD figures

There have been several publications attempting to estimate future numbers of cases of vCJD on the basis of past cases; these are discussed below.

The UK appendicectomy study was a different approach to the prediction of future numbers of cases and produced a result potentially at variance with those from the quarterly case data analysis. In this study, 3 out of 12,674 appendices examined were found to be positive for disease-related PrP. This equates to a LRS positive prevalence of 237 per million of the UK population (95% confidence interval (CI) of 49-692). Therefore the worst case scenario assumes there are 41 520 affected individuals, based on a population of 60 million. 83% of the appendicectomies were carried out in the 10-30 age group. The study results equate to 3,808 LRS positive individuals in this age group in the UK (95% CI: 785-11,128). (Hilton et al 2004a).

Considerations of what may happen in other countries have usually been based on levels of imports of potentially relevant materials (from the UK) and the distribution of cases of BSE.

An essential underlying assumption of these predictive studies has been that vCJD in humans is due to exposure to BSE infectivity in the diet. There is a considerable body of data to support this assumption (Will 1989, Hill et al 1997a, Bruce et al 1997, Knight 1999). Figure 4 shows the temporal relationship between the BSE and vCJD epidemics in the UK.



June 2005

Figure 4. Reported BSE cases (DEFRA) and vCJD onsets in the UK 1980-2005 (NCJDSU, June 2005)

General Review of possible future of vCJD

The published prediction papers have produced a variety of figures, reflecting differing assumptions and methods, given the many uncertainties and unknowns. Over time, however, the estimated maximum number of cases has tended to fall and the range tended to narrow (Figure 5).

Future predictions are obviously at least partly based on the assumption that there is no continuing extensive exposure to BSE.

As detailed above, the UK quarterly analysis estimates the current vCJD incidence at 1.5 deaths/quarter and a total of 5 deaths is predicted in the twelve months from March 2005 (95% CI of 1 to 11). (NJ Andrews, www.cjd.ed.ac.uk).

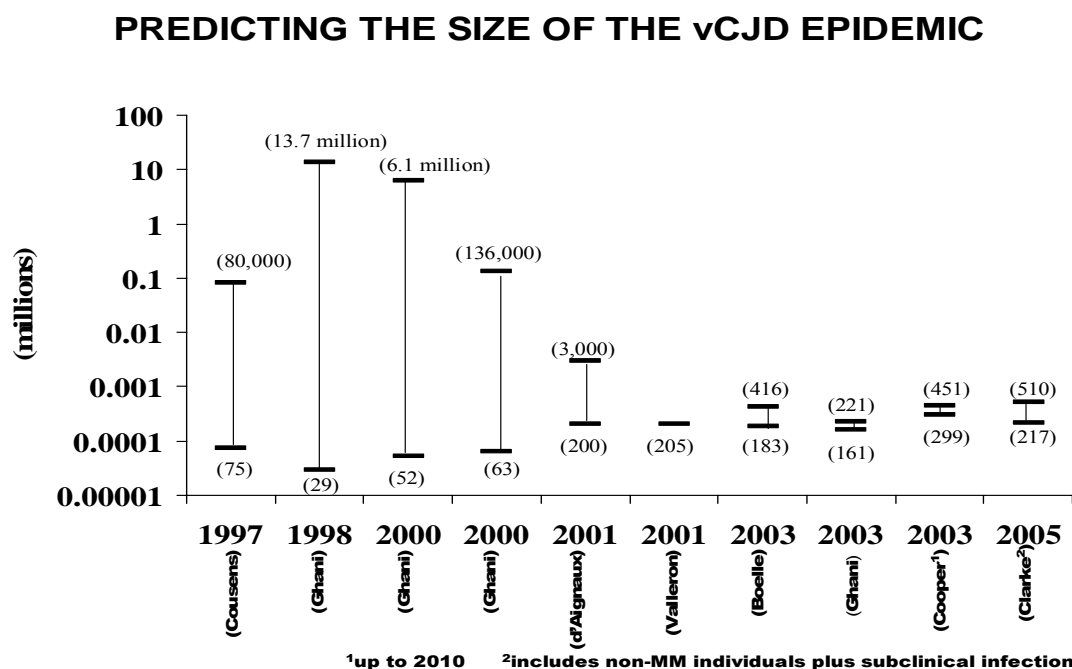


Figure 5. Published estimates for the prediction of the size of the UK vCJD epidemic.

Note: The different predictions have often used different methodologies and different assumptions. The different figures and ranges above are not necessarily directly comparable. The graph is therefore simply to illustrate the general fall in the predicted numbers over time and the general approximate agreement of recent estimates.

A further publication (Ghani et al 2003) gave the best estimate of future cases as 40 (95% CI: 9-540) based on the number of clinical cases at that time.

However, while these data are relatively reassuring, there are a number of caveats:

- All cases, to date, have been in *PRNP*-129 MM individuals (133 tested)
- Other *PRNP* 129 genotypes may be affected with different incubation periods (the report of lymphoreticular prion involvement in an MV blood recipient, is relevant to this consideration).
- There may be other, presently unrecognised, genetic susceptibility/incubation period determinants
- Secondary transmission by blood or surgery may occur (there are two case reports of vCJD infection after transfusion).
- The incubation period (and its variability) of vCJD is unknown and this has implications for possible future numbers. There are publications attempting to estimate the incubation period of dietary-vCJD and the effects of this on future numbers of cases.

In addition, the figures from the UK appendix study are perhaps less reassuring. The paper of Ghani et al (2003) gave a best estimate of future cases of 100 (rather than 40) if the appendix population data are included in the analysis (and the 95% prediction interval becomes 10-2,600 rather than 9-540).

However, there are a number of considerations:

- The confidence intervals in the appendix study are wide
- The *PRNP*-129 genotype of the 3 positive individuals is presently unknown
- There may be subclinical cases of human BSE infection (i.e. infected, with lymphoreticular positivity, but not vCJD). This would have an impact on the number of cases of vCJD itself, but would potentially lead to an increased risk of inadvertent secondary transmission (in the absence a reliable pre-clinical/sub-clinical test).

As far as other countries are concerned, any predictions are yet more problematic. Cases reported to date have not exactly mirrored BSE cases. The occurrence of cases in certain places such as Sicily, Saudi Arabia and Japan are potentially concerning.

3.4.4 Epidemiological approaches to secondary transmission

3.4.4.1 General approaches

Any assessment of possible secondary transmission must take into account non-epidemiological data (such as laboratory experimental studies of infectivity and transmission). However, it can be difficult to know how much one can extrapolate from such data to the human population situation and, therefore, epidemiological approaches are potentially valuable. Obviously there are possible problems including ethical considerations and the long incubation periods associated with such events.

The number of individuals with potentially infective tissues is an important background for secondary infection and this issue has been addressed above. Clearly, there are greater opportunities for secondary transmission from preclinical or subclinical cases than from clinical cases.

3.4.4.2 Identification of possible instances of exposure

Surveillance systems should identify instances of possible risk of secondary transmission from recognised cases of vCJD, but currently there is no means of identifying pre-clinical or subclinical cases. Incidents relating to preclinical cases may be identified retrospectively, after the clinical development of disease. Investigation of such cases is conducted in the UK surveillance system, covering all possible considerations including blood and surgery.

During organised surveillance, vCJD cases are identified and detailed medical histories may be obtained. Such data can give indications of likely problems, both specific and general. For example, if the infected individual had an appendicectomy or donated blood shortly prior to vCJD onset, then others specifically at risk may be identified. Alternatively, to gauge the general nature of the problem, it is useful to know how many vCJD cases did undergo surgery or donate blood during a relevant risk period. However, these data need further refinement as indicated below. The data that are available will depend on the particular surveillance methodology. In the UK, detailed past medical history, including blood donations and blood treatments are recorded in each case.

The follow up of potentially exposed individuals

Direct follow-up of individuals who have been exposed to a potential risk requires them to be notified of the exposure and their consent to be reviewed with the attendant ethical issues.

An alternative is the recording of names of those potentially exposed and passing on these data to surveillance systems that then wait to see if these individuals are later identified as cases. There are ethical issues here as well. The UK TMER study (see below) initially followed the second course, but potentially exposed individuals may now be informed of the incident, depending, to some degree, on the nature of the exposure.

Case-control studies of risk factors

Case-control studies can evaluate risk factors such as exposure to infection via surgery and blood. There are no current data from the UK vCJD case-control study to implicate either of these. However, the potential incubation period of vCJD must be kept in mind and the study is continuing.

Clustering analysis

If cases of vCJD result from point exposures to infection via surgery, then clustering of cases would be expected. Thus use of affected labile blood components might also give rise to clustering of cases, but the distribution of blood-derived products is potentially wide. The analysis of spatiotemporal clustering of cases is a very complex matter. Presently, there are only three verified specific concentrations of cases of vCJD:

- The particular involvement of certain countries (essentially UK & France), thought to reflect BSE and not secondary transmission.
- The higher incidence of cases in the north of the UK compared to the south. The basis of this is uncertain, with no convincing data to implicate factors such as dietary differences. There is no evidence to suggest it is based on secondary transmission factors.
- The 'Leicester cluster'. This is the only statistically significant cluster of cases around a UK city. The basis is uncertain, but it has been attributed to local butchering practices and there is no evidence to suggest it is based on secondary transmission factors.

Continuing surveillance and analyses may provide further data in the future.

3.4.4.3 Vertical Transmission

Vertical transmission is a particular concern, especially given the relatively young age group affected by vCJD. Again, any considerations must take into account experimental evidence, but also again, there are issues concerning the valid extrapolation of such data to the human situation and epidemiological approaches are also needed. The issue of possible vertical transmission in cattle BSE is discussed elsewhere in this report (section 3.6.3) but, currently, there are no data to support an important role for such transmission.

Current epidemiological evidence for vertical transmission

Children have been born to mothers with prion disease (including vCJD), both within any expected incubation period and during clinical illness. In the UK, 114 children have been born to vCJD cases (in 60% the affected parent was the mother). Currently there is no reported instance of prion disease resulting in the offspring. (Knight, personal communication, NCJDSU 2005).

Follow up of children born to affected mothers

There are four ways of following children who have been born to affected mothers: they could be formally reviewed on a regular basis; their medical records could be ‘flagged’ in some way; their names could be kept by a surveillance system in case they later present as a case; there could be no formal system but instead a reliance on an established surveillance system to identify them and their family history if they developed prion disease.

3.4.4.4 Surgery

There are two aspects to possible exposure to infectivity by surgery: accidental exposure related to recognised vCJD cases and the general risk from unrecognised cases (missed diagnoses, pre-clinical and subclinical cases). Only the first aspect is considered here.

Follow up of individual incidents

There are ethical considerations concerning the identification and follow-up of individual potential exposures, as discussed above. In the UK, the surveillance system should successfully identify any exposure instances and the development of vCJD in the exposed. There are no reported instances of surgical transmission at present, but the likely long incubation period of vCJD should be kept in mind.

The practical management of any specific incidents is managed by the CJD Incidents Panel (part of the Health Protection Agency H) in the UK.

Case-control studies

The UK case control study considers surgery as a potential risk factor; so far there is no evidence to implicate surgery.

Organized studies

Organized studies trying to identify specific surgical procedures and their possible spatio-temporal relationship to other individuals and their procedures are logistically (and, potentially, ethically) complex. There are no such studies in the UK concerning vCJD.

3.4.4.5 Blood

The potential risks of blood (labile components) and of (fractionated) blood components are dealt with in section 3.6.

Blood data from routine vCJD surveillance

Routine disease surveillance can provide some general data; this is of limited value but can be helpful. For example, some general evaluation of potential risk can be made from simple data such as the number of blood donations made by vCJD cases and the timing of such donations with respect to disease onset.

Blood as a risk factor in case-control studies

Blood and blood components are potential risk factors considered in the UK case-control studies. There is, currently, no evidence that they are risk factors for vCJD.

UK Transfusion Medicine Epidemiological Review (TMER)

The UK Transfusion Medicine Epidemiological Review (TMER) study was set up in 1997 as collaboration between the NCJDSU and the UK National Blood Authorities (NBAs). Essentially, the NCJDSU identifies cases of vCJD, obtains a detailed blood donation history and passes names of relevant cases to the appropriate NBA(s). The relevant NBA traces the donations and their fates and provides the NCJDSU with names of the recipients which then checks to see if any have developed or develop vCJD (Tables 2 and 3). There is also a 'reverse study' concerning vCJD patients who have a history of receiving blood. This study concerns labile components.

Table 2. vCJD donor summary ("www.cjd.ed.ac.uk/TMER, September 2005)

vCJD DONOR SUMMARY	157 vCJD cases
Eligible to donate	147
Reported by relatives to have donated	31
Numbers where donor records traced*	23
Numbers from whom components actually issued	18
Numbers of recipients identified	66

* donor records were traced on three individuals where the relatives had reported the case had not been a donor

Table 3. Use of blood from vCJD donors ("www.cjd.ed.ac.uk/TMER, September 2005)

USE OF BLOOD FROM vCJD DONORS	157 vCJD cases
Red Cells	27
Leucodepleted red cells	25
Buffy-coat reduced red cells	2
Fresh frozen plasma	3
Fresh Frozen Plasma (leucodepleted)	2
Whole blood	2
Cryo-depleted plasma	1
Cryoprecipitate	1
Platelets (pooled)	1
Platelets (pooled, leucopdepleted)	2

In addition, 9 vCJD donors contributed plasma to 23 plasma pools identified by UK fractionators (BPL & PFC) as having been used for the manufacture of plasma products prior to 1999.

There have been two individual cases of note:

1. A possible case of vCJD transmission by blood.
2. A possible case of transmission of vCJD-infection (but not vCJD)

CASE 1

A 62 year old patient was transfused with 5 units of red cells during surgery in 1996. One unit had been donated by a 24 year old who developed vCJD symptoms 3 years and 4 months following donation (later dying of neuropathologically confirmed vCJD). In 2002, 6.5 years after the transfusion, the recipient developed symptoms of vCJD and the diagnosis was later neuropathologically confirmed (*PRNP* codon 129 MM). The recipient had lived in the UK during the identified dietary risk period but a statistical analysis determined that the chance of this being a dietary case was between 1:15,000-1:30,000.

The affected donor had donated another unit of blood, the red cells of which were transfused to a patient who died of cancer only 5 months after the transfusion. The platelets from this donation were included in a platelet pool that has not been traced to a recipient. Plasma from both donations was included in two different plasma pools for the production of fractionated plasma products (Llewelyn et al 2004)

CASE 2

In 1999, an elderly patient received a unit of non-leucodepleted red blood cells from a donor who had developed symptoms of vCJD (later, neuropathologically confirmed) 18 months after donation. The recipient died 5 years after the transfusion with no evidence of any neurological disorder. As the recipient had been identified as a recipient of potentially vCJD-infected blood, an autopsy was undertaken with specific analyses to detect vCJD. The brain showed no evidence of vCJD, but the spleen was positive for PrP^{Res} with the protein type being typical for vCJD and distinct from the types associated with sporadic CJD. Positivity was found also in a cervical lymph node, but not in tonsil, appendix nor dorsal root ganglion. This therefore represents an instance of either preclinical or subclinical vCJD in a recipient of potentially infected blood. The individual was found to have the *PRNP* codon 129-MV genotype (Peden et al 2004).

Mathematical modelling

Data on the presence and treatment of an infection in the population may be used for modelling disease development (Duerr et al 2005, UK Collaborative Group on HIV Drug Resistance 2005). Such an approach was used for estimation of the fate of vCJD in the population and the risk whether the infection could establish itself (Dietz, personal communication, 2005). In this model data on the blood donors and blood recipients were taken into consideration including the age distribution of both populations. In addition, some assumptions were made on the behaviour of vCJD itself. The model describes the spread of an infection by blood donation after it was introduced into the population via the alimentary route. It predicts that an infection could not become endemic by transfusion alone and that only few cases could be avoided by excluding donors with a transfusion history. The most likely explanation for this is the age dependent effects in the model, i.e. the difference in age distribution between the blood donors, who are generally younger, and the blood recipients, who are generally older (Dietz, personal communication 2005).

3.4.5 Problems & Possible Deficiencies in Current Systems

The complete ascertainment of all cases of vCJD depends on good clinical neurology services and good national surveillance systems. These are mainly dependent on factors internal to each country, but EU-funded collaborative projects have been of enormous value. The declining UK vCJD figures, the lower prediction estimates in recent studies and the non-involvement of many individual countries may lead to lessened interest, reduction of funding and, therefore, less efficient surveillance.

It is important to note that the clinico-pathological phenotype of human prion disease reflects not only the nature of the relevant agent (in this case BSE) but also the genotype of the individual and, potentially, other factors (such as mode of acquisition). Therefore, surveillance systems must be aware of the possibility of new phenotypic expressions of human BSE infection. Current clinical diagnostic criteria are based on the characteristics of previously described cases. Neuropathological studies are of critical importance; relatively low and declining autopsy rates are a potential concern.

Predictions of likely numbers of future cases are limited by the uncertainties in the data that underlie these predictions, such as the incubation period of vCJD and the nature of possible disease susceptibility factors.

3.4.6 Conclusions

The incidence of vCJD in the UK is decreasing but there remain considerable uncertainties and concerns over future numbers of cases.

While other countries have not been involved to the same degree, France continues to identify new cases and new countries have been affected.

With the control of BSE in cattle and the precautions taken to prevent BSE infected material to enter the human food chain, there remains the issue of controlling secondary human-to-human transmission.

There is recent evidence to implicate blood as a potential means of secondary transmission.

Transmission via surgery remains a concern, but to date there is no evidence that it has actually occurred.

Vertical transmission has not yet been shown to be a means of secondary transmission of vCJD. The monitoring of vCJD cases, vigilance for any new phenotypes of human BSE infection and the identification of any actual secondary transmission events are the necessary underpinnings for all predictions and policies; laboratory experiments are crucial but these cannot replace observation and epidemiological studies of human populations.

Model predictions show that it is unlikely that a vCJD infection could establish itself in the population by blood transfusion only.

3.5 Evaluation of prion decontamination procedures for surgical instruments

The countermeasures implemented in response to the BSE epidemic are expected to be effective in preventing further spread of this disease to humans from cattle, thereby minimizing the risk of new primary vCJD infections. However, additional challenges for public health in the context of TSEs arise from the hypothetical as well as the established risks of human-to-human transmission of vCJD and other forms of CJD, respectively (Gibbs et al 1994, Brown et al 2001, Taylor 2003, Beekes et al 2004, Llewelyn et al 2004). The experience with iatrogenic CJD, of which 267 cases were reported until July 2000 (Brown et al 2000), and the detection of infectivity or PrP^{Sc} in a variety of tissues from vCJD patients in addition to the brain and spinal cord (e.g. lymphatic system and peripheral nervous system, Table 1) (Bruce et al 2001, Wadsworth et al 2001, Hilton et al 2002, Haik et al 2003, Ramasamy et al 2003) have led to the formulation of national and international recommendations and guidelines aimed at the prevention of iatrogenic transmission of these diseases (Simon & Pauli 1998, World Health Organization 1999, Abschlussbericht der Task Force vCJD 2002).

In hospitals, it is of the utmost importance to avoid the spread of TSE infectivity via surgical instruments (e.g. by single use or cleaning, followed by chemical disinfection, or sterilization (Beekes et al 2004, Schulster 2004). The potential risks are increased by the fact that PrP^{Sc} has also been detected in skeletal muscles of patients with sCJD, albeit at low levels (Glatzel et al 2003). Infectivity is present in lymphoid tissues, and possibly blood, during the preclinical phase of vCJD (Hilton et al 1998, 2002, 2004a, Llewelyn et al 2004, Peden et al 2004).

3.5.1 Cleaning

Instruments are cleaned before decontamination to remove tissues and fluids. Cleaning of surgical and diagnostic instruments may be difficult because of the shapes and construction of surgical instruments, where the first priority is obviously suitability for surgical use, not ease of cleaning. There may therefore be parts of the instruments which are hard to reach. Instruments that can be disassembled for cleaning, or are designed to have fewer inaccessible parts may help in the future. Studies have implied that in the UK at least, contamination remaining after cleaning is common; an estimate used in risk assessments has been that 10 mg of tissue may remain on a single instrument after routine cleaning (UK Department of Health 2005). For instance in a recent study lymphocytes were observed on 30% of the investigated single use laryngoscope blades (Hirsch et al 2005). In addition, proteinaceous material may remain on instruments after standard cleaning practices (Miller et al 2001) and this is of particular importance in prion diseases. At this moment there is no validated cleaning process available for instruments that might be contaminated with TSE agents like vCJD.

Cleaning or sterilisation procedures may not be consistently applied to high standards. Studies in the UK indicated that in an initial survey only 9% of hospitals investigated reached the appropriate standards in cleaning and 58% in sterilisation. Following extensive investigations, investment and audit, these figures rose to 68% and 93% respectively (UK Department of Health 2005). It is not known how general these findings are, but they suggest that the issue of implementation may be important. One solution has been to propose that single use instruments be used. For complex

instruments this is impractical, and the expense involved for many simpler instruments is likely to be prohibitive.

3.5.2 Decontamination and sterilisation

The high resistance of TSE agents to conventional methods of chemical or thermal inactivation and to UV or ionizing radiation makes decontamination difficult. (Alper et al 1966 and 1967, Brown et al 1982 and 1986, Kimberlin et al 1983, Taguchi et al 1991, Tateishi et al 1991, Ernst & Race 1993, Taylor et al 1994, Manuelidis 1997, Taylor 1999, for review see Taylor 2000). Following exposure to high titre TSE preparations, a considerable residual infectivity can be detected bound to the surface of the steel (Zobeley et al 1999, Flechsig et al 2001), which may affect inactivation procedures so that specific consideration must be given to decontamination procedures in the reprocessing of surgical instruments (Rutala & Weber 2001). The transmission of CJD by neurological probes despite repeated cycles of cleaning and sterilisation is well known.

Solution studies on the inactivation of prion agents by a variety of agents demonstrate a biphasic inactivation kinetics, with a rapid inactivation phase followed by a slower second phase (Brown et al 1986, Ernst & Race 1993, Tateishi et al 1991, Taylor et al 1994, Taylor et al 1999). Most inactivation occurs during the initial rapid phase, and it was proposed that the slower second phase resulted from the presence of a subpopulation of prion agent more resistant to inactivation. Additional studies have since demonstrated that qualities of the spike preparation can have a dramatic effect on the capacity of a given inactivation solution to inactivate prions. Procedures which result in "fixing" of the prion agent, such as drying, treatment with organic solvents or crosslinking with aldehyde based disinfectants results in an increase in the proportion of prion protein demonstrating resistance to inactivation (Taylor 1999). The fixation of the agent probably modulates resistance to inactivation through either preventing access to the inactivation solution (i.e. drying) or by preventing denaturation of the aggregated prion protein (i.e. aldehyde fixation).

The observation of increased resistance to inactivation following certain procedures has implications for the disinfection of surgical instruments suspected of CJD contamination. Allowing instruments to dry before or after the cleaning process, or treatment with organic solvents or fixing agents prior to disinfection is likely to increase the proportion of prion agent resistant to disinfection. On the other hand, procedures capable of reducing protein load at equipment surfaces are likely to considerably increase accessibility of any remaining infectivity to the inactivating agent. In a recent study, bone surfaces devoid of any visible protein or fat deposits, yet containing still 6 logs of prion infectivity, when treated with 0.3M NaOH at ambient temperature resulted in complete inactivation of the remaining infectivity (Taylor 2003). In this instance and in contrast to solution studies, no resistant prion sub-population was observed. Similar results have also been observed with steel wires exposed to prions and washed prior to exposure to inactivating agents (Flechsig et al 2001), reinforcing the importance of cleaning combined with decontamination. Gas plasma treatments used in cleaning metal surfaces industrially have shown promise (Baxter et al 2005). However it must be stressed that the validation of processes for the removal of infectivity from solid surfaces is extremely difficult. While some of the approaches proposed seem promising, no process has yet been validated in accordance with accepted scientific procedures.

Treatments that are considered to result in complete decontamination include use of 1-2 M NaOH solution (for 24h), 2. 5-5% NaOCl solution (for 24h) as well as 3, 4 or 6 M GdnSCN solution (for 24h, 1h or 15 min, respectively), followed by steam sterilization at 134°C for 18 min to 1h (Simon & Pauli 1998, World Health Organization 1999, Hörnlimann et al 2001).

Such stringent conditions are hazardous to both equipment and operators. While they are mandatory for the reprocessing of instruments used in patients with or at risk from CJD they do not offer an option for the routine maintenance of surgical instruments used on patients without a recognizable risk of human TSE. Here, generally applicable decontamination strategies that take into account the theoretical risk of CJD and vCJD transmission from asymptomatic carriers on the one hand, without compromising the conventional processes for cleaning, disinfection and sterilization on the other, are required.

In routine situations it is necessary to use an effective yet instrument-friendly procedure. Some experiments were performed using tests on animals implanted with contaminated steel wires as originally described by Zobeley et al (1999) and Flechsig et al (2001). The use of various decontaminating reagents was investigated showing a decrease of infectivity (Beekes et al 2004). However this correlation needs to be carefully validated as proposed by McLeod et al (2004) who obtained slightly different results with the same agents.

The following conclusions are currently considered justified::

- NaOH and NaOCl have a strong decontaminating activity as previously established by comprehensive infectivity studies (Kimberlin et al 1983, Brown et al 1986, Taylor et al 1999, Taylor 2000, Flechsig et al 2001, Rutala & Weber 2001).
- Certain alkaline cleaners, supplemented with some minor additional components have shown promising activity. This requires further study and a careful examination of the formulation and effect of commercial products (Lemmer et al 2004, Baier et al 2004).
- The efficacy of 4 M GdnSCN and 5% SDS is confirmed (Flechsig et al 2001, Taylor 2004, Lemmer et al 2004).
- The inefficacy of 4 M urea is confirmed (Brown et al 1986, Prusiner et al 1993, Lemmer et al 2004).

Thus associations of SDS (sodium dodecyl sulphate) & NaOH, or peracetic acid & NaOH may be of interest, but more tests both *in vitro* and *in vivo* must be performed. (Baxter et al 2005)

3.5.3 Conclusions

- No procedure for the decontamination of surgical instruments has yet been adequately validated to the extent that its universal introduction can be recommended
- Disinfectants with fixative properties such as those containing aldehydes must not be used for decontamination of instruments suspected to be contaminated with TSEs as they tend to stabilise rather than inactivate prions.
- Drying of instruments before cleaning or decontamination is likely to reduce the effectiveness of the decontamination procedure
- The implementation of whatever procedures are introduced should be monitored.

3.6 Risk assessment on transmission of vCJD

Conventionally a risk assessment comprises the following stages:

- exposure assessment
- hazard identification
- hazard characterization
- risk characterization

This is then followed by an evaluation by the risk manager of the acceptability or otherwise of the risk.

This framework has been followed here while particular emphasis is given to exposure assessment. Although the nature of the hazard is already largely known, there are still a number of uncertainties which remain.

Where fairly reliable quantitative data are available they have been used. However for many of the risk factors discussed above no suitable quantitative information exists. The normal approach in a risk assessment for public health purposes is to make a conservative/worst case assumption in such circumstances. It must be recognized that if a series of worst case assumptions are adopted the final conclusion may be far removed from the real risk. Since the hazards have been well addressed elsewhere, the risk assessment will focus on the possibilities for exposure to the infectious agent by various routes and treatments.

3.6.1 Risk assessment for transmission of vCJD by blood or blood components

3.6.1.1 Identification of risk factors

Before carrying out a risk assessment it is necessary to identify the risk factors involved in the transfer of blood from a blood donor infected with vCJD to a recipient(s). These risk factors include:

A: EXPOSURE FACTORS

- i) Number of blood donors with vCJD as a proportion of all blood donors
- ii) Likelihood that the vCJD donor will not be identified prior to their blood entering the blood bank

-
- iii) Level of vCJD material in the donors blood (degree of infectivity)
 - iv) Dilution/partition factors for the vCJD blood prior to being given to an individual/ group of individuals
 - v) Number of times blood or blood components are administered to a sensitive individual
 - vi) Potential for bioaccumulation of the infectious agent
 - vii) Route of exposure

B: HAZARD FACTORS

- viii) Susceptibility of individuals to vCJD by blood or blood components
- ix) Presence of other factors favouring the development of vCJD

A: EXPOSURE

Number of blood donors with vCJD as a proportion of all blood donors

Blood and blood components tend to be used in the same geographical area in which they are donated. The number of blood donors with vCJD is likely to vary from country to country over a range of two or more orders of magnitude. The highest proportion of blood donors with vCJD is likely to be in the UK.

A distinction needs to be made between the number of individuals in a country with vCJD infection and those with a clinical disease. The number of those with the frank disease appears to be very small even in the UK. It is possible that the prevalence of those with vCJD infection is substantially higher than those with overt disease.

If we base our estimate of the number of infected individuals on the number of clinical cases to date, then the final number is unlikely to be much greater than several hundreds (if we base our estimate of the number of infected people on the results of the UK appendix study, then the total number might be as high as several thousands) (Hilton et al 2004). About 4 % of the eligible population in UK donates blood in any one year (DNV 2003).

Likelihood that that the vCJD donor will not be identified prior to their blood entering the blood bank

The donors of concern are unlikely to be expressing the symptoms of vCJD and will therefore not be identified by routine assessment. Currently there are no available validated laboratory tests to screen potential donors for blood borne CJD prions. At present, therefore such donors can not be identified.

In some countries individuals are excluded from giving blood if they have had a previous transfusion in the UK or have even visited the country for a period during the BSE epidemic. Also people who have ever received blood are now excluded in several Member States. Although the approach may be considered a reasonable one, it is difficult to estimate the risk reduction achieved by these exclusions. The results of a recently developed mathematical model indicate that the exclusion of blood recipients from donation does not result in a considerable reduction of the predicted number of vCJD cases in the model employed (Dietz, personal communication 2005).

Level of CJD material in the donors' blood (degree of infectivity)

The level of TSE infectivity in the blood of animals and humans is likely to be many folds lower than that in brain. Levels of infectivity of vCJD probably vary from individual to individual depending, among other factors, on how long they have been incubating the disease. The amount of prion that needs to be transferred to a susceptible patient in order to initiate CJD is uncertain. It is assumed that there is a threshold dose below which no infection will occur. This is not proven for man.

Rodent experiments suggest that the level of infectivity in blood in the late preclinical stage is about ten infectious units per ml when measured by intracerebral inoculation. This figure is consistent with transmissions of scrapie and BSE by transfusion experiments in sheep. Transmission has also been reported in primates, consistent with infectivity at a level of the same order. These experiments are subject to the reservation that infectivity may be different by different routes.

A unit of blood is about 450ml and could therefore contain 4500 infectious units. Even if the infectivity were 100 fold less a recipient of a whole unit would be exposed to an infectious dose.

In principle a vCJD donor could give whole blood every three months over several years, although in practice for the UK the average number of donations per year is only 1.2 per person (DNV 2003) There is no reason to believe that a preclinical or subclinical vCJD donor would donate blood less frequently than a non-vCJD donor. It is unknown how infectivity changes during the incubation period. In rodent models it gradually increases from about one third through the incubation period, and in the sheep transfusion experiments infection was transmitted by donations taken about half way through the incubation period.

Number of times an individual may receive contaminated blood or blood components

It remains uncertain whether in humans there is a cumulative effect of repeated exposure and if so whether there is a critical interval beyond which a second dose of prions will not supplement the effects of the initial exposure. This issue is not considered relevant because a single donation from an infected individual is thought to contain more than one infectious unit.

However, for certain blood components within one transfusion exposure to multiple donors may occur. As the number of donors is a risk factor the number of donors should be limited. An example is platelet transfusion in which apheresis techniques should be used, allowing harvesting of a sufficient dose of platelets from one donor to be transfused to one individual patient.

B: HAZARD**Susceptibility of individuals to vCJD via blood and blood components**

As relatively few individuals have contracted vCJD it is not surprising that the individual risk factors are not well characterized. Key considerations from a risk assessment point of view are:

- Is there such a thing as a susceptible individual i.e. one who for genetic or environmental reasons is substantially more vulnerable to contracting vCJD than the population at large. And if so is the extent of susceptibility fixed or does it vary substantially with time?

All individuals who have developed disease so far have been homozygous for methionine at codon 129 of the prion gene *PRNP*. If MM homozygosity at codon 129 is the single determinant for vCJD and exposure leads inevitably to disease, about one third of the UK population would theoretically be at risk. The frequency of MM homozygosity within the EU is generally comparable to that observed in the UK (Nurmi et al 2003). However, an individual with codon 129 MV genotype was found to have vCJD infection (Peden et al 2004, section 3.2.4, 3.4.4). vCJD has affected relatively young people, the reason for this is unknown, but various factors (section 3.4.2) have been suggested. However, these do not appear to be relevant for blood transfusion.

A large proportion of the blood used is given to older patients and people with serious illness. Most recipients of blood or blood components are therefore unlikely to fulfil criteria to donate blood in the future for reasons of age or the nature of the disorder for which they are treated. Because of this use of the blood, it seems unlikely that a transmission chain by blood components will be sustained. In this opinion, an overall survival rate of blood recipients is estimated to be 50% over three to five years after transfusion (Wallis et al 2004, Dietz, personal communication, 2005).

3.6.1.2 Quantitative risk assessment for transmission of vCJD by blood or blood components

The term “blood donation” as used throughout this risk assessment means whole blood and blood components collected from an individual and intended for transfusion to another individual. The current state-of-the art in hemotherapy requires to transfuse blood components instead of whole blood. When blood is donated as whole blood, it is separated into the different components *in vitro*. Alternatively, the blood components may be obtained from the donor using apheresis techniques. ‘Apheresis’ is the method of obtaining one or more blood components by machine processing of whole blood in which the residual components of the blood are returned to the donor during or at the end of the process (European Commission, Directive 2004/33/EC). It should be realised that after every separation technique residual material of other components are present.

Blood components for haemotherapy are

1. red blood cells,
2. platelets and
3. plasma.

'Red cells' means the red cells obtained from a single blood donor, with a large proportion of the plasma from the donation removed. 'Plasma' means the liquid portion of the blood in which the cells are suspended. In the context of this opinion we refer only to the use of plasma as single unit, non-pooled fresh frozen plasma. 'Platelets' means a concentrate of blood platelets, suspended in 200-250 ml plasma. A therapeutic unit of platelets contains $2-4 \times 10^{11}$ platelets. A therapeutic unit of platelets may be obtained either by pooling 4-6 platelet preparations obtained from whole blood donations, or by a single aphaeresis procedure. Thus, pooled platelet concentrates contain platelets and plasma from 4-6 different blood donors, whereas a single donor is sufficient to obtain a therapeutic unit of platelets by the apheresis technique.

The risk assessment is in two parts;

- Identification of potential for transfer of infection from a blood donor with vCJD infection
- The relative risk for blood transfusion at the population level

Identification of potential for transfer of infection from a blood donor with vCJD infection

Donor factors

It is reasonable to assume that transmission of vCJD via blood or blood components is possible (Llewelyn et al 2004, Peden et al 2004). From animal studies the estimated range of infectivity in blood ranges from 0.1-310 i/c ID50/ml (Cervenakova et al 2003, Brown et al 1999, Brown et al 1998). The evidence indicates that the lower end of this range is more likely to be realistic for preclinical or sub-clinical donors. The assumed value for calculation purposes is 10 i/c ID50/ml.

These units are expressed as infectivity as measured by the intracerebral route. By the intravenous route based on animal studies, the infectivity is considered to be less, however, there are no relevant human data. Administration via the intravenous route may result in a 1-5 fold lower transmission rate than by the intracerebral route (Brown et al 1998). The worst case scenario would result in an estimated infectivity of 10 iv ID50/ml.

Conclusion on the assumed infectivity levels in whole blood. The typical volume of blood taken from a donor is 450+/-45ml. Thus the total infectivity of a whole blood unit from an infected donor could be of the order of 4500 iv ID50.

Processing factors

a) Distribution of infectivity in blood and blood components

In blood there are a number of estimates of the partitioning of vCJD between the various blood components. However, these estimates are extrapolations from non-vCJD studies (Brown et al 1998). For the purposes of risk assessment it is assumed that:

- 50% (10-60%) of the infectivity is considered to be in plasma, (therefore the infectivity is taken to be 2250 iv ID50) per therapeutic unit.
- 25% is found in packed red blood cells, estimated infectivity 1125iv ID50 per therapeutic unit.

- 25% is associated with the buffy coat (white blood cells and platelets), estimated infectivity 1125iv ID50 per therapeutic unit.

There are theoretical estimates of the level of reduction of infectivity in whole blood achieved by leucodepletion which have suggested that removal of TSE infectivity may be as great as 200-fold (DNV 2003). However, experimentally leucoreduction has failed to demonstrate any removal of plasma borne infectivity (Brown et al 2005) or of spiked model TSE agents (Prowse and Bailey 2000). Leucodepletion of whole blood from infected hamsters showed only a ~2-fold reduction of blood borne infectivity (Gregori et al 2004). Consequently, strategies have been pursued to develop devices for the removal or reduction of prions from blood products by filtration (Eglin and Murphy 2005, Sowemimo-Coker et al 2005). The values and limitations of prion removal devices for blood products as currently evident have recently been reviewed by Ortolano et al (2005). Blood banks may choose or may not choose to adopt prion removal technologies in the future, depending on the assessment of the risk of vCJD transmission by blood products in the different geographical regions worldwide. However, leucodepletion has been introduced widely as an initial treatment process. For risk assessment purposes the lower value of only 25% removal (i.e. removal of only buffy coat associated infectivity-the more conservative approach) is used.

Conclusion Leucodepletion will result in the following infectivity levels in one therapeutic unit:

- Whole blood (3375iv ID50)
- Plasma (2250iv ID50)
- Red blood cells (1125iv ID50)
- Platelets (175iv ID50) assuming 35 ml of plasma is included in each therapeutic unit

b) Risk from transfusion with red blood cells.

Red blood cells tend to be used in the same geographical area in which they are donated. The risk of a recipient receiving an infective dose from transfusion of packed red cells is directly determined by the likelihood that the donor carries an infectious dose of vCJD in their red cells. Buffy coat removal and leucodepletion is unlikely to result in sufficient infectivity reduction to prevent such infection. It is noted that the two UK cases were in elderly individuals receiving transfusion with red blood cells or non-leucodepleted red blood cells.

Recipient factors

The likelihood of infection of an individual depends primarily on the exposure factors as presented above being the level of infectivity in the blood or blood components and the total dose administered. In addition recipient factors may be an influence.

- The frequency of administration of vCJD blood or blood components to the individuals (single versus repeated).
- Genetic background of recipients (*PRNP* gene codon 129 alleles being MM, MV, or VV, respectively)

So far, all clinical cases of vCJD have been expressed MM homozygosity at *PRNP* codon 129. Whether being codon 129 MV or VV really absolutely protects against

developing vCJD or just results in a longer prolonged (very long) incubation period is as yet unknown. However, the latter is generally thought to be most likely. The detection of PrP^{res} in one individual (Peden et al 2004) does show that people heterozygous (MV) at codon 129 can at least become infected.

In conclusion, using a series of conservative assumptions made for the purposes of this risk assessment, there is a considerable risk that a vCJD donor could cause infective material to be passed on to one or more recipients of blood and blood components. These worst case assumptions can be summarized as:

- Each donor with vCJD infectivity confers an infectivity of 10 iv ID50/ml whole blood resulting in an average of 4500 iv ID50 in each therapeutic unit of whole blood
- Leucodepletion produces no more than 25% reduction in infectivity.

If a donor is incubating vCJD it is likely that a potentially infecting dose will be transmitted at some stage during the incubation period. The actual risk will be affected by the number of infected donors and the susceptibility of the recipients.

Risk at the population level

The above estimate of the risk is dependent on the assumption made of the likelihood of a vCJD infected blood donor being a member of the blood donor population. The frequency of this is directly proportional to the incidence of vCJD infected donors in the country. This is certain to vary substantially between Member States.

For the estimation of the number of possible infected individuals in the UK the data of the appendix study (3.4.3) will be used. These data show that 3 out of 12,674 appendices examined were positive for disease related PrP. This equates to 237 (95 % confidence interval of 49-692) individuals with abnormal PrP in the appendix per million of the UK population.

In 1997, 35 million of the UK population were eligible to donate, of these only 4.3 % donated and on average they gave 1.2 times per year (DNV 2003). Using these figures the following calculation could be made to estimate the number of infected donations per year in the UK:

$$692 * 10^{-6} * (1.2 * 35000\ 000 * 0.043) = 1250$$

This results in 1250 infected donations per year in the UK.

For the number of blood recipients in the UK population who could develop vCJD as a consequence of receiving contaminated blood, the following additional points need to be taken into account

- the proportion of susceptible individuals in the recipient population who would develop vCJD following the administration of contaminated blood. It is not possible to give a numerical value for this, although it is unlikely to be 100%.
- the proportion of individuals in the UK receiving blood transfusions who will survive longer than 3-5 years. This is conservatively estimated as no more than 50%.

In addition, elderly patients and those who have poorer prognosis are the most likely to receive multiple transfusions.

In order to assess the potential number of recipients affected it is assumed that the blood or blood components from one donor is received by three recipients, and that the number of infective units transferred is sufficient to initiate the disease. This would result in a worst case estimate of the number of 3750 new infections per year as a consequence of blood transfusions. Eventually this would result in 1875 new individuals who will eventually develop vCJD (assuming that 50% of those receiving blood transfusion will live long enough to develop vCJD (Wallis et al 2004).

This figure is implausible given the current declining trend of vCJD cases in UK. There are number of possible explanations for this:

- this is a worst case assumption
- not all infected people may develop disease
- infectivity in blood may not be uniform during the course of infection
- the presence of abnormal prions in the appendix may not be always associated with blood infectivity
- certain genotypes might not be associated with infectivity in blood
- transmissibility may vary according to the identity of the *PRNP* genotypes of donor and recipient
- extrapolation from animal models concerning blood infectivity of vCJD in terms of units of infectivity present in blood may not be correct

These numbers change considerably when the calculations are performed using the lower end of the confidence interval of the data of the appendix study. In addition, assuming a lower rate of infection has a considerable influence (Table 4).

Table 4. Infected donations in the UK

Infected donors per million population	Donations (million)	p.a %	Infected donations p.a
49	1 806	100	88
237	1 806	100	428
692	1 806	100	1250 (current worst case)
49	1 806	10	9
237	1 806	10	43
692	1 806	10	125

For other countries there are no data available on prevalence of abnormal PrP in lymphoid organs so similar calculations can not be performed. However, based on the current observed number of cases of vCJD within the Member States which stands at 21 non-UK cases as of September 2005), an estimate of the risk for France (14 cases) would be 10% of that calculated for the UK while for other Member States it will be considerably lower.

3.6.2 Risk assessment on transmission by surgical instruments or invasive procedures

There have been no confirmed cases of transmission of TSE by virtue of occupation. There have been a small number of reports of sporadic CJD in healthcare workers (including a neurosurgeon, retired laboratory workers and a pathologist) but their any link with their occupation is speculative. There are around 300 cases of reported iatrogenic CJD, most linked to contaminated growth hormone injections or the surgical use of cadaveric human dura mater; some other cases are described to be have been linked to other surgical procedures, most during neurosurgery.

There is no evidence at present that occupational exposure to BSE is a risk factor for vCJD, and currently there is no described case of a patient with vCJD linked to previous exposure to contaminated surgical instruments.

Thus the risk of transmission of vCJD due to surgical instruments or procedures may not be great, especially if appropriate precautions are taken with clinically evident cases, but there remains the possibility of contamination via pre or subclinical cases of BSE/vCJD infection. The precautionary principle is applied in many countries for reducing the risk of secondary transmission and thus to avoid an outbreak of nosocomial vCJD infection. This is based on the risk assessment procedure that has been performed both in UK and France. Event this risk assessment is of limited value because of the scarcity of adequate information for entering into the classical models.

3.6.2.1 Approach used

A risk assessment for the transmission of vCJD via surgical instruments was performed with a modelling approach and numerical scenarios, that was published in 2001 by the UK Department of Health (2001). It confirms that the risk of surgical transmission cannot be ruled out and that the most risky procedures are those involving the central nervous system and the back of the eye. Procedures involving lymphatic tissues, such as tonsils, and front of the eye tissue are of a lesser order of risk.

The risk of transmission of vCJD due to surgical instruments or procedures may not be great, especially if appropriate precautions are taken with clinically evident cases, but there remains the possibility of contamination via pre or subclinical cases of BSE/vCJD infection. The precautionary principle is applied in many countries for avoiding this risk of secondary transmission and to avoid an outbreak of vCJD disease. This is due to the risk assessment which has been performed both in UK and France. This risk assessment is of limited value because of the scarcity of adequate data to enter in the classical models.

3.6.2.2 Consideration of the individual risk factors

Exposure

According to the CJD section of the last report of the British Department of Emerging Infections and Zoonosis at the Health Protection Agency's Centre for Infections (CFI), there were 183 reported surgical incidents where instruments, that were considered potentially contaminated with a the CJD agent during use on an index patient were subsequently re-used on other patients during 4 years (Aug 2000 – Aug 2004). Of these

183 reports, 61 are linked with vCJD (possible, probable or definite) and 86 linked with sporadic CJD (HPA 2005).

Patients can be categorised as follows, in descending order of risk, according to Table 5. It is, at present, very difficult to clearly differentiate between CJD or vCJD and other forms of human TSEs CJD in these categorisations.

Table 5. Categorisation of patients by risk

1. Symptomatic patients	1.1. Patients who fulfil diagnostic criteria for definite, probable or possible CJD, including vCJD 1.2. Patients with neurological disease of unknown aetiology, not fulfilling current CJD diagnostic criteria, but where a diagnosis of CJD, including vCJD, is being actively considered
2. Asymptomatic patients	2.1. Individuals who have been shown by genetic testing to be at considerable risk of developing CJD or who had two or more relatives affected by CJD or other prion disease or having a genetic mutation indicative of familial CJD 2.2. Recipients of hormone derived from human pituitary glands (e.g. growth hormone.) 2.3. Individuals who have received a graft of <i>dura mater</i> , or neurosurgical procedures or operations before 1992 2.4. Patients with previous exposure to instruments used on, blood, plasma derivatives, organs or tissues donated by a patient who went to develop any form of CJD 'Patients with exposure to instruments previously used on patients with CJD, or who went on to develop CJD. Patients exposed to blood or blood derivatives donated by patients with CJD, or who went on to develop CJD. Patients who have received organs or tissues donated by on patients with CJD, or who went on to develop CJD.'

In these patients it could be assumed, as presented in Table 1, section 3.2.3 that tissue infectivity may be classified, according to WHO (1999) or ACDP/SEAC (2003), ranging from low (dental pulp, peripheral nerve) to medium (anterior eye and cornea) and high (brain, spinal cord and ganglia, cranial nerves, posterior eye). Although such studies are limited with vCJD, the preliminary data are a valuable support as it is believed that their distribution is more or less similar for other forms of CJD although with considerably greater lymphoid involvement. This is especially important in view that lymphoid involvement in vCJD can be preclinical,

There is a lack of direct evidence for how prion material attached to an instrument is likely to behave on re-use. However there are indications that the PrP^{sc} protein remains bound to instrument surfaces. The expectations would then be that only a fraction of it would be detached on re-use. It is expected than 10 % or less may be detached, but

scenarios must consider a wide range from less than 10 % to 100 %. Direct evidence is also lacking about the initial mass of infected tissue potentially adhering to the instrument. For simplification purpose it could be assumed that 10 mg could adhere to each instrument, and across all operations around 20 instruments are used (and 12 during tonsillectomies (section 3.5.1).

Given some infective burden after the first decontamination and sterilization cycle, the eventual number of infections will depend on how much of this is subsequently transferred to other patients rather than being removed or deactivated. Both the transfer percentage and second and subsequent decontamination efficacy are unknown. Thus the worst case scenario must always be employed. Although certain instruments are obviously dedicated to specific procedures, some others may circulate more widely and crossover of instruments between specialities may occur, thus increasing possibilities for transmission, even if such crossover appears to be the exception rather than the rule.

Hazard factors

A titre of 10^8 intracranial ID₅₀/g. could be encountered in brain and other CNS tissue in the pre-clinical stage of the disease. Once the disease reaches its clinical stage, even higher titres may be encountered, but instruments used on such patients should already be quarantined as a matter of routine. Posterior eye tissue should be regarded as having a similar potential infectivity to CNS. However the anterior chamber can be regarded as having a potential titre 1-2 logs less.

Throughout the incubation period, infectivity of 10^6 - 10^7 ic ID₅₀/g may be dispersed through the body in the lymphoreticular system and some other peripheral material.

As previously described PrP^{Sc} protein is very resistant to different decontaminating and "sterilizing" processes, thus it must be considered that only cleaning (2-3 log reduction) and autoclaving (3-6 log reduction in infectivity) are able to induce a reduction of activity.

3.6.2.3 Use of the above risk factors for risk assessment

Assuming that some individuals in the population may currently be incubating vCJD and be potentially infective, a series of models addressed the following key questions:

- how many secondary infections might be transmitted from one operation on an infective patient, as instruments are re-used?
- what would be the resulting rate of secondary infections within the population as a whole ?
- how could the transmission rate develop over time, and how might these infections eventually be translated into additional clinical uses of vCJD?

Uncertainties accumulate in moving from each question to the next, because at each calculation additional key variables need to be estimated each adding to the overall uncertainty. Specific numerical scenarios must therefore be regarded as illustrative only. Given the scarcity of direct data on vCJD in humans, many key inputs are based using scrapie as a model of the disease (implying that infectivity may be widely-distributed through the body, in contrast to classical CJD).

Despite the uncertainties, some interim conclusions may be reached on the basis of current knowledge:

- surgical transmission of vCJD cannot be ruled out as a risk to public health; according to scenarios, with a starting titre log ID₅₀/g of respectively 8 and 5 logs, expected numbers of infections are ranging from 1 to 0.01
- the total number of vCJD cases caused by surgical transmission could be around 5-10 % of the number infected in the primary outbreak, but the absolute scale of such a risk cannot be determined until more is known
- the most important way of reducing the risk is to ensure that decontamination of instruments is as effective as possible
- operations on the Central Nervous System and those ophthalmic procedures involving the posterior eye carry the highest risk of transmitting vCJD (CNS/PO surgery). Results of illustrative scenarios for infections per operation are the following.
 - Inter-CNS/PO¹, initial infectivity 10⁸ ID₅₀/g, 1 to 10⁻³ secondary infections per operation according to effect of cleaning and sterilization (3 logs to 6 logs).
 - Inter-LRS² or anterior eye (10⁶ ID 50/g), 10⁻⁵ to 10⁻² secondary infections per operation (efficiency of reduction from 3 to 6 logs).

3.6.2.4 Conclusions

Because of the actual limitations for conducting a full risk assessment process (lack of knowledge both on the level of contamination of the instruments before reprocessing and on the minimal infectious dose linked to new variants of prion according to the route of transmission) the edited guidances are based on the precautionary principle:

- specific precautions for symptomatic patients (definite, probable and possible) and asymptomatic patients potentially at risk of CJD
- general precautionary measures for surgical procedures and endoscopy.

A patient 'at high risk' is defined as a patient exposed to materials from individuals who are thought to have been infected with vCJD. For all symptomatic patients, use of single-use protective clothing and single-use disposable surgical instruments and equipment are necessary. All single use items must be destroyed by incineration. In some Member States general precautions have been taken in respect to high risk procedures like tonsillectomy.

For asymptomatic patients but who are known to be at risk the same precautions apply, but where single-use instruments are not available, the handling of re-usable items depends on the kind of activity and the tissue in contact with the instrument (tissue considered in the WHO classification as possibly contaminated or not). Thus according to the French SEAC it is possible to consider the following situations and best practice (Table 6).

Table 6. Schedule for cleaning and disinfection treatment of surgical instruments.

¹ CNS/PO Central Nervous System/ Posterior eye

² LRS Lympho Reticular System

	Activity with high risk (nervous system, eye)	Activity with low risk (coeliosurgery, delivery)
Patient with clinical vCJD	Destruction	Destruction
Patient with high risk	Destruction or Procedure I	Procedure II
Patient without risk	Procedure II	Procedure III

Procedure I: Cleaning + chemical inactivation + physical inactivation

Procedure II: Cleaning + chemical inactivation or physical inactivation

Procedure III: Cleaning + classical disinfection or sterilization

In conclusion, the following Figure 6 could be proposed for precautions for surgical procedures on known, suspect or at risk vCJD.

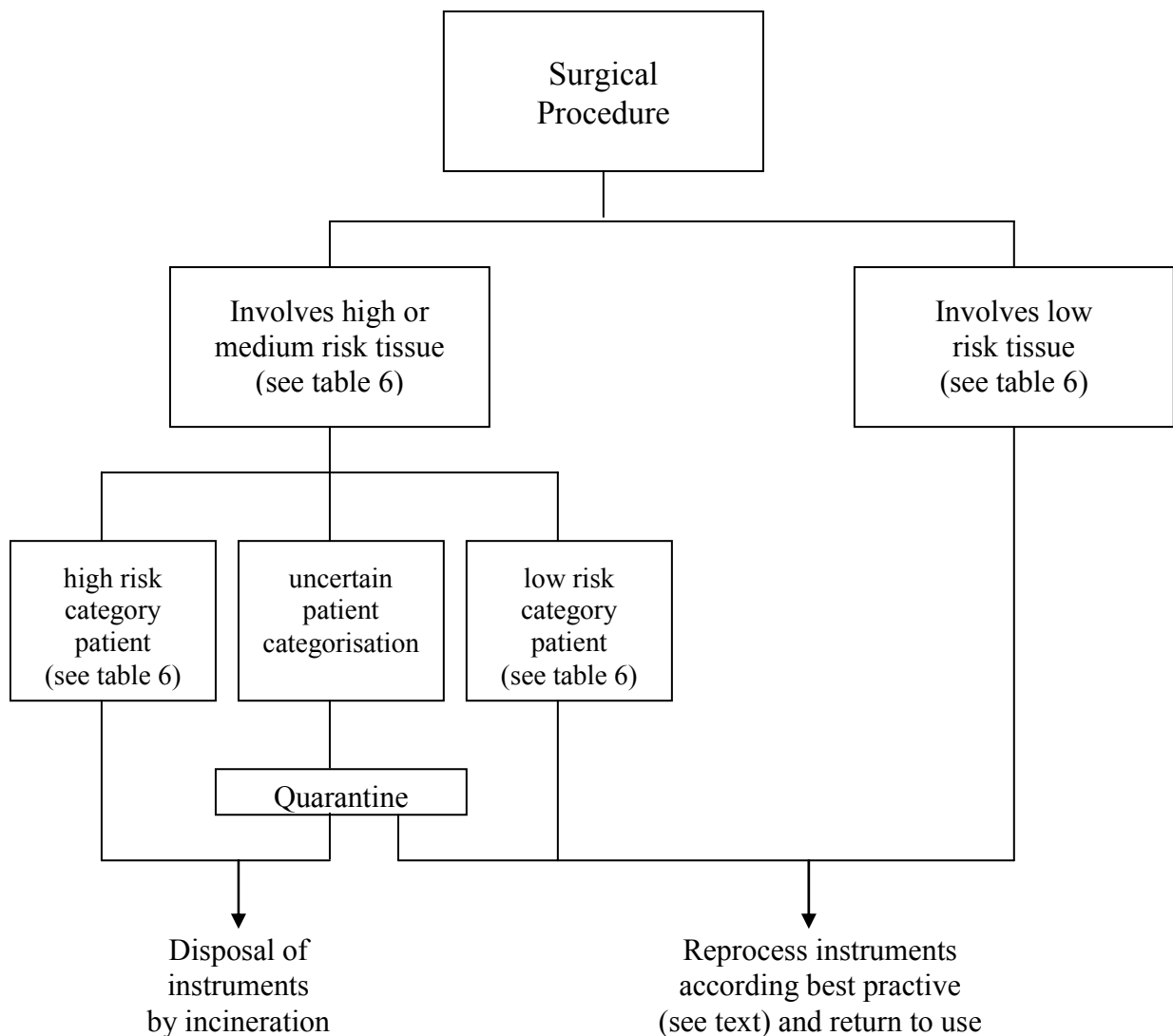


Figure 6. Algorithm chart for precautions for surgical procedures

3.6.3 Risk assessment on vertical transmission

The increase in exposure of humans to transmissible prion agents and the recent possibility of blood borne infection raises the possibility of vertical transmission. This could occur in the uterus, during birth or via breast milk. One important aspect of this question is whether there is any evidence of increased infectivity of the vCJD agent as a result of it being transmitted from human to human, (i.e. the removal of the species barrier).

3.6.3.1 Placental structure

There are marked differences in placental structure among species affected by TSEs – including domestic farm animals, laboratory animals and humans. These differences mean that when attempting to extrapolate between species considerable caution must be exercised.

The placenta of sheep and cattle are of the cotyledonary epitheliochorial type in which the maternal endometrial epithelial surface and the foetal epithelial trophoblast become closely apposed. In cattle there is some fusion of trophoblast and endometrial cells (hence the term “synepitheliochorial” which is sometimes used by Wooding and Flint 1994). In sheep the trophoblast binucleate cells occasionally fuse with maternal epithelial cells to form trinucleate cells. In both species there are zones of maternal haemorrhage where the red cells are lysed and phagocytised by trophoblasts. This facilitates the transfer of iron to the developing foetus.

In commonly used laboratory rodents and man the placentas are discoid and of the haemochorial type. Maternal blood is in intimate contact with the foetal trophoblast. Transfer of gas, nutrient and waste products occurs directly between maternal blood and the trophoblast. In rats and mice this takes place in the labyrinth region of the placenta across a three distinct layers of trophoblast. In humans the structure is of the villous type and there are only 2 layers of trophoblast.

3.6.3.2 Biodistribution of TSE agents

There is considerable variation in the tissues containing infectivity or immunoreactive protein depending on the animal species and the TSE agent used. For example in scrapie infected sheep PrP^{Sc} was widely distributed in nervous and lymphoid tissues but is also found in the placenta (Andreoletti et al 2002). However in BSE infected sheep no immunoreactivity was detected in the placenta, albeit in a very small number of animals tested (Foster et al 2001). In BSE in cattle peripheral deposition was very low (Terry et al 2003). For vCJD, there is no report of any testing of the placenta or mammary tissue.

3.6.3.3 Evidence for vertical transmission in animals

Vertical transmission has been difficult to demonstrate with certainty since it is hard to conclusively exclude transmission from other sources, (contaminated feed or

environment etc). To control for these factors studies were undertaken in which challenged ewes and unchallenged animals were housed together. These studies suggest that transmission by contact is very low (Foster et al 2004) and this is broadly comparable with studies in cattle with BSE (Wilesmith et al 1997). One study has directly addressed the question of *in utero* or perinatal transmission of BSE in susceptible sheep and did not observe any vertical transmission in 29 offspring. The statistical evaluation of the result allowed for an estimation of the occurrence of transmission being at most 1 in 4 (95% CI) (Foster et al 2004). Recently, preliminary results were reported indicating the vertical transmission of BSE in sheep either *in utero* or perinatally (Bellworthy et al, 2005). In this experiment susceptible sheep were dosed orally with 5 gram of BSE cattle brain inoculum. Two lambs developing BSE were born of ewes which developed BSE after the oral administration of BSE cattle brain.

3.6.3.4 Evidence for vertical transmission in man

There are no data available relating to the bio-distribution of PrP^{Res} in human placental tissue. Human and placental tissues vary during gestation and lactation so potential of transmission of infectivity may similarly vary. In addition there is no evidence of vertical transmission of Kuru (Ridley and Baker 1995).

3.6.3.5 Conclusion

Currently there are no proven instances of vertical transmission of any human prion disease. The available animal data are inadequate to allow firm conclusions concerning vertical transmission to be drawn. There are no data indicating that breast milk transmits human prion disease.

3.6.4 Risk assessment on transmission by transplantation of Umbilical Cord Stem Cells

The collection and storage of cells from umbilical cords is becoming increasingly common. These cells can be used as a source of haematopoietic stem cells for transplantation following myeloablation. They can be used in both allogeneic and autologous transplantation in children and adults (Laughlin et al 2001).

The cells that are transplanted are of foetal origin although the possibility of low levels of contamination with maternal blood can not be definitively excluded.

3.6.4.1 The Risk

Umbilical cord blood stem cells are of foetal origin and the likelihood of infection is extremely low, since vertical transmission in humans has not been observed in any prion disease. In addition, it should be recognised that the recipients of the cells may be seriously ill with life-threatening conditions. There remains the possibility that small amounts of maternal blood may be present as a contaminant in cord blood.

3.6.4.2 Conclusion

Since vertical transmission in humans has not been observed in any prion disease, the risk posed by the use of cord blood is considered to be negligible.

3.6.5 Conclusions

Using a series of conservative assumptions made for the purposes of this risk assessment, there is a considerable risk that a vCJD donor could cause infective material to be passed on to one or more recipients of blood and blood components. In the worst case scenario each therapeutic unit of blood donated could contain as much as 4500iv ID50.

In view of the distribution of vCJD infectivity over the various blood compartments (plasma, platelets and leucocytes) leucodepletion may produce no more than a 25% reduction in infectivity. Taken into account the population eligible for blood donation, the number of donations and the percentage of the population actually donating blood, up to 1250 infected donations may occur, per year, in the UK.

A quantitative risk assessment based on the data of the UK appendix study and the worst case scenario, shows that there may occur 3750 new infections each year in the UK. Of these 3750 new infections 1875 new individuals will eventually develop vCJD being the subgroup living long enough after the transfusion to develop vCJD.

There is no evidence that individuals working in hospital settings have developed vCJD by virtue of their profession. Transmission via surgery remains a concern, but to date there is no evidence that it has actually occurred. For prevention of transmission of vCJD by surgical instruments a system is proposed based on the probability of the patient under investigation/treatment being infected with vCJD (or any other TSE), and the precautionary principle.

For use of instruments on clinical vCJD patients single use materials should be used, For patients with high risk, single use materials or alternatively materials followed by specific cleaning plus chemical inactivation plus physical inactivation may be used. For low risk surgical procedures, cleaning plus chemical inactivation or physical inactivation may be used.

For patients without risk the procedure cleaning plus chemical inactivation or physical inactivation, or cleaning plus classical disinfection or sterilization may be used, depending on whether the treatment is an activity with high or low risk, respectively.

There are no proven instances of vertical transmission of any human prion disease. The available animal data are inadequate to allow firm conclusions concerning vertical transmission to be drawn. There are no data indicating that breast milk transmits human prion disease. In view of the lack of vertical transmission in humans the risk posed by the use of cord blood is considered to be negligible.

At this moment there is a declining incidence of trend for the occurrence of vCJD in the UK. All clinical cases to date have been homozygotes MM at codon 129 in the *PRNP* gene. It is unknown whether MV heterozygosity or VV homozygosity protects against developing vCJD or just results in a longer incubation period. However, the latter is generally thought to be most likely and, if so, a second wave of vCJD could develop in the (near) future.

3.7 Evaluation of previous opinions of the SCMPMD on human product safety for vCJD transmission with regard to current scientific knowledge

Three opinions have been issued by SCMPMD on the subject of human derived products and the risk of transmission of vCJD. These opinions are discussed step by step and carefully evaluated which statements no longer are valid due to new scientific data.

3.7.1 Opinion on Quality and Safety of Blood (Adopted by SCMPMD on 16 February 2000)

In this opinion the key elements for establishing high standards of quality and safety for the collection, processing, storage and distribution of whole blood, blood components and blood precursors were identified, namely:

- Inclusion of the complete transfusion chain into the consideration
- Establishment of a Quality System
- Need for a haemovigilance system
- Blood donor recruitment and retention policies
- Need for criteria for the suitability of the donor and for his protection
- Testing requirements
- Storage conditions
- Requirements for labelling
- Introduction of new preparations and products
- Importance of a common terminology
- Mechanisms for establishing and maintaining harmonised quality standards

Comment: The report is very general and the conclusions are still valid. The report stresses the importance of taking into consideration all the steps of the blood transfusion chain. Specifically, in regard to vCJD, it is indicated at page 8, that "although at present testing of vCJD in blood donors is not possible, it is likely that once a sensitive and specific test for vCJD becomes available, such a test will be implemented in all member states". As discussed in section 3.3.6 of the present report, the consequences of a positive test should be evaluated very carefully, taking into account the prevalence of the positive samples, the possible occurrence of false positive tests and the difficulties in determining whether a positive test is a false-positive one.

3.7.2 Opinion on update of the opinion given by SCMPMD on the risk quantification for CJD transmission via substances of human origin (Adopted by SCMPMD on 16 February 2000)

This update concerns an opinion adopted by SCMPMD in October 1998. Three issues are treated in this opinion

1. Leucodepletion. It is stated that the efficiency of leucodepletion is uncertain. TSE infectivity is predominantly associated with the buffy coat but infectivity is also found in plasma. It is speculated that removal of leukocytes might cause shedding of prions and thus increase the infectivity of the plasma. It is recommended to perform further studies and in the meantime introduce leucofiltration which also for other reasons would be of benefit for recipients.

2. Screening assays. SCMPMD encourages efforts to develop easily applicable screening tests for vCJD.
3. Exclusion of donors. The rules applied at that time in USA and Canada are cited: Donors that had stayed in UK more than six months (cumulated) in the period 1980 to 1996 are deferred. It is recommended by SCMPMD that an evaluation is made in Europe on the basis of 1) travel patterns, 2) exposure to UK bovine material in different member states, 3) prevalence of HIV, HBV and HCV in first time donors in member states.

Comment: According to the EMEA report (June 2004, www.emea.eu.int/pdfs/human/press/pos/287902rev1.pdf), studies in UK on leucoreduction show that these procedures do not provoke fragmentation or cell lysis. A study in hamsters indicates that infectivity of whole blood decreases by 42% after leukoreduction, (Gregori et al 2004). Leucodepletion is now adopted in most member countries for other reasons. The recommendations of this report are limited and there are no new data. See section 3.6.1.2. of the present opinion.

3.7.3 Opinion on The Safety Of Human-Derived Products With Regard To TSE's (Adopted by SCMPMD on 18 January 2002)

The working group consisted of members from SCMPMD, the scientific steering committee (SSC) and its TSE/BSE ad hoc group. The report of the working group is divided into the following sections:

- 1) Methods and techniques for the detection of vCJD infectivity
 - a) Bioassays
 - b) In vitro diagnostic assays
 - c) Epidemiology
- 2) vCJD infectivity of blood and other human substances
- 3) Application of vCJD detection tests
 - a) Clinical diagnosis of vCJD (“screening in sufferers of neurodegenerative disease”)
 - b) Surveillance of vCJD
 - c) Donor screening tests for vCJD
- 4) Transmission of vCJD/ TSEs via blood: Risk assessments in different countries
 - a) Europe
 - b) Countries outside Europe (USA, Canada, New Zealand)
 - c) Appendix: Selected official documents (assessments, guidance)
- 5) Compatibility of different risk assessments
- 6) Transmission routes of vCJD: Risk factors in regard to blood donors
- 7) Processing procedures for blood and blood components and vCJD transmission risks
 - a) Leucoreduction of blood components
 - b) Leucoreduction or filtration of plasma for fractionation
 - c) Nanofiltration of plasma derivatives
- 8) Acknowledgement
- 9) Appendix: Selected official documents (assessments, guidance)
- 10) References; Opinions of SSC and SCMPMD; Abbreviations

In the following section 1-7 will be discussed.

1. Methods and techniques for the detection of vCJD infectivity

- a) Bioassays
- b) In vitro diagnostic assays
- c) Epidemiology

The subsections a) and b) are valid; bioassays are still needed in order to detect vCJD infectivity. Subsection c) is no longer correct; it is for instance indicated that epidemiological studies reveal no evidence of human TSE transmission by blood. This statement is in conflict with the probable cases reported recently.

2. vCJD infectivity of blood and other human substances

It is indicated that "currently there is neither a disproof nor a proof for vCJD infectivity of human blood". In section 3.2.4 of the present opinion, the two probable cases of transmission of vCJD or vCJD infection by blood are detailed. It is considered unlikely that these cases were due to dietary transmission. In contrast, there has been no evidence that plasma pools that include donations from patients that later developed vCJD, could transmit the disease.

3. Application of vCJD detection tests

- a) Clinical diagnosis of vCJD
- b) Surveillance of vCJD
- c) Donor screening tests for vCJD

The methods have evolved and the reader is referred to section 3.3 of the present report.

4. Transmission of vCJD/ TSEs via blood: Risk assessments in different countries

- a) Europe
- b) Countries outside Europe (USA, Canada, New Zealand)
- c) Appendix: Selected official documents (assessments, guidance)

This section describes the risk assessment policies applied by European countries (a) and countries outside of Europe. It represents an important work which however needs updating because the situation has evolved since 2002. Table 1 reflects the situation in 2001-2002, the reader is referred to the updated table: risk of vCJD transmission by blood and donor deferrals with data provided by national blood experts as of 6 September 2004 (FMD/D (2004) 360136), see the following link: europa.eu.int/comm/health/ph_threats/human_substance/documents/blood_vcjd_en.pdf

5. Compatibility of different risk assessments

The risk assessments employed by Member States as well as by other countries consist of two separate parts:

1. Assessment of the risk whether or not TSE infectivity can be transmitted by blood (via blood components and plasma derivatives) of individuals infected with the vCJD agent.

-
2. Assessment of the risk that a blood donation was donated from a vCJD infected individual in populations of different geographical areas (prevalence of vCJD infected blood donors).

ad 1.

Recent data demonstrate that TSE infectivity can be transmitted by blood transfusion in animals and probably in man (see section 3.2.4 of present report).

ad 2.

The number of vCJD cases in UK was 115 in the previous report (Dec 2001) and 157 in September 2005 (NCJD surveillance unit, UK). In France the number was 5 in 2001 and is now 14 (Institut de veille sanitaire). In other European countries confirmed vCJD cases are rare (3 in the Republic of Ireland, 1 in Italy, 1 in the Netherlands and 1 in Portugal and 1 in Spain). Outside Europe one case has been reported in each of the following countries: USA, Canada, Japan, Saudi Arabia (see section 3.4.2).

As stated in the report, the estimation of the number of expected vCJD cases in each country is very uncertain, either based on simple extrapolations of UK data or on sophisticated statistical methods. The conclusions of the section are not clear and the reader is referred to the present report.

6. Transmission routes of vCJD: Risk factors in regard to blood donors

As stated in the report, there are in principle two different possible ways to contract vCJD: exposure to bovine material carrying BSE infectivity (primary transmission) and exposure to human derived material carrying vCJD infectivity (secondary transmission). The secondary transmission also includes surgical and investigative diagnostic instruments that have been in contact with infected tissues and there is a risk that individuals infected secondarily become themselves a source for secondary transmission. In addition, these also pose a risk for other forms of CJD transmission. Measures have been taken to interrupt primary transmissions, notably the removal of specified risk material at slaughtering and the exclusion of animals scoring positive in a BSE rapid test.

The first round of secondary vCJD transmissions depends on the prevalence of individuals infected by primary transmissions. If there are differences between different areas in the prevalence of those individuals it might be sensible to prevent, in lower risk areas, the use of human material originating from higher risk areas. This consideration may justify attempts to evaluate or even quantify the extent of the primary vCJD epidemic in different Member States.

The rest of the section concerns considerations about the primary exposure in different countries taking into account trade, extent of BSE epizootic and different genetic susceptibility (methionine homozygosity for codon 129). This is out of scope of the present opinion that only concerns secondary transmission.

7. Processing procedures for blood and blood components and vCJD transmission risks

- a. Leucoreduction of blood components

- b. Leucoreduction or filtration of plasma for fractionation
- c. Nanofiltration of plasma derivatives

In experimental rodent systems, 90% of the vCJD infectivity was estimated to be found in the white cells, while a more recent work reported that only about 42% of the infectivity was in the buffy coat (Gregori et al 2004). As indicated in the report, leucoreduction has other benefits and is applied in most member states.

The subsections on leucoreduction for plasma fractionation and nanofiltration have already been discussed by EMEA and will not be commented upon.

In conclusion, most conclusions and recommendations of the previously published Opinions of the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) and the Scientific Steering committee (SSC) are still valid. Two important issues do need re-evaluation/consideration, the first being the possible transmission of vCJD by blood, and the second being the presence of vCJD in the UK population as indicated by a large scale study of surgical appendix specimens .

4 COMMITTEE OPINION

In 2004 two cases were reported indicating the possible transmission of variant Creutzfeldt -Jakob Disease by blood transfusion (Llewelyn et al 2004, Peden et al 2004). Llewelyn et al (2004) reported on a survey of recipients who were identified as having received blood from donors who developed vCJD. One of the recipients developed symptoms of vCJD 6.5 years after receiving blood transfusion.

Peden et al (2004) reported on an asymptomatic individual who had received a blood transfusion from a donor who subsequently developed vCJD. Protease resistant protein (PrP^{res}) was detected by immunohistochemistry in the spleen and cervical lymph node of the blood recipient who was found to be an MV heterozygote at the prion gene *PRNP* codon 129. All clinical cases to date have been *PRNP* codon 129 MM homozygotes. Whether being codon 129 MV or VV absolutely protects against developing vCJD or just results in a longer incubation period is as yet unknown. However, the latter is generally thought to be most likely and, if so, a second wave of vCJD may develop in the future.

These two human cases could not be definitively proven to be caused by the preceding blood transfusion. However, they raise serious concern on the possibility of infection by blood transfusion from preclinically or subclinically infected donors. It seems unlikely that two dietary-related cases of vCJD infection had previously received blood from vCJD donors, therefore the most likely explanation is that the infection was caused by the blood transfusions. Consequently, it is assumed that in an asymptomatic vCJD infected individual the prion is likely to be present in peripheral blood. This is consistent with animal experimental data.

The present risk assessment of exposure to vCJD infectivity in whole blood and in blood components allows a rationale to define precautionary measures to prevent vCJD transmission within the human species by the intravenous or other routes.

The evidence that potential vCJD infectivity may be also present in MV heterozygotes at codon 129 poses an additional serious threat, since the infectivity may be present in a larger part of the population and the possibility of minimizing risk through a donor group exclusion no longer exists. Concern over possible transmission by blood is increased by the fact that an assay for routine screening with respect to vCJD is not yet available. This has important implications for possible iatrogenic transmission by blood and/or cells and organs from donors in the incubation phase or with subclinical disease, and transmission through surgical instruments used in invasive procedures.

These two cases of a possible transmission of vCJD by blood transfusion initiated several questions which were presented to the Scientific Committee on Emerging and Newly Identified Risks (SCENIHR). These questions (section 2) were as follows:

- to review the previous scientific SCMPMD Opinion on the ‘Safety of Human-Derived Products with regard to TSEs’ adopted on 18 January 2002, and the SCMPMD Opinion on ‘Quality and Safety of Blood’ adopted on 16 February 2000 in order to quantify, if possible, the nature and magnitude of the risks of transmission of

the vCJD agent through blood donated by preclinical cases of vCJD, through surgical instruments or by instruments used in invasive procedures,

- to evaluate the risk of vertical transmission of vCJD in pregnant women and
- to evaluate the risk of vCJD transmission by the tissues stored in umbilical cord cell banks.

The pathogenesis of prion-related diseases is widely considered to be related to defective protein folding resulting in abnormal protein conformation. Normal cellular protein is converted into an insoluble, aggregated, beta-sheet rich form which is deposited in the brain. The mechanism of prion-related diseases is believed to involve disease transmission by replication of this protein conformation. The accumulation of protease resistant prion protein (PrP^{Res}) in tissues is a characteristic feature of prion disease. vCJD is a typical prion disease in this respect.

Current evidence on the pathogenesis of vCJD indicates that orally ingested prions enter mainly via the gut lymphoid tissue, and are transported to the other lymphoid organs. Prions are replicated in the periphery in lymphoid tissues such as the spleen, the appendix and tonsils, and are then transported to the brain mainly by peripheral nerves. Direct penetration into the brain across the blood-brain barrier may occur. Thus, peripheral blood may be involved in propagation and transport of vCJD prions, although abnormal PrP (PrP^{Sc} or PrP^{Res}) has not yet been detected in peripheral blood of vCJD patients. Although the immune system may facilitate vCJD infection, an immune response against vCJD prions is not initiated. However, antibodies against either the normal or disease related forms of PrP can protect against TSE disease in animals. vCJD protein has been found to be present in various tissues including brain, spinal cord, spinal ganglia, cranial nerves and ganglia, posterior eye, tonsil, appendix, spleen and thymus and other lymphoid tissues, with varying degrees of infectivity.

Important advances in test methodologies for prion detection have been made in recent years, and the application of these advances in the diagnosis of vCJD has, in particular, been fruitful. However, no diagnostic system has yet emerged with the high level of sensitivity and specificity required for routine screening of blood or urine. It is essential that confirmatory assays are available, and all ethical implications are considered and carefully taken into account before implementing testing. Independent validation of any new methodology should be mandatory prior to implementation, and it is recommended to adopt the EU procedure used for the BSE testing. For validation carefully controlled vCJD reference materials should be used. The issue of false positives needs careful consideration, as even minute percentages of these may actually involve a large number of individuals. The ethical issues of informing an individual of test results, without providing any certainty as to the likelihood of progression to clinical disease, should be considered seriously.

The incidence of vCJD in the UK is decreasing but there remain considerable uncertainties and concerns over future numbers of cases. While other countries have not been involved to the same degree, France continues to identify new cases and additional countries have been affected. The control of BSE in cattle and the precautions taken to prevent BSE infected material entering the human food chain, there remains the issue of controlling secondary human-to-human transmission. The monitoring of vCJD cases, vigilance for any new phenotypes of human TSE infection and the identification of any actual secondary transmission events are essential. Human population studies are entirely dependent on having

efficient and appropriate surveillance systems in all relevant countries. It is therefore essential that the EU wide surveillance for human TSEs is continued.

For surgical instrument cleaning and disinfection there is a need to validate the effectiveness of the methodology employed. The animal assay with intracerebral inoculation is still the best method for determining TSE infectivity. No procedure for the decontamination of surgical instruments has yet been validated to the extent that its universal introduction can be recommended. Protein removal from the instruments is essential. Drying of instruments before cleaning or decontamination is likely to reduce the effectiveness of the decontamination procedure. Disinfectants with fixative properties such as those containing aldehydes must not be used for decontamination of instruments suspected to be contaminated with TSEs as they tend to stabilise rather than inactivate prions.

The specific answers to the questions presented to SCENIHR are presented below:

Question 1:

- to review the previous scientific SCMPMD Opinion on the ‘Safety of Human-Derived Products with regard to TSEs’ adopted on 18 January 2002, and the SCMPMD Opinion on ‘Quality and Safety of Blood’ adopted on 16 February 2000 in order to quantify, if possible, the nature and magnitude of the risks of transmission of the vCJD agent through blood donated by preclinical cases of vCJD, through surgical instruments or by instruments used in invasive procedures.

The general conclusions and recommendations of the previous Opinions on the safety of human derived products including blood and blood components are still valid. Research on detection methodology has further evolved and an update is presented. Two aspects need our current attention; the possibility of transmission of vCJD by blood and blood components, and the presence of vCJD infected individuals in the population. The possibility for transmission by blood or blood products seems likely in view of the two cases of vCJD infection in persons who received previously a blood donation from a donor who developed vCJD. In addition, the UK study evaluating anonymised routine appendicectomy samples (Hilton et al, 2004a) indicates that vCJD infectivity may be present in the UK population at higher levels than the present numbers of identified clinical cases suggest.

There is a risk that a vCJD infected donor could pass infective material to one or more recipients of blood and blood components. In the worst case scenario each therapeutic unit of blood donated could contain as much as 4500 infectious units.

In view of the distribution of vCJD infectivity over the various blood compartments, leucodepletion may produce no more than a 25% reduction in infectivity. Based on the data of the UK appendix study and the worst case scenario, and taking into account the population eligible for blood donation, the number of donations and the percentage of the population actually donating blood, up to 1250 infected donations may occur, per year, in the UK.

If there are 1250 infected donations per year they will result in 3750 new infections each year in the UK assuming that donations are typically split between 3 recipients. Of these 3750 new infections the subgroup living long enough after the transfusion to develop vCJD is approximately 50 %, so 1875 new individuals are eventually expected to develop vCJD. However, this worst case scenario does not fit the current data on vCJD case trends in the UK and may therefore largely overestimate vCJD transmission. It is unknown how vCJD may

develop in heterozygotes at codon 129 of the *PRNP* gene. They may be not susceptible for developing clinical vCJD, but they also could have a prolonged incubation period. Regarding persistence of such a vCJD infection in the population by blood transfusion, preliminary data from one mathematical model suggest that it is unlikely that a vCJD infection could establish itself in the population by blood transfusion only.

The risk assessment is directly applicable only to the UK situation. In order to perform similar risk assessments for other Member States, it is recommended that data are collected on the presence of infection (PrP^{Sc} or PrP^{Res}) in the general population similar to the UK appendix study.

The current decline in the onset of clinical vCJD in the UK and the general low number of cases in the older age groups that comprise the majority of blood recipients, strongly indicate that this worst case scenario considerably overestimates transfusion-related vCJD disease development.

There are several potential explanations, namely:

- infections may have a very long incubation period so that the individual dies before disease develops,
- infections in some groups such as the MV heterozygotes may not be associated with blood infectivity,
- different genotypes may not transmit efficiently to each other even if the blood unit transfused is infectious.

Taking the lower limit of the confidence interval of the prevalence from the UK appendix study and assuming that only ten percent of infectious donations actually transmit the infectious agent, the number of infections resulting would be 9 per year in contrast to the 1250 predicted by the worst case scenario. Independent of the method of calculation transmission by blood transfusion may occur. Based on current data, the frequency cannot be reliably estimated, but even in the UK it is low. The frequency is largely dependent on the number of asymptomatic infected individuals in the general population which is likely to differ from one Member State to another.

There is no evidence that individuals working in hospitals have developed vCJD by virtue of their occupation. Transmission of TSEs during surgical procedures remains a concern, but to date there is no evidence that it has actually occurred in relation to vCJD. To minimize the risk of transmission of vCJD by surgical instruments, procedures are recommended based on the probability of the patient under investigation/treatment being infected with vCJD (or any other TSE).

- For clinical vCJD patients potentially contaminated instruments must be destroyed.
- For patients at high risk single use instruments are recommended, alternatively cleaning plus chemical inactivation plus physical inactivation, or cleaning plus chemical inactivation or physical inactivation may be used, depending whether the treatment is an activity with high or low risk, respectively.
- For patients not deemed to be at risk for TSE, cleaning plus chemical inactivation or physical inactivation, or cleaning plus classical disinfection or sterilization may be used, depending on whether the treatment is an activity with high or low risk, respectively.

Question 2:

- to evaluate the risk of vertical transmission of vCJD in pregnant women

There are no proven instances of vertical transmission of any human prion disease. The available animal data are inadequate to allow firm conclusions concerning vertical transmission to be drawn. It is recommended that there is a follow-up for children that are born to mothers who had or developed clinical vCJD. There are no data indicating that breast milk transmits human prion disease.

Question 3:

- to evaluate the risk of vCJD transmission by the tissues stored in umbilical cord cell banks.

Current data do not indicate that prion infectivity is transmitted by cord blood cells.

In conclusion, as long as there is a risk that infectious prion protein (PrP^{Sc} or PrP^{Res}) is present in blood and blood components, there will be a risk of transmission of vCJD disease by transfusion. Blood transfusion appears the most likely route for inter-human transmission of vCJD. The Committee does not consider that specific measures are needed to reduce the risk from vCJD infectivity in blood. However, it recognises that there are good practices to reduce any risk for transmission of infectious diseases such as optimal use of the transfusion to reduce the number of patients exposed, and optimal blood donation techniques and blood transfusion practices which minimize the number of blood donors to which an individual patient is exposed.

5 MINORITY OPINION

Not applicable.

6. REFERENCES

ACDP/SEAC joint TSE working group Transmissible spongiform encephalopathy agents: safe working and the prevention of infection. *UK Department of Health* 2003, 124 pp, updated oct 2004. Available at <http://www.doh.gov.uk/cjd/tseguidance>

Abschlussbericht der Task Force vCJK. Die Variante der Creutzfeldt-Jakob-Krankheit (vCJK). *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 2002, 45, 376-394.

Aguzzi, A. and Heikenwalder, M. Prions, cytokines, and chemokines: a meeting in lymphoid organs. *Immunity* 2005, 22, 145-154.

Aguzzi, A., Heikenwalder, M. and Miele, G. Progress and problems in the biology, diagnostics, and therapeutics of prion diseases. *J Clin Invest* 2004, 114, 153-160.

Aguzzi, A. and Sigurdson, C.J. Antiprion immunotherapy: to suppress or to stimulate? *Nat Rev Immunol* 2004, 4, 725-736.

Alper, T., Cramp, W.A., Haig, D.A. and Clarke, M.C. Does the agent of scrapie replicate without nucleic acid. *Nature* 1967, 214, 764-766.

Alper, T., Haig, D. A. & Clarke, M. C. The exceptionally small size of the scrapie agent. *Biochem Biophys Res Commun* 1966, 22, 278-284.

Andreoletti, O., Lacroux, C., Chabert, A., Monnereau, L., Tabouret, G., Lantier, F., Berthon, P., Eychenne, F., Lafond-Benestad, S., Elsen, J. M. & Schelcher, F. PrP(Sc) accumulation in placentas of ewes exposed to natural scrapie: influence of foetal PrP genotype and effect on ewe-to-lamb transmission. *J Gen Virol*. 2002, 83, 2607- 2616.

Aucouturier, P., Geissmann, F., Damotte, D., Saborio, G.P., Meeker, H.C., Kascsak, R., Carp, R.I. and Wisniewski, T. Infected splenic dendritic cells are sufficient for prion transmission to the CNS in mouse scrapie. *J Clin Invest* 2001, 108, 703-708.

Baier, M., Schwarz, A. & Mielke, M. Activity of an alkaline ‘cleaner’ in the inactivation of the scrapie agent. *J Hosp Infect* 2004, 57, 80-84.

Baskakov, I.V., Legname, G., Gryczynski, Z. and Prusiner, S.B. The peculiar nature of unfolding of the human prion protein. *Protein Sci* 2004, 13, 586-595.

Baxter HC, Campbell GA, Whittaker AG, Jones AC, Aitken A, Simpson AH, Casey M, Bountiff L, Gibbard L, Baxter RL. Elimination of transmissible spongiform encephalopathy infectivity and decontamination of surgical instruments by using radio-frequency gas-plasma treatment. *J Gen Virol*. 2005, 86, 2393-2399.

Beekes, M., Mielke, M., Pauli, G., Baier, M. & Kurth, R. Aspects of risk assessment and risk management of nosocomial transmission of classical and variant Creutzfeldt-Jakob disease with special attention to German regulations. In Prions. A Challenge for Science, Medicine and the Public Health System. *Contributions to Microbiology* 2004, vol. 11, pp. 117-135. Edited by H. F. Rabenau, J. Ciantl & H. W. Doerr. Basel: Karger.

Bellon A, Seyfert-Brandt W, Lang W, Baron H, Groner A, Vey M. Improved conformation-dependent immunoassay: suitability for human prion detection with enhanced sensitivity. *J Gen Virol*. 2003, 84, 1921-1925.

Bellworthy S, dexter G, Stack M, Chaplin M, Hawkins SAC, Simmons MM, jeffrey M, Martin S, Gonzalez L, Hill P. Natural transmission of BSE between sheep within an experimental flock. *Veterinary record* 2005, 157, 206.

Bieschke J, Giese A, Schulz-Schaeffer W, Zerr I, Poser S, Eigen M, Kretzschmar H. Ultrasensitive detection of pathological prion protein aggregates by dual-color scanning for intensely fluorescent targets. *Proc Natl Acad Sci U S A* 2000, 97, 5468-5473.

Boelle P-y et al Modelling the epidemic of variant Creutzfeldt-Jakob disease in the UK based on age characteristics: updated, detailed analysis. *Statistical Methods in Medical Research*. 2003, 12, 221-233.

Boelle P-Y, Cesbron J-Y, Valleron A-J. Epidemiological evidence of highersusceptibility to vCJD in the young. *BMC Infectious Diseases* 2004, 4, 26-32.

Bolton, D.C., McKinley, M.P. and Prusiner, S.B. Identification of a protein that purifies with the scrapie prion. *Science* 1982, 218, 1309-1311.

Brown P. Blood infectivity, processing and screening tests in transmissible spongiform encephalopathy. *Vox Sang*. 2005, 89, 63-70.

Brown, P., Gibbs, C. J., Jr, Amyx, H. L., Kingsbury, D. T., Rohwer, R. G., Sulima, M. P. & Gajdusek, D. C. Chemical disinfection of Creutzfeldt-Jakob disease. *N Engl J Med* 1982, 306, 1279-1282.

Brown, P., Rohwer, R. G. & Gajdusek, D. C. Newer data on the inactivation of scrapie virus or Creutzfeldt-Jakob disease virus in brain tissue. *J Infect Dis* 1986, 153, 1145-1148.

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

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

Brown, P., Preece, M., Brandel, J. P. et al. Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology* 2000, 55, 1075-1081.

Brown, P., Cervenakova, L. & Diringer, H. Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease. *J Lab Clin Med* 2001, 137, 5-13.

Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H, Bostock CJ. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* 1997, 389, 498-501.

Bruce, M. E., McConnell, I., Will, R. G. & Ironside, J. W. Detection of variant Creutzfeldt-Jakob disease infectivity in extra-neural tissues. *Lancet* 2001, 358, 208-209.

Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C.M. and Stefani, M. (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature*, 2002, 416, 507-511.

Cashman, N.R.: Transmissible spongiform encephalopathies: vaccine issues. *Dev Biol (Basel)*, 2001,106, 455-459, discussion 460-451, 465-475.

Castilla J, Saa P, Hetz C, Soto C. In vitro generation of infectious scrapie prions. *Cell* 2005, 121,195-206.

Cervenakova L, Brown P, Soukharev S, Yakovleva O, Diringer H, Saenko EL, Drohan WN. (2003b): Failure of immunocompetitive capillary electrophoresis assay to detect disease-specific prion protein in buffy coat from humans and chimpanzees with Creutzfeldt-Jakob disease. *Electrophoresis* 2003, 24, 853-859.

Cervenakova L, Yakovleva O, McKenzie C, Kolchinsky S, McShane L, Drohan WN, Brown P. (2003a): Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. *Transfusion*. 2003, 43, 1687-1694.

Circulaire DGS/SC/DHD/E22001/138 du 14 mars 2001 Précautions à observer lors des soins en vue de réduire les risques de transmission d'agents transmissibles non conventionnels. Ministère de la Santé, France.

Clarke P. and Ghani AC. Projections of the future course of the primary vCJD epidemic in the UK: inclusion of subclinical infection and the possibility of wider susceptibility. *J R Soc Interface* 2005, 2, 19-31.

Collinge J, Sidle KC, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. *Nature* 1996, 383, 685-690.

Cooper JD, and Bird SM. Predicting incidence of variant Creutzfeldt-Jakob disease from UK dietary exposure to bovine spongiform encephalopathy for the 1940 to 1969 and post-1969 birth cohorts. *Int J Epidemiol*. 2003, 32, 784-791.

Cousens SN, Vynnycky E, Zeidler M et al Predicting the CJD epidemic in humans. *Nature* 1997, 385, 197-198.

D'Aignaux JN, Cousens SN, Smith PG. Predictability of the UK variant Creutzfeldt-Jakob disease epidemic. *Science*. 2001, 294, 1729-1731.

Devereux G, Stellitano L, Verity CM et al Variations in neurodegenerative disease across the UK: findings from the national study of Progressive Intellectual and Neurological Deterioration (PIND). *Arch Dis Child* 2004, 89, 8-12.

Det Norske Veritas Report: Risk Assessment of Exposure to vCJD Infectivity in Blood and Blood Products; Job No. 716108; Revision D; February 2003

Duer H-P, Dietz K, Eichner M. Determinants of the eradicability of filarial infections: a conceptual approach. *Trends in Parasitology* 2005, 21, 88-96.

Eglin, R.P. and Murphy, W.G. Beyond leukodepletion: removing infectious prions by filtration (editorial), *Transfusion* 2005, 45, 1836-1838.

Ernst, D. R. & Race, R. E. Comparative analysis of scrapie agent inactivation methods. *J Virol Methods* 1993, 41, 193-202.

European Commission, Directive 2004/33/EC

Fevrier, B., Vilette, D., Archer, F., Loew, D., Faigle, W., Vidal, M., Laude, H. and Raposo, G. Cells release prions in association with exosomes. *Proc Natl Acad Sci U S A* 2004, 101, 9683-9688.

Flechsigg, E., Hegyi, L., Enari, M., Schwarz, P., Collinge, J. & Weissmann, C. Transmission of scrapie by steel-surface-bound prions. *Mol Med* 2001, 7, 679-684.

Foster, J. D., Parnham, D. W., Hunter, N. & Bruce, M. Distribution of the prion protein in sheep terminally affected with BSE following experimental oral transmission, *J Gen Virol.* 2001, 82, 2319-2326.

Foster, J. D., Goldmann, W., McKenzie, C., Smith, A., Parnham, D. W. & Hunter, N. Maternal transmission studies of BSE in sheep, *J Gen Virol.* 2004, 85, 3159-3163.

Furukawa H, Doh-ura K, Okuwaki R, Shirabe S, Yamamoto K, Udono H, Ito T, Katamine S, Niwa M. A pitfall in diagnosis of human prion diseases using detection of protease-resistant prion protein in urine. Contamination with bacterial outer membrane proteins. *J Biol Chem.* 2004, 279, 23661-23667.

Ghani AC, Donnelly CA, Ferguson NM, Anderson RM. Updated projections of future vCJD deaths in the UK. *BMC Infect Dis.* 2003, 3, 4.

Ghani AC, Ferguson NM, Donnelly CA et al Epidemiological determinants of the pattern and magnitude of the vCJD epidemic in Great Britain. *Proc R Soc Lond B* 1998, 265, 2443-2452.

Ghani AC et al Predicted vCJD mortality in Great Britain. *Nature* 2000, 406, 583-584

Gibbs CJ Jr, Asher DM, Kobrine A, Amyx HL, Sulima MP, Gajdusek DC. Transmission of Creutzfeldt-Jakob disease to a chimpanzee by electrodes contaminated during neurosurgery. *J Neurol Neurosurg Psychiatry.* 1994,57,757-758.

Glatzel, M., Abela, E., Maissen, M. & Aguzzi, A. Extraneural pathologic prion protein in sporadic Creutzfeldt-Jakob disease. *N Engl J Med* 2003, 349, 1812-1820.

Goodall CA, Head MW, Everington D, Ironside JW, Knight RSG, Green AJE. Elevated CSF phosphorylated tau in variant CJD: a potential discriminator between variant and sporadic Creutzfeldt-Jakob disease. *J Neurol Neurosurg Psychiatry* 2005 (in press).

Green AJE, Thompson EJ, Stewart GE, Zeidler M, McKenzie JM, MacLeod M-A, Ironside JW, Will RG, Knight RSG. Use of 14-3-3 and other brain-specific proteins in CSF in the diagnosis of variant Creutzfeldt-Jakob disease. *J. Neurol. Neurosurg. Psychiatry* 2001, 70, 744-748.

Gregori L, McCombie N, Palmer D, Birch P, Sowemimo-Coker SO, Giulivi A, Rohwer RG Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood. *Lancet* 2004, 364, 529-531.

Grosset A., Moskowitz K., Nelsen C., Pan T., Davidson E., Orser CS rapid presymptomatic detection of PrPSc via conformationally responsive palindromic PrP peptides. *Peptides* 2005 (in press).

Haik, S., Faucheux, B. A., Sazdovitch, V., Privat, N., Kemeny, J. L., Perret-Liaudet, A. & Hauw, J. J. The sympathetic nervous system is involved in variant Creutzfeldt-jakob disease. *Nat Med* 2003, 9, 1121-1123.

Head MW, Bunn TJ, Bishop MT, McLoughlin V, Lowrie S, McKimmie CS, Williams MC, Mccardle L, MacKenzie J, Knight R, Will RG, Ironside JW. Prion protein heterogeneity in sporadic but not variant Creutzfeldt-Jakob disease: UK cases 1991-2002. *Ann Neurol.* 2004, 55, 851-859.

Head M.W., Kouverianou E., Taylor L. Green A, Knight R Evaluation of urinary PrPsc as diagnostic test for sporadic variant and familial CJD. *Neurology* 2005, 64, 1794-1796.

Heikenwalder, M., Zeller, N., Seeger, H., Prinz, M., Klohn, P.C., Schwarz, P., Ruddle, N.H., Weissmann, C. and Aguzzi, A. Chronic lymphocytic inflammation specifies the organ tropism of prions. *Science* 2005, 307, 1107-1110.

Heppner, F.L. and Aguzzi, A. Recent developments in prion immunotherapy. *Curr Opin Immunol* 2004, 16, 594-598.

Heppner, F.L., Christ, A.D., Klein, M.A., Prinz, M., Fried, M., Kraehenbuhl, J.P. and Aguzzi, A. Transepithelial prion transport by M cells. *Nat Med* 2001, 7, 976-977.

Herzog, C., Sales, N., Etchegaray, N., Charbonnier, A., Freire, S., Dormont, D., Deslys, J.P. and Lasmez, C.I. : Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. *Lancet*, 2004, 363, 422-428

Hill AF, Butterworth RJ, Joiner S, Jackson G, Rossor MN, Thomas DJ, Frosh A, Tolley N, Bell JE, Spencer M, King A, Al-Sarraj S, Ironside JW, Lantos PL, Collinge J. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. *Lancet.* 1999, 353, 183-189.

Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, Collinge J. The same prion strain causes vCJD and BSE. *Nature* 1997a 389, 448-450

Hill AF, Zeidler M, Ironside J, Collinge J. Diagnosis of new variant Creutzfeldt-Jakob by tonsil biopsy *Lancet* 1997b 349, 99-100 .

Hill AF, Collinge J. Species-barrier-independent prion replication in apparently resistant species. *APMIS.* 2002, 110, 44-53.

Hillier CEM, Salmon RL, Neal JW, Hilton DA. Possible underascertainment of variant Creutzfeldt-Jakob disease: a systematic study. *J Neurol Neurosurg Psychiatry* 2002, 72, 304-309

Hilton DA, Fathers E, Edwards P et al Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt-Jakob disease. *Lancet* 1998, 352, 703-704.

Hilton, DA., Ghani, A. C., Conyers, L., Edwards, P., McCardle, L., Penney, M., Ritchie, D. & Ironside, J. W. Accumulation of prion protein in tonsil and appendix: review of tissue samples. *BMJ* 2002, 325, 633-634.

Hilton DA, Sutak J, Smith MEF et al Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt-Jakob disease. *J Clin Pathol* 2004b, 57, 300-302.

Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, Ritchie D, Penney M, Hegazy D, Ironside JW. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol.* 2004a , 203, 733-739.

Hirsch N, Beckett A, Collinge J, Scaravelli F, Tabrizi S, Berry S. Lymphocyte contamination of laryngoscope blades--a possible vector for transmission of variant Creutzfeldt-Jakob disease. *Anaesthesia.* 2005, 60, 664-667.

Hörnlimann, B., Pauli, G., Harbarth, S., Widmer, H.-R. & Simon, D. (2001). Die Prävention von Prionkrankheiten im medizinischen Bereich. In Prionen und Prionkrankheiten, pp. 415-422. Edited by B. Hörnlimann, D. Riesner & H. Kretzschmar. Berlin, New York: de Gruyter,

Houston F, Foster JD, Chong A, Hunter N, Bostock CJ, Transmission of BSE by blood transfusion in sheep. *Lancet* 2000, 356, 999-1000

HPA, Health Protection Agency (online) CJD Incidents Panel. London: HPA 2005 Available at http://www.hpa.org.uk/infections/topics_a7/cjd/incidents-panel.htm

Hsich G, Kenney K, Gibbs CJ, Lee KH, Harrington MG. The 14-3-3 brain protein in cerebrospinal fluid as a marker for transmissible spongiform encephalopathies. *N Engl J Med.* 1996, 335, 924-930.

Huang, F.P., Farquhar, C.F., Mabbott, N.A., Bruce, M.E. and MacPherson, G.G. Migrating intestinal dendritic cells transport PrP(Sc) from the gut. *J Gen Virol* 2002, 83, 267-271.

Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, Eaton S, MacKenzie C, Houston F, Transmission of prion diseases by blood transfusion. *J Gen Virol* 2002, 83, 2897-2905.

Ironside JW, Hilton DA, Ghani A et al Retrospective study of prion-protein accumulation in tonsil and appendix tissues. *Lancet* 2000, 355, 1693-1694.

Jackson, G.S., Beck, J.A., Navarrete, C., Brown, J., Sutton, P.M., Contreras, M. and Collinge, J. HLA-DQ7 antigen and resistance to variant CJD. *Nature* 2001, 414, 269-270.

Jones, E.M. and Surewicz, W.K. Fibril conformation as the basis of species- and strain-dependent seeding specificity of mammalian prion amyloids. *Cell* 2005, 121, 63-72.

Kaesler, P.S., Klein, M.A., Schwarz, P. and Aguzzi, A. Efficient lymphoreticular prion propagation requires PrP(c) in stromal and hematopoietic cells. *J Virol* 2001, 75, 7097-7106.

Kimberlin, R. H., Walker, C. A., Millson, G. C., Taylor, D. M., Robertson, P. A., Tomlinson, A. H. & Dickinson, A. G. Disinfection studies with two strains of mouse-passaged scrapie agent. Guidelines for Creutzfeldt-Jakob and related agent. *J Neurol Sci* 1983, 59, 355-369.

Klein, M.A., Frigg, R., Flechsig, E., Raeber, A.J., Kalinke, U., Bluethmann, H., Bootz, F., Suter, M., Zinkernagel, R.M. and Aguzzi, A. A crucial role for B cells in neuroinvasive scrapie. *Nature* 1997, 390, 687-690.

Klein, M.A., Kaeser, P.S., Schwarz, P., Weyd, H., Xenarios, I., Zinkernagel, R.M., Carroll, M.C., Verbeek, J.S., Botto, M., Walport, M.J., Molina, H., Kalinke, U., Acha-Orbea, H. and Aguzzi, A. Complement facilitates early prion pathogenesis. *Nat Med* 2001, 7, 488-492.

Klohn PC, Stoltze L, Flechsig E, Enari M, Weissmann C. A quantitative, highly sensitive cell-based infectivity assay for mouse scrapie prions. *Proc Natl Acad Sci U S A*. 2003, 100, 11666-11671.

Knight R. Epidemiology of variant CJD. In: Brown F, Seitz R, editors. *Advances in Transfusion Safety*. Basel: Karger, 2002, 87-92.

Knight R The Relationship between New Variant Creutzfeldt-Jakob Disease and Bovine Spongiform Encephalopathy. *Vox Sag* 1999, 76, 203-208

Korth C, Stierli B, Streit P, Moser M, Schaller O, Fischer R, Schulz-Schaeffer W, Kretschmar H, Raeber A, Braun U, Ehrensperger F, Hornemann S, Glockshuber R, Riek R, Billeter M, Wuthrich K, Oesch B. Prion (PrP^{Sc})-specific epitope defined by a monoclonal antibody. *Nature* 1997, 390, 74-77.

Korth C, Streit P, Oesch B. Monoclonal antibodies specific for the native, disease-associated isoform of the prion protein. *Methods Enzymol.* 1999, 309, 106-122.

Korth C, Kaneko K, Groth D, Heye N, Telling G, Mastrianni J, Parchi P, Gambetti P, Will R, Ironside J, Heinrich C, Tremblay P, DeArmond SJ, Prusiner SB. Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. *Proc Natl Acad Sci U S A*. 2003 Apr 15, 100, 4784-4789.

Kuczius T and Groschup MH. Differences in proteinase K resistance and neuronal deposition of abnormal prion proteins characterize bovine spongiform encephalopathy (BSE) and scrapie strains. *Mol Med.* 1999, 5, 406-418.

Lane A., Stanley CJ, Dealer S, Wilson SM. Polymeric ligands with specificity for aggregated prion protein. *Clin. Chem.* 2003, 49, 1774-1775

Laplanche JL, Lepage V, Peoc'h K, Delasnerie-Laupretre N, Charron D. HLA in French patients with variant Creutzfeldt-Jakob disease. *Lancet* 2003, 361, 531-532.

Laughlin MJ, Barker J, Bambach B, Koc ON, Rizzieri DA, Wagner JE, Gerson SL, Lazarus HM, Cairo M, Stevens CE, Rubinstein P, Kurtzberg J. Hematopoietic

engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med* 2001, 344, 1815-1822.

Legname G, Baskakov IV, Nguyen HO, Riesner D, Cohen FE, DeArmond SJ, Prusiner SB. Synthetic mammalian prions. *Science* 2004, 305, 673-676.

Lemmer, K., Mielke, M., Pauli, G. & Beekes, M., Decontamination of surgical instruments from prion proteins: in vitro studies on the detachment, destabilization and degradation of PrP^{Sc} bound to steel surfaces. *J Gen Virol* 2004, 85, 3805-3816.

Llewelyn, C. A., Hewitt, P. E., Knight, R. S., Amar, K., Cousens, S., Mackenzie, J. & Will, R. G. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 2004, 363, 411-412.

Lundmark, K., Westermark, G.T., Nystrom, S., Murphy, C.L., Solomon, A. and Westermark, P. Transmissibility of systemic amyloidosis by a prion-like mechanism. *Proc Natl Acad Sci U S A* 2002, 99, 6979-6984.

Mabbott, N.A., Bruce, M.E., Botto, M., Walport, M.J. and Pepys, M.B. Temporary depletion of complement component C3 or genetic deficiency of C1q significantly delays onset of scrapie. *Nat Med* 2001, 7, 485-487.

Majeed A, Lehmann P, Kirby L, Knight R, Coleman M. Extent of misclassification of death from Creutzfeldt-Jakob disease in England 1979-96: retrospective examination of clinical records. *BMJ* 2000, 320, 145-147

Manuelidis, L. Decontamination of Creutzfeldt-Jakob disease and other transmissible agents. *J Neurovirol* 1997, 3, 62-65.

Miele G, Manson J, Clinton M. A novel erythroid-specific marker of transmissible spongiform encephalopathies. *Nat Med.* 2001, 7, 361-364.

Miller DM, Youkhana I, Karunaratne WU, Pearce A. Presence of protein deposits on 'cleaned' re-usable anaesthetic equipment. *Anaesthesia.* 2001, 56, 1069-1072.

Minor PD. Technical aspects of the development and validation of tests for variant Creutzfeldt-Jakob disease in blood transfusion. *Vox Sang.* 2004, 86, 164-170.

Nikles, D., Bach, P., Boller, K., Merten, C.A., Montrasio, F., Heppner, F.L., Aguzzi, A., Cichutek, K., Kalinke, U. and Buchholz, C.J. Circumventing tolerance to the prion protein (PrP): vaccination with PrP-displaying retrovirus particles induces humoral immune responses against the native form of cellular PrP. *J Virol* 2005, 79, 4033-4042.

Ortolano, G., Wilkins, K., Cervia, J. 2005: Characterization of Prion Removal Devices for Blood Products, *Transfus Med Hemother* 2005, 32, 245-251

Otto M, Wiltfang J, Tumani H, Zerr I, Lantsch M, Kornhuber J, Weber T, Kretschmar HA, Poser S. Elevated levels of tau-protein in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *Neurosci Lett.* 1997, 225, 210-212.

Pan et al 2004 (Abstract), Keystone Symposium on TSE Diseases, December 2004

Pan, T., Li, R., Kang, S.C., Pastore, M., Wong, B.S., Ironside, J., Gambetti, P. and Sy, M.S. Biochemical fingerprints of prion diseases: scrapie prion protein in human prion diseases that share prion genotype and type. *J Neurochem* 2005, 92, 132-142.

Paramithiotis E, Pinard M, Lawton T, LaBoissiere S, Leathers VL, Zou WQ, Estey LA, Lamontagne J, Lehto MT, Kondejewski LH, Francoeur GP, Papadopoulos M, Haghghat A, Spatz SJ, Head M, Will R, Ironside J, O'Rourke K, Tonelli Q, Ledebur HC, Chakrabartty A, Cashman NR. A prion protein epitope selective for the pathologically misfolded conformation. *Nat Med.* 2003, 9, 893-899.

Peden AH, Head MW, Ritchie DL et al Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 2004, 364, 527-529.

Pepys, M.B., Bybee, A., Booth, D.R., Bishop, M.T., Will, R.G., Little, A.M., Prokupek, B. and Madrigal, J.A. MHC typing in variant Creutzfeldt-Jakob disease. *Lancet* 2003, 361, 487-489.

Prowse CV and Bailey A. Validation of prion removal by leucocyte-depleting filters: a cautionary tale. *Vox Sang.* 2000, 79, 248.

Prusiner, S.B. Novel proteinaceous infectious particles cause scrapie. *Science* 1982, 216, 136-144.

Prusiner, S. B., Groth, D., Serban, A., Stahl, N. & Gabizon, R. Attempts to restore scrapie prion infectivity after exposure to protein denaturants. *Proc Natl Acad Sci U S A* 1993, 90, 2793-2797.

Raeber, A.J., Klein, M.A., Frigg, R., Flechsig, E., Aguzzi, A. and Weissmann, C. PrP-dependent association of prions with splenic but not circulating lymphocytes of scrapie-infected mice. *Embo J* 1999, 18, 2702-2706.

Ramasamy, I., Law, M., Collins, S. & Brooke, F. Organ distribution of prion proteins in variant Creutzfeldt-Jakob disease. *Lancet Infect Dis* 2003, 3, 214-222.

Ridley, R. M. & Baker, H. F. The myth of maternal transmission of spongiform encephalopathy, *BMJ.* 1995, 311, 1071-1075, discussion 1075-1076.

Rubenstein, R., Carp, R.I. and Callahan, S.M. In vitro replication of scrapie agent in a neuronal model: infection of PC12 cells. *J Gen Virol* 1984, 65, 2191-2198.

Rutala, W. A. & Weber, D. J. Creutzfeldt-Jakob disease: recommendations for disinfection and sterilization. *Clin Infect Dis* 2001, 32, 1348-1356.

Saborio, G.P., Permanne, B. and Soto, C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* 2001, 411, 810-813.

Sachse C, Groschup MH, Warzok R, Greinacher A. Role of variant Creutzfeldt-Jakob disease for safety of treatment with blood components: screening of lymphatic tissue is a potential tool for risk assessment. *Eur J Haematol.* 2003, 70, 11-16.

Safar JG, Scott M, Monaghan J, Deering C, Didorenko S, Vergara J, Ball H, Legname G, Leclerc E, Solfrosi L, Serban H, Groth D, Burton DR, Prusiner SB, Williamson RA. Measuring prions causing bovine spongiform encephalopathy or chronic wasting disease by immunoassays and transgenic mice. *Nat Biotechnol.* 2002, 20, 1147-1150.

Safar JG, Geschwind MD, Deering C, Didorenko S, Sattavat M, Sanchez H, Serban A, Vey M, Baron H, Giles K, Miller BL, Dearmond SJ, Prusiner SB. Diagnosis of human prion disease. *Proc Natl Acad Sci U S A.* 2005, 102, 3501-3506.

Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature.* 2001, 411, 810-813.

Schmerr MJ, Jenny AL, Bulgin MS, Miller JM, Hamir AN, Cutlip RC, Goodwin KR. Use of capillary electrophoresis and fluorescent labeled peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy. *J Chromatogr A.* 1999, 853, 207-214.

SCMPMD 1998 Opinion on the Risk Quantification for CJD Transmission via Substances of Human Origin (adopted on 21 October 1998)
(http://europa.eu.int/comm/health/ph_risk/committees/scmp/documents/out12_en.pdf)

SCMPMD 2000a Opinion on Quality and Safety of Blood (adopted on 16 February 2000)
(http://europa.eu.int/comm/health/ph_risk/committees/scmp/documents/out29_en.pdf)

SCMPMD 2000b Opinion on update of the Opinion on the Risk Quantification for CJD Transmission via Substances of Human origin (adopted on 16 February 2000)
(http://europa.eu.int/comm/health/ph_risk/committees/scmp/documents/out28_en.pdf)

SCMPMD 2002 Opinion on the Safety of Human derived Products with regard to TSE's (adopted on 18 January 2002)
(http://europa.eu.int/comm/health/ph_risk/committees/scmp/documents/out40_en.pdf)

Scott, M.R., Peretz, D., Nguyen, H.O., Dearmond, S.J. and Prusiner, S.B. Transmission barriers for bovine, ovine, and human prions in transgenic mice. *J Virol* 2005, 79, 5259-5271.

Seeger H, Heikenwalker M, Zeller N, Kranich J, Schwarz P, Gaspert A, Seifert B, Miele G, Aguzzi A. Coincident scrapie infection and nephritis lead to urinary prion excretion. *Science* 2005, 310, 324-326.

Schulster L. M. Prion inactivation and medical instrument reprocessing: challenges facing healthcare facilities. *Infect Control Hosp Epidemiol* 2004, 25, 276-279.

Shaked GM, Shaked Y, Kariv-Inbal Z, Halimi M, Avraham I, Gabizon R. A protease-resistant prion protein isoform is present in urine of animals and humans affected with prion diseases. *J Biol Chem*. 2001, 276, 31479-31482.

Shiga Y, Miyazawa K, Sato S, Fukushima R, Shibuya S, Sato Y, Konno H, Doh-ura K, Mugikura S, Tamura H, Higano S, Takahashi S, Itoyama Y. Diffusion-weighted MRI abnormalities as an early diagnostic marker for Creutzfeldt-Jakob disease. *Neurology* 2004, 63, 443-449.

Simon, D. & Pauli, G. Krankenversorgung und Instrumentensterilisation bei CJK-Patienten und CJK-Verdachtsfällen. *Bundesgesundheitsblatt* 1998, 7, 279-285.

Soto, C. Protein misfolding and disease; protein refolding and therapy. *FEBS Lett* 2001, 498, 204-207.

Soto, C. Diagnosing prion diseases: needs, challenges and hopes. *Nat Rev Microbiol* 2004, 2, 809-819.

Soto, C. and Castilla, J. The controversial protein-only hypothesis of prion propagation. *Nat Med* 2004, 10 Suppl, S63-67.

Soto C, Anderes L, Suardi S, Cardone F, Castilla J, Frossard MJ, Peano S, Saa P, Limido L, Carbonatto M, Ironside J, Torres JM, Pocchiari M, Tagliavini F. Pre-symptomatic detection of prions by cyclic amplification of protein misfolding. *FEBS Lett*. 2005, 579, 638-642.

Sowemimo-Coker, S., Kascsak, R., Kim, A., Andrade, F., Pesci, S., Kascsak, R., Meeker, C., Carp, R., Brown, P. Removal of exogenous (spiked) and endogenous prion infectivity from red cells with a new prototype of leukoreduction filter. *Transfusion* 2005, 45, 1839-1822.

Taguchi Y, Mohri S, Ironside JW, Muramoto T, Kitamoto T. Humanized knock-in mice expressing chimeric prion protein showed varied susceptibility to different human prions. *Am J Pathol*. 2003, 163, 2585-2593.

Taguchi, F., Tamai, Y., Uchida, K., Kitajima, H., Kojima, H., Kawaguchi, T., Ohtani, Y. & Miura, S. Proposal for a procedure for complete inactivation of the Creutzfeldt-Jakob disease agent. *Arch Virol* 1991, 119, 297-301.

Tanaka, M., Chien, P., Yonekura, K. and Weissman, J.S. Mechanism of cross-species prion transmission: an infectious conformation compatible with two highly divergent yeast prion proteins. *Cell* 2005, 121, 49-62.

Tateishi, J., Tashima, T. & Kitamoto, T. Practical methods for chemical inactivation of Creutzfeldt-Jakob disease pathogen. *Microbiol Immunol* 1991, 35, 163-166.

Taylor, D. M., Fraser, H., McConnell, I, Brown, D. A., Brown, K. L., Lamza, K. A. & Smith, G. R. Decontamination studies with the agents of bovine spongiform encephalopathy and scrapie. *Arch Virol* 1994, 139, 313-326.

Taylor, D. M. Inactivation of prions by physical and chemical means. *J Hosp Infect* 1999, 43, S69-S76.

Taylor, D. M. Inactivation of transmissible degenerative encephalopathy agents: a review. *Vet J* 2000, 159, 10-17.

Taylor, D. M. Preventing accidental transmission of human transmissible spongiform encephalopathies. *Br Med Bull* 2003, 66, 293-303.

Taylor, D. M. Resistance of transmissible spongiform encephalopathy agents to decontamination. *Contrib Microbiol* 2004, 11, 136-145.

Terry, L. A., Marsh, S., Ryder, S. J., Hawkins, S. A., Wells, G. A. & Spencer, Y. I. Detection of disease-specific PrP in the distal ileum of cattle exposed orally to the agent of bovine spongiform encephalopathy, *Vet Rec.* 2003, 152, 387-392.

Turner, M. The risk of transmission of nvCJD by blood transfusion and the potential benefits of leucodepletion. *Transfus Sci* 1998, 19, 331-332.

Turner, M. Universal leucodepletion to reduce potential risk of transmission of new-variant Creutzfeldt-Jakob disease. *Br J Haematol* 2000, 110, 745-748.

UK Collaborative Group on HIV Drug Resistance. Estimating HIV-1 drug resistance in antiretroviral-treated individuals in the United Kingdom. *J Inf Dis* 2005, 192, 967-973.

UK Department of Health 2001: Risk assessment for the transmission of vCJD via surgical instruments: a modelling approach and numerical scenarios. www.dh.gov.uk published 16th March 2001

UK Department of Health 2005: The decontamination of surgical instruments in the NHS in England update report: a step change. www.dh.gov.uk published 15th June 2005

Valleron A-J et al Estimation of Epidemic Size and Incubation Time Based on Age Characteristics of vCJD in the United Kingdom. *Science* 2001, 294, 1726-1728

Vorberg I, Raines A, Story B, Priola SA. Susceptibility of common fibroblast cell lines to transmissible spongiform encephalopathy agents. *J Infect Dis.* 2004, 189, 431-439.

Wadsworth, J.D., Asante, E.A., Desbruslais, M., Linehan, J.M., Joiner, S., Gowland, I., Welch, J., Stone, L., Lloyd, S.E., Hill, A.F., Brandner, S. and Collinge, J.: Human prion protein with valine 129 prevents expression of variant CJD phenotype. *Science* 2004, 306, 1793-1796.

Wadsworth, J. D. F., Joiner, S., Hill, A. F., Campbell, T. A., Desbruslais, M., Luthert, P. J. & Collinge, J. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet* 2001, 358, 171-180.

-
- Wallis JP, Wells AW, Matthews JN, Chapman CE. Long term survival after blood transfusion: a population based study in the North of England. *Transfusion* 2004, 44, 1025-1032.
- Ward H, Cousens S, Everington D et al Vaccines and Creutzfeldt-Jakob disease (CJD). International Conference on Transmissible Spongiform Encephalopathies Book of Abstracts 2003, 57.
- Ward HJT, Cousens SN, Smith-Bathgate B et al Obstacles to conducting epidemiological research in the UK general population. *BMJ* 2004, 329, 277-279.
- Weissmann, C. and Aguzzi, A. Approaches to therapy of prion diseases. *Annu Rev Med* 2005, 56, 321-344.
- WHO, World Health Organization Infection Control Guidelines for Transmissible Spongiform Encephalopathies. Report of a WHO consultation, Geneva, Switzerland, 23-26 March 1999. WHO/CDS/CSR/APH/2000, 3.
- WHO 2002 The Revision of the Surveillance Case Definition for variant Creutzfeldt-Jakob Disease (vCJD) WHO 2002
- Wilesmith, J. W., Wells, G. A., Ryan, J. B., Gavier-Widen, D. & Simmons, M. M. A cohort study to examine maternally-associated risk factors for bovine spongiform encephalopathy, *Vet Rec.* 1997, 141, 239-243.
- Will, R.G., Ironside, J.W., Zeidler, M., Cousens, S.N., Estibeiro, K., Alperovitch, A., Poser, S., Pocchiari, M., Hofman, A. and Smith, P.G. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996, 347, 921-925.
- Will RG, Zeidler M, Stewart GE et al Diagnosis of new variant Creutzfeldt-Jakob disease. *Ann Neurol* 2000, 47, 575-582.
- Will RG, Knight RSG, Zeidler M et al Reporting of suspect new variant Creutzfeldt-Jakob disease. *Lancet* 1997, 349, 847.
- Will R *The Darlington Postgraduate Journal* 1989, 17, 35-42.
- Wilson S: Presentation at IBC conference on Transmissible Spongiform Encephalopathies, Reston, VA, December 2004.
- Wooding, F.B.P. and Flint, A.P.F.: Placentation. Chapter 4 (pp.233-460) in, G.E. Lamming, ed. Marshall's Physiology of Reproduction, 4th ed. Vol. 3, Part 1. Chapman & Hall, London, 1994
- Yakovleva O, Janiak A, McKenzie C, McShane L, Brown P, Cervenakova L. Effect of protease treatment on plasma infectivity in variant Creutzfeldt-Jakob disease mice. *Transfusion.* 2004, 44, 1700-1705.

Yang WC, Schmeerr MJ, Jackman R, Bodemer W, Yeung ES. Capillary electrophoresis-based noncompetitive immunoassay for the prion protein using fluorescein-labeled protein a as a fluorescent probe. *Anal Chem.* 2005, 77, 4489-4494.

Zeidler M, Sellar RJ, Collie DA, Knight R, Stewart G, Macleod MA, Ironside JW, Cousens S, Colchester AC, Hadley DM, Will RG. The pulvinar sign on magnetic resonance imaging in variant Creutzfeldt-Jakob disease. *Lancet.* 2000, 355, 1412-1418.

Zeidler M, Collie DA, Macleod MA, Sellar RJ, Knight R. FLAIR MRI in sporadic Creutzfeldt-Jakob disease. *Neurology.* 2001, 56, 282.

Zerr I, Bodemer M, Gefeller O, Otto M, Poser S, Wiltfang J, Windl O, Kretschmar HA, Weber T. Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt-Jakob disease. *Ann Neurol.* 1998, 43, 32-40.

Zetterberg H, Hammarin AL, Nilsson P, Andersson E, Lind B, Blennow K. New investigations in suspected Creutzfeldt-Jakob disease. Analysis of 14-3-3 protein and T-tau in cerebrospinal fluid for safer diagnosis. *Lakartidningen.* 2005, 102, 956-958, 960-961.

Zobeley, E., Flechsig, E., Cozzio, A., Enari, M. & Weissmann, C. Infectivity of scrapie prions bound to a stainless steel surface. *Mol Med* 1999, 5, 240-243.

Zwald et al: A novel blood based TSE diagnostic test, oral presentation First International Conference of the European Network of Excellence, Paris, May 2004.

7 ACKNOWLEDGEMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion. The members of the working group are:

SCENIHR members:

Dr. Wim De Jong (chair and rapporteur)

Prof. Jim Bridges

Prof. Philippe Hartemann

PD. Dr. med Dorothea Stahl

Dr. Mogens Thomsen

External experts:

Dr. Andrew Bailey³, Austrianova, Austria

Dr. Stephen Charnock-Jones, University of Cambridge, United Kingdom

Dr. Richard Knight, National CJD Surveillance Unit, United Kingdom

Prof. Dr. Hans Kretzschmar, Centre for Neuropathology and Prion Research, Germany

Dr. Philip Minor, National Institute for Biological Standards and Control (NIBSC), United Kingdom

Dr. Mikko Paunio, Ministry of Social Affairs and Health, Finland

Prof. Jean-Hugues Trouvin, AFSSAPS, Direction de l'évaluation des médicaments et des Produits biologiques, France

³ Declared Interest (see minutes of the SCENIHR plenary meeting of 28-29 September 2005 : http://europa.eu.int/comm/health/ph_risk/committees/04_scenihhr/docs/scenihhr_mi_007.pdf).

GLOSSARY

CJD Creutzfeldt-Jakob Disease

BSE Bovine spongiform encephalopathy

TSE Transmissible spongiform encephalopathy

sCJD Sporadic CJD, the commonest form of human prion disease

vCJD Variant CJD, the human prion disease thought to be due to transmission of BSE from cattle to man

Prion Infectious agent of TSE

PrP (prion protein) A protein of uncertain function that exists in a normal form (in health) and also an abnormal form (in prion diseases)

PrP^C The normal prion protein (c=cellular)

PrP^{Sc} The abnormal form of the prion protein associated with prion diseases (Sc= scrapie)

PrP^{Res} The protease resistant core of PrP^{Sc} that remains after treatment with proteases such as proteinase K. Immunocytochemical detection methods generally detect PrP^{Res}, rather than PrP^{Sc}, but the presence of PrP^{Res} is obviously a marker of the presence of PrP^{Sc},

NCJDSU The UK National CJD Surveillance Unit