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CHMP position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products

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This CHMP position statement replaces the CHMP position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products (EMA/CHMP/BWP/303353/2010).

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CHMP position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products

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This is the third revision of the CHMP Position Statement on “Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products”. It was originally published in February 2003 (EMEA/CPMP/BWP/2879/02), replacing the CPMP Position Statement on “New variant CJD and plasma-derived medicinal products” (CPMP/201/98) from February 1998. EMEA/CPMP/BWP/2879/02 was revised in June 2004 (EMEA/CPMP/BWP/2879/02 rev 1.) and in June 2011 (EMA/CHMP/BWP/303353/2010).

Summary

The purpose of this revision is to account for scientific developments since the last revision in 2011. The scientific information has been updated. However, there is no change in the regulatory recommendations regarding exclusion, potential testing of donors, the need to evaluate the prion reduction capacity of the manufacturing process and batch recalls.

Emergence of variant CJD (vCJD) was noted in UK in 1996. Although the number of cases has been in decline in the UK since 2001, isolated cases of vCJD are still being identified since 2011 in the UK as in other countries and there is still uncertainty about the future number of cases. Studies on appendix tissues from the UK indicate a potential high prevalence (about 1:2000 in the people examined) of infected persons and this is of concern considering potential human-to human transmissions. However, there are some uncertainties about the significance of the results and their correlation to the BSE epizootic. The recent appendix tissue studies from 2013 have not produced a clear answer to the question of whether abnormal prion in the British population is limited to those exposed to the BSE epizootic. Residence in the UK has been a recognised risk factor for vCJD and there is no change to the recommendations for country-based donor exclusion. It is recommended that donors who have spent a cumulative period of 1 year or more in the UK between the beginning of 1980 and the end of 1996 are excluded from donating blood/plasma for fractionation. In addition, there is no change in the recommendations for precautionary recall of batches of plasma-derived medicinal products where a donor to a plasma pool subsequently develops vCJD.

Originally, a wider distribution and higher level of infectivity or abnormal prion protein in human peripheral tissues, including the lymphoreticular system was found in patients with vCJD compared with sporadic CJD. However, recent studies indicate that the prion-levels in peripheral tissue may vary in individual vCJD patients, and some cases of vCJD and sporadic CJD have been found with equal amounts of abnormal prion protein or seeding activity in peripheral tissue. Moreover, infectivity was detected in the plasma of two in four sCJD infected patients tested by bioassay in human PrP transgenic mice. These findings raise a concern that sCJD could be present in plasma from donors incubating sCJD. However, a direct link between sCJD cases and treatment with plasma-derived medicinal products or blood has not been established and there is still no firm epidemiological evidence that sporadic, genetic or iatrogenic forms of human TSEs have been transmitted from person to person through exposure to blood, plasma products or urinary-derived medicinal products. Therefore, at this stage, the recommendation not to recall batches of plasma-derived medicinal products where a donor is later confirmed as having sporadic, genetic or iatrogenic CJD is maintained, provided the manufacturer has demonstrated using appropriate methodology, that the process includes steps which significantly minimize the risk of prion contamination of the final product.

No recommendation for testing of donors was made in the former version of this position statement and this policy is maintained. Significant progress has been made in developing sensitive in vitro assays for prion detection in blood and some methods offer the possibility for screening of blood donors. However, these tests have not yet been completely validated according to the current

requirements of specificity as defined in the Common Technical Specifications for *in vitro* diagnostics. Comparison and validation of potential screening tests has been considerably confounded by the paucity of blood samples from confirmed cases of clinical prion disease and very limited samples available from asymptomatic individuals who later developed vCJD.

No requirement for leucoreduction of plasma was made in the former version of this position statement and this policy is maintained. Experience in TSE animal models indicates that leucodepletion reduces the risk for transmission by blood transfusion. However with respect to plasma-derived medicinal products, the same models indicate no clear evidence that leucoreduction of plasma significantly reduces the risk of prion disease transmission.

Taking account of the available data concerning potential contamination of blood donations with vCJD or CJD agents, assuring an adequate prion reduction capacity of the manufacturing process is considered crucial for the TSE safety of plasma-derived medicinal products. Available data indicate that the manufacturing processes for plasma-derived medicinal products would reduce TSE-infectivity if it were present in human plasma. Manufacturers have been required to estimate the potential of their specific manufacturing processes to reduce infectivity using a step-wise approach and it has been recommended that manufacturers consult the relevant competent authorities at each of the milestones in this estimation. This policy is maintained.

In support of this recommendation, CHMP and BWP, with the involvement of external experts, developed guidance on how to investigate manufacturing processes with regard to vCJD risk. Since publication of this Guideline in 2004, the methods for prion detection, the knowledge about infectivity in prion area in general and, prion infectivty in the blood have significantly evolved. Experimental studies highlighted the fact that prion removal capacity may vary directly according to the spiking preparation (dispersion and TSE agents strains) particularly for steps based on retention mechanisms.

There is no change to the recommendations for urine-derived medicinal products. Low levels of infectious TSE agents were first detected in the urine of scrapie-infected rodents and in the urine of deer with chronic wasting disease raising concerns about the possibility of infectious agents in human urine. Recent investigations on human urine have produced diverse results. While one study failed to detect infectivity by bioassay in the urine from 3 sCJD patients using sensitive assays, abnormal prion protein has recently been detected in urine from 8 out of 20 sCJD patients, 1 of 2 iatrogenic cases as well as in 1 of 13 vCJD patient urine samples using a highly sensitive immunoassay. There is still no epidemiological evidence of CJD or vCJD transmission by urine-derived medicinal products and prion reduction capacity of the manufacturing processes has been indicated. Therefore, the recommendation to apply exclusion criteria for selection of a urine donor panel from the former version of the position statement is maintained. The same exclusion criteria should be applied with respect to sJD and vCJD as used for blood/plasma donors providing starting material for the manufacture of plasma-derived medicinal products and manufacturers should follow up these criteria at defined intervals.
Manufacturers of urine-derived medicinal products are recommended to estimate the potential of their manufacturing processes to reduce infectivity by following a similar general stepwise approach as recommended for plasma-derived medicinal products.

1. Introduction

Creutzfeldt-Jakob disease (CJD) is a rare neurodegenerative disease belonging to the group of human Transmissible Spongiform Encephalopathies (TSEs) or prion diseases. The mortality rate of TSEs ranges approximately from 1.5 to 2 persons per million population per year. TSEs can occur sporadically (sporadic CJD (sCJD), variably proteinase sensitive prionopathy and sporadic fatal insomnia), be associated with mutations of the prion protein gene (genetic TSEs (gTSE)), or result from medical exposure to infectious material (iatrogenic CJD (iCJD)). In 1996, a variant form of CJD (vCJD) was identified[1]. There is strong evidence that vCJD is caused by the agent responsible for bovine spongiform encephalopathy (BSE) in cattle[2,3,4]. The most likely hypothesis is that vCJD has occurred through exposure to BSE contaminated food.

Human TSEs, including in particular vCJD, were addressed in expert meetings/workshops at the EMA in January 1998, January 1999, December 1999, May 2000, and December 2000[5c, 5d, 5e]. A CPMP Position Statement on variant CJD and plasma-derived medicinal products was issued in February 1998 [5b] and the outcome of the subsequent meetings was published on the EMA website. An EMA Expert Workshop on Human TSEs and Medicinal Products was held on 19-21 June 2002. This provided the scientific basis for a new CPMP Position Statement issued in 2003[5b]. A further EMA Expert Workshop was held in January 2004 to review the current state of knowledge of vCJD, in the light of a report of a possible human transmission by blood transfusion[6]. In addition, the Workshop discussed the CPMP Discussion document on the investigation of manufacturing processes with respect to vCJD[5a]. In October 2005, a follow-up workshop was held to discuss the number of vCJD cases reported in France and other European countries and the potential effect of additional donor exclusion measures. Urine-derived medicinal products were specifically discussed at an EMA expert workshop in July 2007[5g] after publication of experiments indicating transmission of infection via urine using a hamster model. A revised version of the CPMP position statement was published in 2011[5h].

Blood and blood components for transfusion are outside the scope of this Position Statement. Recommendations on the suitability of blood and plasma donors and the screening of donated blood in the European Community were described in Council Recommendation 98/463/EC[7c]. European legislation on human blood and blood components entered into force on 8 February 2003[7a]. Under this legislation, a Commission Directive on certain technical requirements for blood and blood components, including eligibility criteria for donors, entered into force in April 2004[7b].

In addition, Council of Europe Recommendation No. R (95) 16 contains a technical appendix on the use, preparation and quality assurance of blood components and details the current requirements for donors[8].

In December 2003, following the announcement of a possible case of vCJD transmission by blood transfusion, Commissioner Byrne made a statement highlighting EU activities in the area of vCJD and announcing a meeting of the Working Group of the Blood Regulatory Committee to consider the latest information available from the UK[7d]. The meeting took place in January 2004 and a summary statement was produced[7e].

The Scientific Steering Committee (SSC), the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) of the European Commission have published a number of opinions relating to TSEs, which are of relevance to blood and blood components for transfusion, as well as to plasma-derived medicinal products[9]. WHO Guidelines on TSEs are also of relevance to both blood components for transfusion and plasma-derived medicinal products as well as urine-derived medicinal products[10]. The Council of Europe has made recommendations for blood and blood components for transfusion[11].

The purpose of this revision is to update the position statement according to the recent scientific developments since the last revision in 2011. This included developments in detection techniques, epidemiological studies/findings, studies on the tissue distribution of (v)CJD agent, and a study indicating blood from some patients with sCJD might be infectious.

2. Human TSEs current status

2.1. Sporadic, genetic and iatrogenic forms of human TSEs

There is no firm evidence that sporadic, genetic or iatrogenic forms of human TSEs have been transmitted from person to person through exposure to plasma products or urinary derived medicinal products. Systematic surveillance for CJD of all types has been undertaken in a number of countries, including a collaborative study in the EU since 1993,[12,13] and no case of sporadic, genetic or iatrogenic CJD has been causally linked to prior treatment with plasma products. Two plasma product recipients in the UK have been diagnosed with sporadic CJD[14]. Both were aged 64 years and had been exposed to UK sourced plasma products, one for the treatment of von Willebrand's disease and the other Haemophilia B. Both patients had received, in addition to plasma products, multiple blood transfusions, but a partial look-back study performed for one patient has not identified a donor with either sCJD or vCJD. A causal link between the treatment with plasma products and the development of sCJD has not yet been established and there is a possibility that both cases may reflect a chance event in the context of systematic surveillance of CJD in large populations[14].

Cases of sporadic CJD with a history of drug treatment for infertility have not been identified but there is uncertainty about the validity of this observation (see the report of the 2007 EMA expert meeting for further details) [5g]. The strength of epidemiological evidence excluding transmission by urinary derived medicinal products is less secure than in other forms of human prion disease.

Variably proteinase sensitive prionopathy (VPSPr) is an idiopathic disorder with patients having no known risk factors for acquired or genetic prion disease. Recent laboratory studies have indicated limited transmissibility to transgenic mice, with transmission characteristics distinct from sporadic CJD[15, 16].

2.2. Variant CJD

The official UK figures for vCJD at the end of May 2016 were a total of 178 definite or probable vCJD cases[17]. (One case diagnosed in Hong Kong was classified as a UK case and is included in the UK figures.) Outside of the UK, there have been 27 cases in France[18], 5 in Spain, 4 in the Republic of Ireland and the USA, 3 in the Netherlands and Italy, 2 in Portugal and Canada and single cases in Saudi Arabia, Japan and Taiwan. Some of these cases, 2 of the Irish cases, 2 of the US cases, 1 French case, 1 Canadian case and the Taiwanese case had spent more than 6 months in the UK during the period 1980-1996 and were probably infected while in the UK[19]. The third and fourth US cases and the second Canadian case have been reported as most likely infected when living outside the USA. The possibility of cases occurring in other countries cannot be excluded.

Two cases of vCJD identified in Spain occurred in the same family. No family links have been reported in any other vCJD cases to date.

All definite and probable cases genotyped had been Met-Met homozygotes at codon 129 of the prion protein (PrP) gene[20]. In 2016, a definite case of variant CJD was reported in the UK with a heterozygous codon 129 genotype, raising the possibility of a further outbreak of cases in this genetic background[21].

Analysis of the figures indicates that vCJD incidence in the UK and internationally is in decline. However, single cases of vCJD have been identified in the UK and Italy[22] in 2016 and there may be a long tail or more than one peak to the epidemic.

A UK study screening specimens from surgically removed appendices and tonsils for accumulation of disease related prion protein in the lymphoreticular system, has been carried out in order to try and obtain some estimation of the number of people that might be incubating vCJD in the UK[23]. Three positive appendix specimens have been found as a result of the screening of 12,674 appendix and tonsil specimens. However, the pattern of lymphoreticular accumulation in two of these samples was dissimilar from that seen in known cases of vCJD, raising the possibility that they may be false positives. With respect to this possibility, the authors comment that although it is uncertain whether immunohistochemical accumulation of disease-related prion protein in the lymphoreticular system is specific for vCJD, it has not been described in any other disease, including other forms of human prion disease or a range of inflammatory and infective conditions. Subsequent genetic analysis of residual tissue samples from these 2 cases found that both were valine homozygotes at codon 129 in the prion protein gene[24]. This finding might account for the immunohistochemical features in these cases; none of the patients who have developed vCJD and have undergone a comparable genetic analysis have been valine homozygotes at codon 129 in the prion protein gene.

Statistical analysis on this finding of 3 positive specimens gives the following estimations of numbers who may be incubating vCJD in the UK:

237 infections per million population (95% confidence interval (CI): 49-692 per million)

These estimations are higher than predictions from modelling of the clinical data (upper 95% confidence interval of 540 future cases) [25]. It is not known whether those incubating vCJD will eventually develop clinical disease. However, estimates of numbers possibly incubating are important with respect to any potential for secondary transmission (e.g. by blood donation, surgical instruments) while individuals are in the incubation phase. It should be noted that plasma-derived medicinal products have not been manufactured from donations collected in the UK since 1998.

A larger study of an archive of tonsil tissue from 63,007 people of all ages removed during routine tonsillectomies has been published[26]. In this study, 12,753 samples were from the 1961- 1985 birth cohort in which most cases of vCJD have arisen and 19,808 were from the 1986-1995 birth cohort, that may also have been orally exposed to bovine spongiform encephalopathy. None of the samples were unequivocally reactive to two enzyme immunoassays and none of the initial reactive samples were positive for disease-related PrP by immunohistochemistry or immunoblotting. The estimated 95% confidence interval for the prevalence of disease-related PrP in the 1961-1995 birth cohort was 0-113 per million and in the 1961-1985 birth cohort, 0-289 per million. These estimates are lower than the previous study of appendix tissue, but are still consistent with that study. To confirm the reliability of the results from the 1961-85 birth cohort, 10,075 of these samples were investigated further by immunohistochemistry on paraffin-embedded tonsil tissues using two anti-PrP monoclonal antibodies[27]. One specimen showed a single positive follicle with both antibodies on 2 slides from adjacent sections, although the earlier enzyme immunoassays and immunoblotting studies on the frozen tissue samples from this case were negative[26, 27]. If this case is now accepted as positive for abnormal PrP (since the findings were similar to those of the three positive cases in the earlier study of Hilton et al in 2004[23]), it gives a prevalence of disease-related PrP in the UK population of 109 per million, with a 95% confidence interval of 3-608 per million, which is not statistically significantly different (exact p = 0.63) from the population prevalence based on the finding of 3 positives in the Hilton et al study[23, 27] . If the case is not accepted as a positive, this gives a prevalence of 0 out of 9160, with a 95% confidence interval of 0-403 per million for the 1961-85 cohort, which is also not significantly different (exact p = 0.25) from the findings of the Hilton et al study[23]. A more recent study from 2013 included 32,441 appendix samples and 16 were positive leading to an estimated prevalence in the UK population of 492 cases per million, with wide confidence intervals. All three PRNP codon 129 genotypes were identified among the 16 positive samples with a relative excess of the VV genotype[28].

The results of further UK prevalence studies of appendix tissues derived from individuals either before the BSE epidemic or after the introduction of further measures to restrict BSE in the food chain have recently been published[29]. Positives were found in both groups and the report concluded: "the Appendix-III survey data have not produced a clear answer to the question of whether abnormal prions detected by immunohistochemistry n the British population is limited to those exposed to the BSE epizootic, and various interpretations are possible[29].

3. Human tissue distribution of infectivity/abnormal prion protein.

Tissue distribution has been investigated by detection of the abnormal prion protein (PrP^{TSE}) or by infectivity assays. Detection of PrP^{TSE} in tissues has often been associated with infectivity, however it should be noted that infectivity can be present without detection of PrP^{TSE},[30] or PrP^{TSE} be present in the absence of infectivity[31] and that the relation between the amount of PrP^{TSE} and infectivity is strain dependent[32]. The reason for this finding is not known but may be related to limitations of assay methods for PrP^{TSE} or different ratios between protease-resistant and protease-sensitive PrP^{TSE} isoforms[33,34]. It is thus recommended that any study on tissue or fluid distribution of the abnormal prion protein be confirmed with an infectivity assay.

A wider distribution and higher level of PrP^{TSE} in human peripheral tissues, including the lymphoreticular system, has been found in vCJD compared with sporadic CJD[35, 36, 37]. The magnitude of PrP^{TSE} may vary however, as a recent case of vCJD reported extremely low levels of PrP^{TSE} in lymphoreticular tissues[38] and recent data showed equal amounts of PrP^{TSE} in vCJD and sporadic CJD[39]. Limited data from infectivity assays of vCJD tissues are consistent with the PrP^{TSE} findings[40]. In clinical vCJD cases, high titres of infectivity are found in the brain and spinal cord and lower levels in spleen and tonsil[40, 41]. Infectious vCJD infectivity was detected in spleen but not in the brain from an individual with the methionine-valine (MV) genotype[42]. While PrP^{TSE} and infectivity are occasionally found in the spleen of sporadic CJD, the levels of PrP^{TSE} are lower than in vCJD. PrP^{TSE} accumulations have been observed in muscles of some patients with both sporadic and variant CJD[43].

One study reported that the distribution of PrP^{TSE} in iCJD is more similar to sCJD than vCJD[36]. Data are lacking for gCJD.

4. Infectivity in blood and transmissibility via blood

4.1. Animal blood

In early 2000, most of the knowledge relating to the presence of prion infectivity in blood relied on information from rodent prion disease models. In these experimental systems, prion infectivity titres were reported to vary between 1 and 10 ID_{50}/mL of blood during the asymptomatic phase and up to 100 ID_{50}/mL during the clinical phase of the disease[44, 45]. In these bioassays, infectious prion titres were measured bybioassay perfoming intracerebral inoculation of blood, or blood fractions from the same animal species to indicator animals, (i.e. autologous combinations of inocula and animal bioassay). The observed infectious prion titres were equivalent to the level of infectivity found in 10^{-6} - 10^{-8} g of brain tissue from animals at the terminal stage of prion disease. It was found that approximately 40% of the prion infectivity was associated with the buffy coat fraction, the remainder was found principally in plasma[46, 47]. Importantly, buffy coat-associated prion infectivity was reportedly washed off these cells by rinsing with PBS.[48] Platelets were shown to have little, if any, prion infectivity[49].

Subsequent experiments in other animal species, whereby donor blood material was assessed by bioassay in a host via intracerebral inoculation, have investigated the distribution of prion infectivity in various blood fractions. Infectivity has also been detected in buffy coat of a prosimian microcebe[50] and in whole blood of a macaque experimentally infected with a macaque-adapted BSE strain[51] and in red blood cells of two macaques experimentally infected with a macaque-adapted vCJD strain[51]. In sheep, naturally or experimentally infected with scrapie, infectious prion titres in whole blood were similar to those observed in rodents (<35 ID_{50}/mL) when measured by bioassay in reporter ovine PrP transgenic mice[52]. Prion infectivity was detected in plasma from scrapie-infected sheep, but at a lower proportion to that found in the blood of prion-diseased mice and hamster models[53]. Moreover, a substantial level of prion infectivity was detected in sheep platelets and infectivity associated with leukocytes was not reduced by washing of these cells[52]. Similar observations were reported in deer naturally infected with chronic wasting disease[54].

The intracerebral inoculation of prions is unlikely to recapitulate the cellular and molecular events that occur as a consequence of prion infection by blood transfusion, a process that involves the administration of large numbers of viable cells and/or a large volume of material intravenously injected into the recipient.

The relative similarity in size between sheep and humans allows the transfusion of ruminant blood volumes that are relevant to human medicine. In addition, the pathogenesis of vCJD mirrors features similar to natural classical scrapie in sheep, for example the presence of prions in peripheral lymphoid tissue of affected individuals. Consequently, sheep prion disease models were considered to be relevant models for the assessment of the risks associated with vCJD blood-borne transmission[55].

In early 2000, transfusion of whole blood collected from asymptomatic sheep infected with either natural scrapie or experimental BSE resulted in prion transmission to recipient sheep[56, 57].

Using the sheep transfusion model, it was also confirmed that RBCs, plasma, platelets and buffy coat prepared by similar protocols to those used in transfusion medicine can transmit prion disease[58, 59]. In two different sheep scrapie models, the transfusion of 200 mL of whole blood collected during the early preclinical phase of the condition (3 months post infection) was able to transmit the disease with 100% efficacy[52, 58]. However, in two other sheep prion disease studies, the efficacy to transmission after transfusion of ca. 400 mL of whole blood at a late stage of incubation of the disease was limited to 19%[57] or 40%[59] respectively[57]. Features of the different sheep prion disease models, such as age of animals used, PrP genotype of the animals and/or the prion strain used for inoculation could contribute to an explanation for the discrepancies between the results of these different models. However, these sheep blood transfusion studies collectively suggest that in a proportion of prion-infected blood donors, the level of prionemia may be insufficient to allow prion disease transmission by blood transfusion[60].

Transfusion experiments carried out in a sheep scrapie model demonstrated that the transfusion of 200 µL of prion-infected whole blood has an apparent 100% efficacy for disease transmission and that 100µL blood transfusion is still sufficient to transmit the disease in a proportion of the recipients[53]. These experiments also indicated that, despite their apparent low infectious titre, the intravenous administration of white blood cells (WBC) resulted in efficient disease transmission. The intravenous administration of 10^{5} WBCs were sufficient to cause scrapie in recipient sheep. Cell-sorted CD45R+ (predominantly B lymphocytes), CD4+/CD8+ (T lymphocytes) and CD14+ (monocytes/macrophages) blood cell sub-populations were all shown to contain prion infectivity by bioassays in ovine PrP transgenic mice[61]. However, while the intravenous administration of 10^6 CD45+ or CD4/8+ living cells were able to transmit the disease, similar numbers of CD14+ failed to infect any of their recipients. These indicated that blood cell populations display different abilities to transmit TSE by the transfusion route.

PrP^{TSE} has been detected in blood components of TSE-infected animals by different techniques. In TSE-infected rodents, PrP^{TSE} positivity has been reported in buffy coat[62] and plasma exosomes[63] by Protein Misfolding Cyclic Amplification PMCA), whole blood by Real-Time Quaking induced Conversion Assay (RT-QuIC) [64], and by steel-binding assay[65] and in plasma exosomes by standard Wetsern Blot (WB) procedures. [66] Abnormal PrP conformers can be detected throughout the whole incubation period of the disease[65].

In pre-clinical and clinical scrapie-infected or BSE infected sheep, PrP^{TSE} positivity has been reported in platelets and WBC by PMCA or infectivity assay[52,67,68] or surface-FIDA (fluorescence intensity distribution analysis) [68]. In chronic wasting disease (CWD)-infected deer, whole blood resulted PrP^{TSE} positive by RT-QuIC in both animals in both, pre-clinical and clinical phases of disease[63]. Plasma, buffy coat and WBC tested PrP^{TSE} positive by PMCA in vCJD-infected macaques during the earliest pre-clinical and clinical phases of disease[66,70, 71] .

4.2. Human blood

The tracing of recipients of blood transfusion from UK donors who have subsequently developed vCJD (the Transfusion Medicine Epidemiology Medicine Review, TMER study) has revealed four instances of secondary transmission[72]. These individuals had received transfusion of non-leucodepleted red cells from donors who were clinically healthy at the time of donation but subsequently (17–40 months later) developed variant CJD. Three of the four patients developed disease after incubation periods ranging from 6.5 to 8.5 years; the fourth died of an illness unrelated to prion disease 5 years after transfusion. This asymptomatic prion-infected patient was heterozygous (methionine/valine) at codon 129 of the *PRNP* gene. However the spleen and lymph nodes tested positive[73] and the prion agent was experimentally transmitted from brain and spleen to humanised transgenic mice[74]. Taken together, these instances are strong evidence that vCJD is transmissible through blood transfusion.

In 2010, another presumed case of asymptomatic vCJD infection was identified in an elderly haemophilia patient who was heterozygous at codon 129 in the prion protein gene[75]. The patient, who died of unrelated pathology, had received large quantities of UK-sourced fractionated plasma products (i.e. FVIII), including some units derived from plasma pools which contained plasma from a donor who later developed variant CJD. This patient was identified through an intensive search for PrP^{TSE} positivity in a range of post-mortem tissues, although only 1 of 24 samples taken from the spleen tested positive. Whether someone with this limited distribution of PrP^{TSE} would be infectious is unknown, but from a public health perspective, this patient represents a warning that some plasma-derived products might contain residual prion infectivity.

The surveillance described above emphasises the importance of the TMER study for identifying the risk of blood transfusion in transmitting vCJD. Moreover, national databases of blood donors and the maintenance of traceability from donor to recipient and vice versa are essential to establish whether a vCJD case has been a blood donor (UK experience has shown that questioning of family members is unreliable for establishing whether a patient has been a blood donor). Traceability is a specific requirement in Article 14 of Directive 2002/98/EC.

In a conventional mouse model (RIII mice), infectivity was not detected in the blood of two vCJD cases but the bioassay had limited sensitivity to detect infectivity in peripheral tissues such as tonsil or spleen[40]. Bioassays carried out in PrP transgenic mice using blood harvested post mortem from a vCJD-affected patient have shown the presence of prion infectivity in red blood cells, plasma and white blood cells[74]. The blood fractions used in these assays had been prepared in 2000 using laboratory-scale haematological protocols but did not include leukoreduction. The infectious titre of whole blood in the bioassayed vCJD sample was estimated to be approximately 4.45 ID_{50}/mL, which is 10^{-6} - 10^{-7} lower than that found in one gram of brain from a vCJD-affected patient at terminal stage of disease. Importantly, the leukocyte-associated prion infectivity of the vCJD blood sample could not be reduced by rinsing of the cells, similar to that found in ruminant animal models. These data support the view that prion infectivity levels in the blood of vCJD patients and different animal prion disease models are similar. They also demonstrated that interspecies variations exist with regards to distribution of infectivity in different blood fractions.

Look-back studies in the UK[77] and USA[78] have not revealed any possible case of sporadic CJD linked to blood transfusion. However, current data are too scant to unequivocally exclude the possibility that such an event could occur in a small number of cases with a long (10 or more years) incubation period.

A review of transmission studies to detect infectivity in the blood of humans with sporadic and iatrogenic CJD shows that experimental transmissions in animal models have occasionally been reported in some studies[79-83] but not in others.[84] It is possible that PrP^{TSE} is present at low levels in the blood of clinically affected cases of sCJD. Recently, intracerebral inoculation of plasma from two of four sporadic CJD patients transmitted disease into human PrP transgenic mice. The relative infectivitity between brain and plasma was the same in sCJD and vCJD[76]. Data are lacking for gCJD and iCJD.

PrP^{TSE} was detected in WBC of a single vCJD patient, in buffy coat of 2 out of 3 vCJD patients by PMCA[67] and in the blood of 15 out of 21 vCJD cases by steel binding assay[85].

For the purpose of risk assessments, it is recommended that, as a worst case assumption, a relative efficiency of the intravenous and intracerebral routes of 1:1 should be used.[86] This is because the accumulated information now available from animal studies indicates that the intravenous route can be an efficient route of transmission and in certain cases can give a transmission rate and/or an incubation period similar to the intracerebral route (see also 4.1).

5. Detection techniques

A donor screening test could provide an improved level of safety. The development of blood tests for vCJD remains a strategic priority but has suffered from declining efforts from an assumption that the technical challenges are insurmountable, an assumption that has seen commercial bodies abandoning test development[87].

As unique biological agents mammalian prions provide many research challenges. Not least is the ability to detect and quantify their presence in tissue and fluid samples. The severity of pathology associated with clinical prion disease suggests markers for infection and disease progression other than abnormal PrP may exist. Numerous studies by groups worldwide[88-94] have applied ’omics’ approaches to discovery of alternative markers. Several differential changes between baseline and disease states have been demonstrated but they lack the specificity required for use in screening or diagnostic tests[8]. In contrast the deposition of $PrP^{TSE}$is the archetypal marker of prion disease. Whilst moderately abundant in the tissues of the central nervous system and lymphoreticular tissue in cases of vCJD, the concentration of infectivity, and by inference PrP^{TSE}, is very low in blood and cerebrospinal fluid (CSF).

This situation is further complicated by the large background excess of normal non-pathogenic cellular protein PrP^C associated with the cellular compartment of blood.

A conceptually obvious approach to overcome the problems of abnormal PrP detection is to exploit the innate propensity of amyloid to self-propagate. This approach has been developed in a variety of formats of which two: QuIC[95] and PMCA[96] have seen widespread adoption and development for research. The adoption of QuIC for the diagnosis of sporadic CJD using CSF samples has been successful with excellent although not perfect performance characteristics[97]. However, adaptation of this methodology to the testing of blood samples has yet to be convincingly demonstrated. PMCA has been shown to be capable of detecting vCJD infection in blood[67] and urine[98]. However, the specificity of such an assay is generally considered to be a frailty of this approach. Two recent studies using PCMA showed 100% sensitivity at identification of blood samples from 14 [99]or 18 [100] clinical vCJD cases and indicated specificities in the range as required in the EU Common technical specification (CTS) [101]. However, full validation according to the CTS has not yet been performed.

As an alternative to amplification strategies, enrichment by capture using stainless steel beads has allowed the direct immunoassay of captured material, detecting a signal in blood in 71% (15 out of 21) of vCJD patients[85] whilst being highly specific[102].

It is clear that there several methods in research and development that offer possibilities for routine screening and confirmatory assays but they have not yet completely demonstrated the current requirements of sensitivity and specificity as defined in the Common Technical Specifications.[101] Comparison and validation of potential screening tests is considerably confounded by the paucity of blood samples from confirmed cases of clinical prion disease and very limited samples available from asymptomatic individuals who later developed vCJD.

6. Leucoreduction and specific prion affinity filters

Leucodepletion was introduced in the UK in 1999 as a precautionary measure in transfusion medicine to reduce the risk of iatrogenic transmissions of vCJD. The rationale was based upon evidence to suggest the majority of infectivity in whole blood is associated with ‘buffy coat’ fractions or mononuclear cells.

Despite widespread exposure to potentially contaminated blood transfusions in the UK, Europe and the wider world, confirmed cases of vCJD resulting from exposure to contaminated blood or blood products are small[75, 103, 104]. This may be partly attributed to the rapid introduction of leucodepletion.

In addition to the potential protection afforded against vCJD transmission, leucodepletion has other benefits in transfusion medicine including reduced risk of HLA alloimmunisation with the potential for refractoriness to platelet transfusion, reduction in specific viral transmission risk, the disappearance of transfusion-related graft versus host disease and a significant decrease in cases of post-transfusion purpura[105].

Experience from animal models indicates that leucodepletion is highly effective for prion safety of blood transfusion. Taken together with the additional benefit of improved red blood cell and platelet quality it is clear that leucodepletion is advantageous and is likely to remain in place irrespective of prion transmission risk assessments.

The Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) opinion on leucoreduction[9a, 9b] for blood and blood components for transfusion stated that it might be a precautionary step to remove white cells as completely as possible. For plasma for fractionation the opinion stated the following:

'Taken together, there is no compelling scientific evidence to date for the introduction of leucoreduction of plasma for fractionation, or other methods aiming at removal of cells and debris, as a precaution against vCJD transmission. The question should be further explored by suitable experiments.'

Results reported at the 2002 EMEA Workshop, suggested that leucodepletion does not cause fragmentation of cells and lysis. Results of a comprehensive study involving a number of different filters and procedures indicate that leucodepletion is not detrimental in terms of the generation of microvesicles or the release of prion proteins[106].

Specific affinity ligands that bind prion proteins have been evaluated for their ability to further reduce TSE infectivity present in blood and plasma. Exogenous spiking experiments have suggested prion-specific filters could be effective. However, such studies do not provide a good model of infectivity distribution in blood and endogenous validation experiments have indicated the efficiency of prion removal is not very effective with an overall logarithmic reduction value of only 1.22 from infectivity assay in a hamster model[107].

In October 2009, the UK Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) stated that there was sufficient evidence that a specific affinity ligand filter reduces infectivity and recommended the use of prion filtration of red cell components administered to children born since 1 January 1996. This recommendation was subject to the satisfactory completion of the PRISM clinical trial to evaluate the safety of prion filtered red blood cells[108].

Despite the fact that PRISM has indicated that the use of commercially available prion filters was not detrimental to the quality or safety of filtered red blood cells, the use of prion reduction filters has not been recommended. This decision has been based upon the need for independent studies to replicate the findings of these studies since the studies involved the filter manufacturers.

Two such studies were commissioned and finally published in 2015. One, using a hamster model of prion disease concluded that the majority of infectivity was removed using leucodepletion alone, with filtration using the CE marked prion filter P-Capt (MacoPharma, France) achieving a further reduction in titre of around only 0.2 ID/ml.[109] The study was compromised by the low dynamic range afforded by the input material, however, residual infectivity was still present following combined leucodepletion and prion filtration and the low concentration was not statistically different from the residual levels following leucodepletion alone. The second study involved transfusion from scrapie-infected sheep and recipients received either leucodepleted blood or sequentially leucodepleted and P-Capt prion filtered blood[110]. This study also concluded that there was no significant difference in residual titre following only leucodepletion or leucodepletion and prion filtration. However, this study was also flawed in that all transfused materials were leucodepleted and the genotypes of recipient sheep were not disclosed so the possibility of resistant genotypes being transfused cannot be excluded. As a result, despite the large number of sheep used in the study, only two recipient animals were considered transfusion positive; one having received leucodepleted blood and the other receiving blood following combined leucodepletion and prion filtration. In conclusion, both studies failed to demonstrate a clear effect of the prion affinity filters.

The prion binding capacity of another affinity ligand chromatography step has been investigated in the processing of a plasma medicinal product using hamster brain derived spiking material.[111, 112] These data require further evaluation before conclusions can be drawn on possible efficacy.

7. Manufacturing processes for plasma-derived medicinal products

Despite the fact that there is no firm evidence of transmission of CJD through plasma-derived medicinal products, infectivity has been detected in the plasma of both vCJD and sCJD affected patients[76].

Taking account of the available data concerning blood infectivity, it is of utmost importance to investigate the capacities of the manufacturing process (fractionation) to eliminate/inactivate the infectious material potentially present in the plasma pool used as the starting material for preparation of plasma-derived products.

Initial results from animal studies, using blood from rodents, indicated that the fractionation process contributes to the decrease of infectivity in some fractionated products[44, 46].

However, information reported at the EMA Workshops in 2002 and 2004 suggested that endogenous, rodent blood-associated infectivity might persist through the fractionation process to a greater extent than would be expected from spiking studies using brain-derived prion preparations, possibly because of the differing physical and biochemical properties of the associated infectious particles.

A significant number of studies aimed at following the partition/removal of PrP^{TSE} and/or infectivity during plasma fractionation process have been carried out using such spiking approaches[113, 114].

The vast majority used rodent-adapted TSE agent (263K hamster strain) brain homogenate and microsomal brain fractions as a spike. They relied on direct PrP^{TSE} immunodetection tools (western blot or conformation dependent immunoassay) to demonstrate a drop in the TSE agent content in processed fractions and on bioassay infectivity measurements to confirm the results. Generally, the limited sensitivity of these immuno-detection methods made necessary the use of a massive amount of TSE agent in the spike.

These studies established the potential contribution of the various manufacturing steps to the reduction of TSE agents (including precipitation followed by centrifugation or depth filtration, specific chromatographic steps and nanofiltration).

However since 2004 and the publication of the EMA guideline on *The investigation of manufacturing processes for plasma-derived medicinal products with regards to vCJD risk* (October 2004), the knowledge of the prion area in general and the endogenous infectivity in blood in particular, have significantly evolved. Moreover, experimental studies highlighted the fact that prion removal capacity may directly vary according to the spiking preparation (dispersion and TSE agents strains) particularly for steps based on retention mechanisms[115].

These new elements raise questions about the final relevance of certain experimental approaches that were used for characterizing prion removal capacities of plasma manufacturing steps. Consequently there is still a need to perform research on the best experimental approach for evaluation of the partitioning or removal capacities of the various fractionation steps used in the preparation of plasma-derived medicinal products.

It is recommended to use various forms of spike preparations in order to obtain an insight into their influence on prion reduction at the specific investigated step as compared to what has been published in the literature. In specific cases, it might be worth considering the use of blood from infected animals as an alternative material for investigation of early plasma processing steps, where feasible and where the overall prion reduction capacity seems limited or questionable. There is still further need for research to gain better knowledge of the form of infectivity present in blood (or in intermediates from manufacture) in order to confirm the relevance of the spiking material used in the validation studies.

8. Infectivity in urine

8.1. Animal urine

Low levels of infectivity have been detected in urine of scrapie-infected rodents by several research groups and in the urine of deer with CWD[5g]. Accordingly, urine has been reclassified among the category of "lower-infectivity tissues" by WHO[10c].

Seeger *et al*.[116] have studied transmission via urine using mouse models of chronic inflammation. They have detected prionuria in scrapie infected mice with coincident chronic lymphocytic nephritis. Transmission has been shown upon intracerebral inoculation of purified proteins from pooled urine collected from scrapie sick or presymptomatic mice. In contrast, prionuria was not observed in scrapie infected mice displaying isolated glomerulonephritis without interstitial lymphofollicular foci or in scrapie infected wild type mice lacking inflammatory conditions.

Gregori *et al*.[117] demonstrated that the disease could be transmitted by intracerebral inoculation of pooled urine from scrapie-sick hamsters. The infectivity titre of the urine was calculated to be around 3.8 infectious doses/ml. Titration of kidney and urinary bladders from the same animals gave 20,000-fold greater concentrations. Histologic and immunhistochemical examination of these tissues showed no indication of inflammation or other pathologic changes, except for occasional deposits of disease-associated prion protein in kidneys.

Prionuria was also detected in CWD of deer. Experiments by Haley *et al*.[118] provided evidence that concentrated urine from deer at the terminal stage of the disease, that also showed mild to moderate nephritis histopathologically, was infectious when inoculated into transgenic mice expressing the cervid PrP gene. In addition, the urine collected from the CWD sick deer that was used for mouse inoculation, showed positive results when assayed for PrP^{TSE} by serial rounds of PMCA assay. The concentration of abnormal prion protein was very low as indicated by undetectable PrP^{TSE} by traditional assays and prolonged incubation periods and incomplete TSE attack rates in the transgenic mice.

Using the highly sensitive PMCA or RT-QuIC technologies, PrP^{TSE} have been detected in urine of scrapie sick hamsters,[119, 120, 121] cervids with preclinical and clinical CWD[122-125] and sheep with at preclinical and clinical stages of scrapie disease scrapie[125]. The concentration of PrP^{TSE} in urine is, on average, 10-fold lower than in blood[119].

8.2. Human urine

Epidemiological evidence in the last 25 years, during which urinary-derived medicinal products and particularly gonadotrophins have been widely used, does not suggest, at present, a risk from sporadic CJD. Since epidemiological evidence has identified the few cases of iatrogenic transmission of CJD through the use of pituitary-derived gonadotrophins, it is possible that transmission from urinary-derived gonadotrophins would have been detected if it had occurred. This is further supported by a recent study, in which prion infectivity in urine from a sCJD patient was not detected using bioassays in transgenic mice suggesting that prion infectivity in urine is either not present or was below the detection limit of 0.38 infectious units/ml [126].

Recently, PrP^{TSE} has been detected in the urine of patients with vCJD by using the highly sensitive PMCA technique[98,] but not in urine of sporadic CJD patients[39, 98]. However the sensitivity of the PMCA detection for sCJD remained unassessed in these studies, raising concern about the significance of these negative results. More recently, abnormal PrP conformers were also detected in the urine of sCJD patients using an enrichment technique followed by an immunoassay. In this study, 8 of 20 sCJD cases tested positive while the analysis of 125 control samples (comprising 91 normal control individuals and 34 neurological disease control individuals), remained negative[127].

9. Recommendations and proposals

9.1. Sporadic, genetic and iatrogenic CJD and plasma-derived medicinal products

There is no change in the recommendations for donor selection. There is also no change in the recommendations for batch recalls. However the importance of the prion-reducing capacity of the manufacturing process is emphasised.

Donor selection criteria include criteria to exclude donors who might be at higher risk of developing CJD. The following permanent deferral criteria are specified in Commission Directive 2004/33/EC: Persons who have a family history which places them at risk of developing a TSE, or persons who have received a corneal or dura mater graft, or who have been treated in the past with medicines made from human pituitary glands. Precautionary recalls of batches of plasma-derived medicinal products after post-donation reports of CJD or CJD risk factors in a donor contributed to severe shortages of certain products[10a].

The perception that plasma products and blood of sporadic CJD patients might contain prion infectivity has increased because of the recent transmission study with human blood in transgenic mice and the occurrence of two cases in plasma product recipients. However, cumulative epidemiological evidence does not support transmission of sporadic, genetic and iatrogenic CJD by blood, blood components or plasma-derived medicinal products, although the statistical power of these epidemiological studies for tracing blood-related sCJD cases may not be sufficient to definitively exclude the possibility of blood transmission in a small number of cases. Therefore, the CHMP recommendation that recall of plasma derived medicinal products is not justified where a donor is later confirmed as having sporadic genetic or iatrogenic CJD or risk factors is maintained provided the manufacturer has demonstrated using appropriate methodology that the process includes steps which will minimize any risk of prion contamination of the final product.

The implementation of appropriate actions in relation to CJD depends on accurate diagnosis in suspected cases. There is still potential for diagnostic confusion between sporadic and variant CJD, particularly in younger age groups[128].

9.2. Variant CJD and plasma-derived medicinal products

There is no change in the recommendations for vCJD. Although the number of cases is in decline in the UK and France, isolated cases of vJCD are still being reported and there is still uncertainty about the future number of cases. Variant CJD has a wide distribution of infectivity in tissues outside the central nervous system.

There is strong epidemiological evidence of human to human transmission of vCJD by blood transfusion (see Section 4.2). In addition, one vCJD infection was detected in a patient with haemophilia treated with high doses of intermediate purity factor VIII. Estimates of the relative risks of exposure through diet, surgery, endoscopy, blood transfusion and receipt of UK-sourced plasma products suggest that the most likely route of infection in the patient with haemophilia was receipt of UK plasma products. At least one batch came from a pool containing a donation from a donor who later developed vCJD.

The following measures are aimed at minimising the risk of transmission of the agent by plasma-derived medicinal products.

9.2.1. Exclusion Criteria

a) Consideration of Country-based exclusions

There is currently no screening test to detect donors who may be incubating the disease or in the early clinical stages. Therefore, other approaches are considered in order to try and identify donors who may present a higher risk.

UK plasma

Residence in the UK is a recognised risk factor for vCJD and has led to the UK deciding no longer to fractionate from UK plasma.

Exclusion of donors based on cumulative period of time spent in the UK

Since UK donors are excluded from donating plasma for the manufacture of plasma-derived medicinal products in the UK, it is consistent to exclude donors who have spent long periods in the UK. This is supported by the finding of vCJD cases, which have a risk factor of long periods spent in the UK, in other countries.

It is therefore recommended that donors who have spent a cumulative period of 1 year or more in the UK between the beginning of 1980 and the end of 1996 are excluded from donating blood/plasma for fractionation. Countries are highly encouraged to choose their national cumulative period limit for plasma-derived medicinal products according to a nationally calculated benefit/risk balance, which will take into account the endogenous risk of BSE exposure (and introduction in the food chain) and the risk of shortages of blood and plasma for the manufacture of medicinal products. The national limit is recommended to be of cumulative periods in the UK below or equal to 1 year.

Countries may still apply a stricter limit than 1 year for exclusion of donors for blood/plasma collected for fractionation within the country (e.g. 6 months) but will accept plasma-derived medicinal products from other countries provided that at least the one-year time limit is applied.

The rationale for this recommendation is to exclude donors who have the highest individual risk from stays in the UK and to be consistent with the UK decision to no longer fractionate from UK plasma. This is further explained in the first version of this Position Statement published in February 2003[5b].

French plasma and plasma from other BSE-exposed European countries

France published an analysis of the risk of transmission of vCJD by blood and its derivatives sourced from French plasma in December 2000[129g]. This concluded that plasma collected in France could continue to be used for fractionation. The safety margin for plasma-derived medicinal products was considered to be sufficient. However, introduction of additional steps to further increase the safety margin of some products was recommended (e.g. nanofiltration of Factor VIII introduced in January 2001). Leucodepletion for plasma for fractionation, as for plasma for transfusion products, was also recommended in 2001 as a precautionary measure. The subsequent risk-analyses published in 2002, 2003, 2004, 2005, 2007 and 2009 re-confirmed these conclusions and acknowledged that the estimated size of the epidemic had been reduced by more recent modelling, and the risk associated with collecting blood from vCJD-incubating donors was lower than previously estimated[129].

Based on the limited data on human exposure to BSE-risk materials in other European countries, it is still difficult to estimate the epidemiological risk in those countries which have a small number of vCJD cases or have not yet reported any vCJD cases.

Donors who have spent a cumulative period of time in France and other BSE-exposed countries

Exclusion of donors who have spent a cumulative period of time in France is not recommended because of the lower risk associated with time spent in France compared with time spent in the UK (the risk in France is estimated to be 1/10 of that in the UK) [129b]. Endogenous vCJD cases occurred in some other countries (see Section 2. Human TSEs current status) placing them close to or lower than France in terms of incidence and ratio of risk in comparison to UK. Exclusion of donors who have spent time in other countries having a risk ratio in the same order of magnitude as France is not recommended.

Concluding remarks

Country-based exclusions may appear unjustified in the sense that the vast majority of donors who will be excluded will not develop the disease. There is a lack of spare plasma capacity to make up for shortfalls if countries that are major producers of plasma-derived medicinal products discontinue the use of nationally collected plasma for fractionation.

b) Other possible exclusion criteria

Commission Directive 2004/33/EC indicates that further deferral criteria for vCJD may be recommended as a precautionary measure.

Other possible exclusion criteria that could be considered include permanent exclusion of recipients of blood transfusion in UK.

Caution is needed because of the risk of loss of donors and consequent supply problems. Since such criteria could apply to both blood and blood components, and plasma-derived medicinal products, this is kept under review within the scope of Directive 2002/98/EC. The Competent Authorities for blood and blood components expressed the need to have scientific evidence on the safety impact of possible additional exclusion criteria, as well as to make a national assessment on the expected impact of these criteria on donation volumes, before implementing additional exclusion criteria.

The opinion of May 2006 from the Scientific Committee on Emerging and Newly Identified health Risks (SCENHIR) stated that it did not consider that additional specific measures were needed to reduce the risk from vCJD infectivity in blood. When there is a concern for spreading vCJD by blood transfusion, donor exclusion of blood transfusion recipients is the appropriate measure[9i].

9.2.2. Leucoreduction and specific prion affinity filters

The benefit of inclusion of leucoreduction to improve the safety of plasma has not been demonstrated.

At present it is not appropriate to recommend the introduction of leucoreduction for the safety of plasma-derived products.

Efficacy of introducing recently developed affinity media / filters to blood or plasma has been investigated. Although they might have some effect in reducing prion loads, clear evidence for their use in providing protection against transmission is still uncertain.

9.2.3. Manufacturing processes for plasma-derived medicinal products

The available data support the reduction of infectivity by steps in the manufacturing process. Manufacturers are required to estimate the potential of their specific manufacturing processes to reduce infectivity. This should follow a step-wise approach as described below and illustrated in the accompanying flow diagram. It is recommended that manufacturers consult the relevant competent authorities at each of the milestones in this estimation. A decision to add a further manufacturing step(s) to increase reduction capacity should only be made after careful consideration of all benefit-risk factors for a certain product.

Firstly, manufacturers should compare their own processes to those with published data on reduction of infectivity in order to estimate the theoretical potential of their specific manufacturing processes to reduce infectivity. *(Flow diagram, step 1)*

Whereas the general information available on manufacturing processes provides useful background information, the actual effectiveness of a manufacturing process might be dependent on the specific process conditions. Manufacturers should consider the relevance of the published data to their specific manufacturing processes and whether the removal capacity can be expected to be comparable.

If it cannot be concluded that the removal capacity would be expected to be comparable, it is recommended that manufacturers undertake product-specific investigational studies on key steps in their manufacturing processes using biochemical assays. Priority should be given to studies on products with the lowest potential removal capacity. *(Flow diagram, step 2)*

Investigations using biochemical assays may be sufficient if a clear correlation with infectivity data has already been established for similar processes (e.g. ethanol fractionation). If such a correlation is not established (e.g. a novel step) and the step is considered critical for removal of infectivity for the specific product (e.g. it is the only step for removal), the investigations should be confirmed using an infectivity assay for the critical step(s). *(Flow diagram, step 3)*

The above steps will allow manufacturers to estimate the reduction capacity of their manufacturing processes. *(Flow diagram, step 4)*

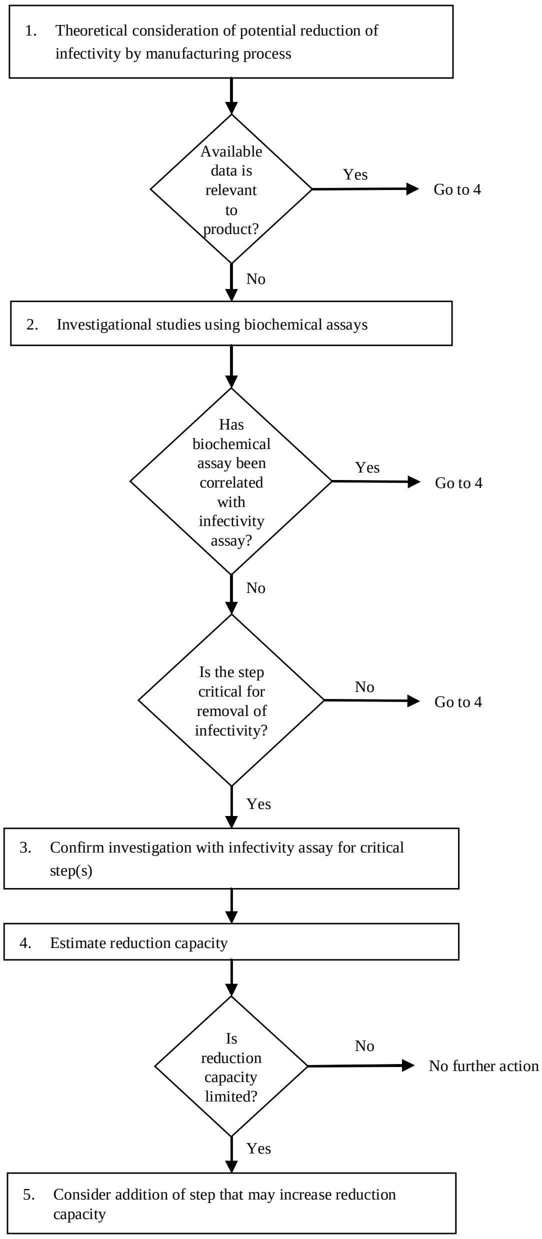
In cases where the overall reduction capacity is limited, manufacturers should consider the addition of steps that may increase the removal capacity where this is feasible without compromising the safety, quality and availability of the existing products. Discussion with the relevant competent authorities is recommended. *(Flow diagram, step 5)*

The outcome of the estimates of the theoretical potential of manufacturing processes to reduce infectivity and the results of product-specific investigational studies should be reported to the relevant competent authorities for the medicinal products concerned, as information becomes available. Applicants submitting new marketing authorisation applications for plasma-derived medicinal products will be expected to include such information in the application dossier. The outcome of the estimation of the theoretical potential to reduce infectivity should always be included in the application.

In support of these recommendations, CHMP's Biologics Working Party, with the involvement of external experts, has developed guidance on how to investigate manufacturing processes with regard to vCJD risk[5a].

Figure 1: Plasma-Derived Medicinal Products: estimation of potential reduction capacity of specific manufacturing processes

Important Note: this flow diagram should be read in conjunction with the preceding text in 9.2.3. It is recommended to consult the relevant competent authorities at the milestones in this estimation. Give priority to studies on products with the lowest potential removal capacity.



9.2.4. Recall of batches where information becomes available post-donation

In view of the lack of adequate information on vCJD, it is prudent to recall batches of plasma-derived medicinal products where a donor to a plasma pool subsequently develops vCJD. Recall should also include medicinal products containing plasma-derived products as excipients (see also 9.2.5). However, in both cases, consequences for essential medicinal products where alternatives are not available will need careful consideration by the competent authorities.

A case-by-case consideration would be appropriate where plasma-derived products have been used in the manufacture of other medicinal products. This consideration would include the nature of the product, the amount used, where it is used in the manufacturing process and the downstream processing.

Look-back to identify the fate of donations should be taken as far as possible. Regulatory authorities, Official Medicines Control Laboratories, surveillance centres and the supply chain should be informed of all batches of product and intermediate implicated whether or not supplies of the batch are exhausted.

There is no recommendation to recall batches if information becomes available post-donation, which would have excluded a donor based on his/her stay in the UK (see 9.2.1).

9.2.5. Albumin used as an excipient or in manufacturing processes

The available data on the removal of infectivity during the fractionation process used in the manufacture of albumin indicates that the risk of transmission of infectivity by albumin would be particularly low. Where a donor to a plasma pool subsequently develops vCJD in the case of albumin used as an excipient, a recall should be considered. However, a careful case-by-case risk analysis taking into account the estimated capacity of the process to remove infectivity and the amount of albumin incorporated in the medicinal product could justify not needing a recall. A single batch of albumin may be used to produce a number of batches of a medicinal product because of the small amounts that are typically used as an excipient. As a consequence, a recall could affect complete stocks of a product and create severe shortages. Therefore, to avoid a negative impact on supply, companies should consider the origin of plasma and select countries where the probability of having to recall batches is as limited as possible.

Use of substitutes for plasma-derived albumin used as an excipient or in manufacturing processes is encouraged and should be considered as a long-term approach.

9.2.6. Substitution with alternative products

Use of alternative products to plasma-derived medicinal products could be considered, where these are available. It is felt that this choice should remain with users, taking into account the needs of the individual patient. It should be noted that plasma-derived products such as albumin may be used in the manufacture of recombinant products.

9.2.7. Optimal Use

Optimal use of plasma-derived medicinal products is encouraged, as this will maximise the benefits of the products compared with any potential risk.

9.3. Urine-derived medicinal products

The recommendations for urine-derived medicinal products are based on the following considerations:

- There is at present no epidemiological evidence of CJD and vCJD transmission by urine-derived medicinal products.
- TSE infectivity in urine has been reported in some animal models.
- Abnormal PrP has been detected by different methods in 40% of sCJD patient urine samples and 93% of vCJD samples.

- The review of manufacturing processes is described below.

Urine should be collected from countries where there is a surveillance system for both human and animal TSEs unless otherwise justified. It is noted that urine-derived medicinal products are not sourced from urine collected in the UK. Based on the limited data on human exposure to BSE-risk materials in other countries, it is still difficult to estimate the epidemiological risk in those countries which have a small number of vCJD cases or may have a TSE exposure risk.

For particular products, such as hormones from a relatively small well-defined donor population, some manufacturers have put in place limited exclusion criteria for the selection of a donor for inclusion in a donor panel. For other products manufactured from very large donor pools (e.g. urokinase), such measures are more difficult to apply. The use of exclusion criteria for selection for a donor panel is encouraged. The same exclusion criteria should be applied with respect to CJD and vCJD as used for blood/plasma donors providing starting material for the manufacture of plasma-derived medicinal products. Manufacturers should follow up the donor criteria at defined intervals. The exclusion of donors with known inflammation of kidney and/or chronic renal inflammatory diseases is encouraged.

Manufacturers are required to estimate the potential of their specific manufacturing processes to reduce infectivity following the same general, stepwise approach as recommended for plasma derived medicinal products (see Section 9.2.3). Extrapolation of results for plasma-derived medicinal products is not justified particularly for chromatographic steps at the beginning of the manufacturing process because of the high protein content in plasma. Investigational studies of infectivity reduction by the manufacturing processes should address potential accumulation of infectivity/PrP^{TSE} on chromatographic columns or a potential batch to batch contamination due to carry-over of infectivity/PrP^{TSE}. For inactivation studies, investigation of different TSE strains should be considered as they may vary in resistance.

General review of the manufacturing processes indicates that, in each manufacturing process, there is at least one step that might be theoretically capable of reducing infectivity if it were present in the starting material. In cases where the reduction capacity is limited, manufacturers should consider the addition of steps that may increase the overall removal capacity.

Record keeping for traceability is recommended for products where it is possible to trace back to donor level.

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