Pathogen safety of plasma-derived products – Haemate® P/Humate-P®

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Summary. Plasma-derived factor VIII (FVIII) and von Willebrand Factor (VWF)/FVIII concentrates have been successfully used to treat haemophilia since the late 1960s. These products are derived from pools of plasma donations that may contain viral contaminants – including hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) – and may therefore present a transmission risk to recipients. To ensure the safety of Haemate® P/Humate-P®, a plasma-derived VWF/FVIII concentrate, donors of plasma are carefully selected and all donations are screened for viral antigens (HBV), virus-specific antibodies (HIV-1/2, HCV) and genomic material [hepatitis A virus, HBV, HCV, HIV-1 and high titres of human parvovirus B19 (B19V)]. As a quality control measure, plasma pools for fractionation are only released for further processing when non-reactivity has been demonstrated in serological and genome amplification assays. The manufacturing process for plasma-derived products, especially the fundamental procedure of pasteurization, is effective in inactivating and/or removing a wide variety of viruses that may potentially be present despite the screening process. This has been demonstrated in virus validation studies using a range of different viruses. New emerging infectious agents, including prions, which potentially pose a threat to recipients of plasma derivatives, are also the subject of safety evaluations. The multiple precautionary measures that are inherent in the overall production process of Haemate P/Humate-P have resulted in an excellent safety record, documented during 25 years of clinical use, and will help to maintain the high safety margin in the future.

Keywords: manufacturing process, nucleic acid amplification technique, pasteurization, plasma-derived product, variant Creutzfeldt-Jakob disease, virus validation study

Introduction

Haemate® P/Humate-P® is a plasma-derived product used for prophylaxis and the treatment of bleeding in haemophilia A and von Willebrand's disease. It can also be used for prophylaxis and the treatment of bleeding in patients with other diseases resulting from a factor VIII (FVIII) deficiency. The key constituents of Haemate P/Humate-P are human von Willebrand factor (VWF) and the coagulation factor, FVIII. The Haemate P/Humate-P manufacturing process is designed to purify, concentrate the desired VWF and FVIII proteins and to inactivate/ remove viruses that may potentially be present in the starting material i.e. the plasma pool for fractionation. Haemate P/Humate-P is a lyophilized product that is reconstituted with water for injection, prior to administration, to facilitate appropriate blood coagulation in patients.

When Haemate P/Humate-P [formerly known as 'FVIII HS' (hepatitis safe) and 'Haemate® HS'] was licensed and introduced into the German market more than 25 years ago, the production process included a pasteurization step to inactivate transfusion-relevant viruses. The pasteurization step involved heat-treating of the stabilized product intermediate in aqueous solution at 60°C for 10 h. Since that time, several other virus reduction measures have been implemented to increase the safety margin of the final product, including the stringent selection of all donors and screening of all donations for markers and genomes of transfusion-relevant

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viruses. Any seropositive or virus-reactive donations are discarded. The plasma pool for fractionation is then rechecked for the presence of viruses prior to further stages of the manufacturing process. Various steps in the Haemate P/Humate-P manufacturing process inactivate or remove remaining viruses; this has been confirmed in carefully planned virus-validation studies using viruses of diverse physico-chemical characteristics, including enveloped and non-enveloped viruses. The concept of a virus-safe, plasma-derived product is in line with the European guideline CPMP/BWP/268/95 [1], which addresses three complementary approaches to control potential viral contamination of biological products. These include: (i) the selection and testing of source plasma for the absence of viruses, (ii) testing the ability of the production process to remove or inactivate viruses and (iii) testing the product, at appropriate stages of production, for any contaminating viruses. In addition, the impact of new pathogens, including prions [(the causative agent of variant Creutzfeldt-Jakob disease (vCJD)], which may enter the plasma pool for fractionation and present a risk to the recipients of the product, is carefully assessed. Virus validation and prion evaluation studies are performed to assess how effectively these new pathogens can be removed and/or inactivated during the manufacturing process. The main procedures aimed at reducing the load of infectious viruses emanating from plasma during the Haemate P/Humate-P manufacturing process are outlined in Fig. 1.

Selection and assessment of plasma for the Haemate P/Humate-P manufacturing process

Selection of starting material

The selection and control of starting material is a major factor in the quality assurance of the Haemate P/Humate-P manufacturing process. By carefully choosing the starting material, the potential load of hazardous viruses can be reduced to a minute amount or even eliminated. Improvements that ensure the safety of the starting plasma have been achieved in the collection process itself, for example, the careful selection of donor centres and donors. Plasma is either collected by plasmapheresis (source plasma) at designated collection centres – most of them owned by and all of them audited by CSL Behring and inspected by the relevant authorities – or from whole blood donations (recovered plasma) collected by transfusion services. All donors are required to undergo a physical examination and to provide answers to a predefined questionnaire at the time of each donation. The questionnaire is used to identify any donor who could pose a risk, in terms of transmitting pathogens, and to reject their donation and defer the donor either temporarily or indefinitely depending on the risk. For source plasma donations, the first donation from an 'applicant' donor (first time donor) is initially quarantined. This quarantine can only be lifted when the donor has successfully passed a second medical history assessment and a second donation has been screened and shown to be
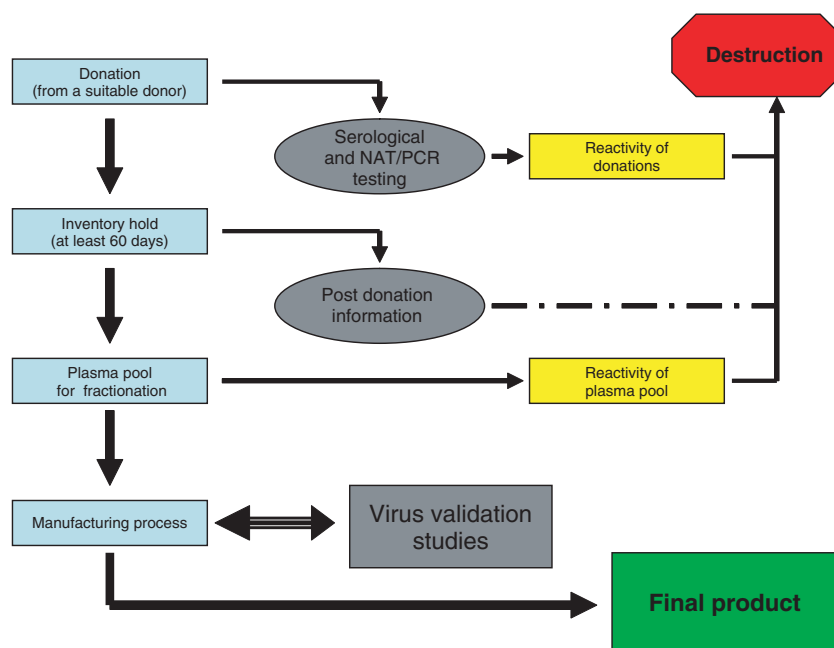

Fig. 1. The virus safety procedure of Haemate P/Humate-P, a plasma-derived product. NAT, nucleic acid amplification technique; PCR, polymerase chain reaction.

free of transfusion-relevant viruses. These measures result in a healthy population of donors (known as 'qualified' donors) committed to donating plasma. Source plasma from donors who have not reached the status of qualified donors is not processed for further manufacturing and is therefore destroyed. Qualified donors who have not made a donation for a period of 6 months or more are considered to be applicant donors again. All donations are screened for viruses using currently approved serological and nucleic acid amplification assays.

As Haemate P/Humate-P was licensed in 1981 as 'Faktor FVIII HS', several screening assays used to detect viral markers (such as virus-specific antibodies or viral antigens) have been established or significantly improved. The first-generation assay to detect antibodies against human immunodeficiency virus (HIV) was introduced in 1985 [2,3]. Since then, the sensitivity of the assay has been greatly improved. The HIV screening assays currently licensed in Europe are highly effective in identifying any donors infected with HIV-1, HIV-2 and HIV-1 group O, and the screening assays currently licensed for use in the USA are highly effective in detecting HIV-1 and HIV-2.

After years of unsuccessful attempts to identify the agent responsible for parenterally transmitted non-A, non-B hepatitis [now known as hepatitis C virus (HCV)], a cDNA clone expressing a protein allowing the detection of antibodies in the majority of non-A, non-B serum specimens was identified [4,5]. A first-generation screening assay used to detect antibodies against HCV, based on the C 100-3 clone, was introduced in early 1990s followed by both screening and confirmation assays employing other recombinant viral antigens [6]. Currently, a highly sensitive, third-generation assay is used to detect any donors who have previously been infected with HCV [7].

The screening assay for hepatitis B virus (HBV) infection has also been improved from the early detection systems [8], which utilized agar gel diffusion or counter-electrophoresis, as opposed to the current third-generation serology-based detection assays. Assays licensed in Europe have a detection limit of less than 0.15 ng HBsAg mL^{-1} in plasma or serum [9], whilst assays licensed for use in the USA have a slightly lower, but comparable detection limit [10]. All plasma/whole blood donations used for the production of plasma-derived products must be screened using sensitive, specific and validated assays. These assays must have been approved for screening blood donations for the purpose of transfusion in the country where the donations are collected. Plasma/whole blood donations must be negative for:

1 HBsAg,
2 antibodies against HIV-1/-2 and
3 antibodies against HCV.

Inventory hold and post-donation information

Testing for viral markers (antibodies against HIV-1/-2 and HCV encoded antigens) exhibits both a sensitivity limit and a temporal dependency (Fig. 2). This time-period, known as the 'diagnostic window', is particularly relevant for tests based on antibody detection techniques. It encompasses the period during which serological screening of a donation is negative despite the sample actually containing infectious virus. This diagnostic window occurs because there is a time lapse between an individual being infected with a virus and their body's ability to recognize the virus and subsequently generate enough specific antibody-producing cells (i.e. time to seroconvert). Therefore, plasma donations are stored in an inventory hold to address post-donation information. Source plasma, which
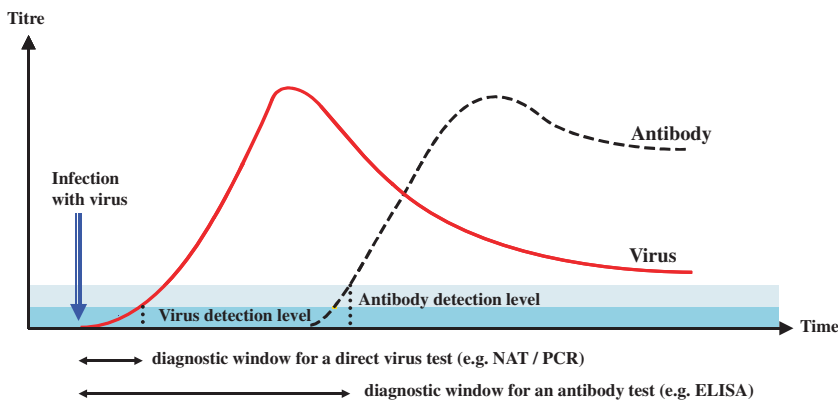

Fig. 2. Reduction of the 'diagnostic window' for antibody screening by implementation of a nucleic acid amplification technique (NAT) and polymerase chain reaction (PCR) testing. Serologically negative donations may be window donations posing a considerable risk; NAT/PCR non-reactive donations pose a very small risk. ELISA, enzyme-linked immunoabsorbant assay.

can be obtained several times per month from each donor, is stored for at least 60 days in the inventory hold before further processing occurs. During the inventory hold period, all post-donation information relating to the donor is assessed with regard to the suitability of the stored plasma. The most important information is whether seroconversion is detected in a subsequent donation from the same donor. If seroconversion has occurred, all stored donations from that donor are removed from the inventory hold and destroyed, thus preventing potentially virus contaminated, but seronegative donations, from being processed into the plasma pool for fractionation. Any post-donation information from a whole blood donor, such as revealing a blood-borne virus infection in a recipient or components of that donation, would also result in the destruction of that donor's stored plasma. Post-donation information also includes the results of testing for genomic material of transfusion-relevant viruses. Furthermore, if new information on the suitability of a donor is gathered during the inventory hold period, a risk assessment is performed and stored donation(s) may then be destroyed based on the assessment outcome. Therefore, by utilizing an inventory hold, donations that may present a virus-transmission risk can be identified and eliminated from the production process (Fig. 3). Donations from applicant donors are stored in quarantine until a second donation confirms the health of the donor; the first donation is subsequently forwarded to the inventory hold.

Serological screening of donations for hepatitis A virus (HAV) and human parvovirus B19 (B19V) has not been established. This is because both viruses have a short viremic phase resulting in the production of protective neutralizing antibodies, which clear the virus from the body and can potentially confer life-long protection. Therefore, antibody screening similar to that for HIV and HCV would not be appropriate for these particular viruses, and discarding antibody-reactive donations would deplete immunoglobulin preparations from these clinically relevant antibodies.

To improve the sensitivity of screening assays, an assay based on detecting antigen (such as for HBV) or viral nucleic acid (discussed below) should be employed, enabling the virus itself to be detected rather than the host response to the virus. This approach offers another clear advantage over an antibody-based detection assay because it also allows the diagnostic window to be significantly reduced (Fig. 2).

Nucleic acid amplification technique/polymerase chain reactions

For quality control purposes, i.e. production of plasma-derived products with no (or minimal) contamination with blood-borne viruses, a screening programme utilizing a nucleic acid amplification technique (NAT) in the form of a sensitive polymerase chain reaction (PCR) designed for industrial use, was implemented at CSL Behring. NAT/PCR testing has been used by CSL Behring to detect HBV, HCV and HIV-1 nucleic acid since 1997, and HAV and high titres of B19V since 2000. This NAT/PCR testing scheme shortens the estimated diagnostic window from around 59 to 34 days for HBV, 82

Inventory hold for source plasma donations (example)

Fig. 3. Inventory hold of source plasma donations. Donations from qualified donors remain in the inventory hold for at least 60 days before fractionation. Any donation that is reactive in either serology or nucleic acid amplification technique (NAT)/polymerase chain reaction (PCR) is interdicted and destroyed together with any previous donations being held in the inventory hold as these donations may pose an infection risk.
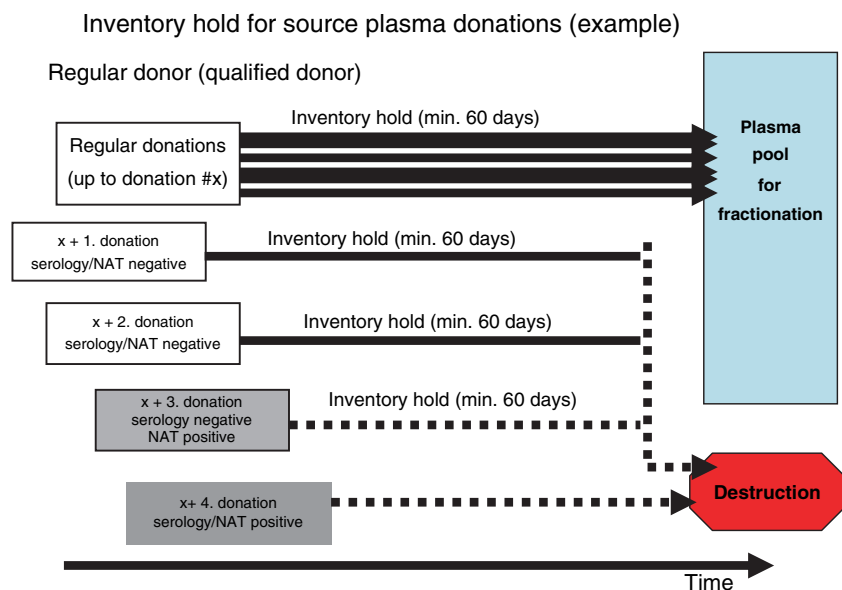
to 23 days for HCV and 22 to 11 days for HIV-1 (Fig. 2) [11]. Therefore, all donations entering the manufacturing process are non-reactive in the sensitive NAT/PCR assay and consequently contain either no blood-borne viruses or a very low-virus load that is below the detection limit of the assay.

A strategy for creating mini pools for NAT/PCR testing was implemented to avoid the unnecessary plasma loss that would be caused by disposing of large plasma pools for fractionation if NAT/PCR reactivity was detected. During plasma collection, an additional sample is obtained and used to prepare sample mini pools, specifically for NAT/PCR testing. This enables any NAT/PCR-reactive mini pools to be detected and the causative NAT/PCR-positive donation(s) to be individually discarded, enabling a NAT/PCR non-reactive plasma pool to be created for fractionation. According to the European guideline (CPMP/BWP/390/97), non-reactivity must be demonstrated by utilizing a validated NAT/PCR test (this guideline is only relevant for HCV) [12]. The CPMP guideline is used in conjunction with the European Directorate for the Quality of Medicines (EDQM) and the US Food and Drug Administration (USFDA) to provide guidance for using NAT to detect nucleic acid from HCV and HIV-1/-2 [13–15]. The effectiveness of NAT/PCR testing on all source plasma donations collected for fractionation at the facilities in Marburg, Germany and Kankakee, Illinois, USA (and Bern, Switzerland from 2004) is shown in Table 1. The NAT/PCR testing scheme is used to screen for HBV, HCV, HIV-1, HAV and high-titre B19V in window donations. Any positive donations are discarded, preventing them from entering the plasma pools for fractionation. Any previous donations from the same donor that are being stored in an inventory hold are also discarded (Fig. 3).

The degree of virus reduction that can potentially be achieved by discarding any NAT/PCR-reactive donations is shown in Table 2. These data show that NAT/PCR testing performed by CSL Behring has a significant impact on the reduction of the virus load

Table 2. Effectiveness of NAT/PCR in reducing the virus load in plasma pools for fractionation by discarding one virus-positive window donations.

Virus	Virus removal because of NAT/PCR screening (GE log$_{10}$)*	Reference
HBV	7.6	[73]
HCV	12.6	[73]
HIV-1	9.4	[74]
HAV	9.9	[28]
B19V	16.9	[75]

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus 1; NAT, nucleic acid amplification technique; PCR, polymerase chain reaction; B19V, parvovirus B19; GE, genome equivalent.
*The quantity of virus particles that can be harboured by one-window donation (approximately 800 mL).

in plasma pools for fractionation and can be considered an effective virus reduction step.

To ensure that the processes for testing donations, discarding reactive donations and pooling of non-reactive plasma, have been performed successfully, the final plasma pool for fractionation is again tested for the absence of viral markers (HBsAg, antibodies against HIV-1/-2) and genomic material (for HAV, HBV, HCV, HIV-1 and high titres of B19V) based on regulatory requirements and CSL Behring's voluntary standards. Plasma pools are released for further processing only if the test results show that the pools are non-reactive. The quality control measure implemented by CSL Behring for HCV detection is based on an analytical sensitivity of the NAT/PCR assay for plasma pool release that is considerably lower than the assay sensitivity required by the European Medicines Agency (EMEA; CPMP/BWP/390/97) for HCV [12]. Although no regulatory requirements are implemented for other viruses, CSL Behring's release criteria for the HAV, HBV and HIV-1 are set at similarly low assay sensitivities as for HCV (20 IU mL^{-1} plasma pool for fractionation). In comparison, blood for transfusion is only released if the loads of HCV and HIV-1 are less than 5 000 to 10 000 IU per mL of donated plasma [16–18].

Table 1. Results of NAT/PCR testing of plasma donations at CSL Behring (data until end of October 2007).

Number of donations	HBV	HCV	HIV-1	HAV	B19V^{HTS}
Tested*	29 751 454	29 751 454	29 751 454	24 234 274	24 966 274
Positive	823	4 505	220	78	4 068
Positive per 10^5 donations tested	2.77	15.14	0.74	0.32	16.29

B19V, parvovirus B19; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus 1; HTS, high-titre screening; NAT, nucleic acid amplification technique; PCR, polymerase chain reaction.
*Number of donations from applicant and qualified donors. Because of the testing scheme (pooling of donations) and subsequent screening by NAT/PCR, more than one donation from a source plasma donor may be interdicted; therefore, the number of reactive donations is considerably higher than the number of reactive donors.

By implementing such a rigorous testing scheme, a significant reduction in the virus load of the starting material for Haemate P/Humate-P production is achieved. This preproduction screening process facilitates the virus inactivation/elimination steps of the manufacturing procedure, as the virus reduction process during production will be exposed to only very limited amounts (if any) of the transfusion-relevant viruses.

Virus inactivation during the Haemate P/Humate-P manufacturing process

Pasteurization

The development of a pasteurized FVIII product began in mid-1970 at Behringwerke in Marburg, a predecessor company of CSL Behring. The introduction of the pasteurization process was based upon the excellent virus safety record of human serum albumin – a plasma-derived product manufactured by subsequent cold ethanol precipitation steps [19] or by modification of the Cohn process [20,21]. The pasteurization process, which involves heat-treatment of the product in aqueous solution at 60°C for 10 h, takes place in the final container. In contrast to albumin, FVIII is heat-sensitive and therefore susceptible to damage by the pasteurization step. A pasteurized FVIII product was, nevertheless, successfully developed following the discovery of the proprietary stabilizer – a combination of sucrose and glycine. The stabilizer is added to the aqueous solution to protect FVIII from (partial) denaturation whilst still allowing effective virus inactivation.

When production of the pasteurized FVIII product was in early development, the main goal was to create a product that would not transmit hepatitis-causing viruses. At that time, only three hepatitis viruses had been discovered and of these, HCV was still known as hepatitis non-A, non-B virus. This nomenclature was used because non-A, non-B virus was recognized as a causative agent of hepatitis, but it was also known to be distinct from both HAV and HBV despite the fact that the infectious agent itself was unknown. Neither HBV nor HCV can be propagated in cell culture, and no small animal model exists for infectivity assays because these viruses have a very narrow host range. Infectious virus can only be detected and quantified in either humans or chimpanzees, which serve as the only animal model for HBV and HCV infection. Consequently, all virus inactivation studies included chimpanzees.

Two HBV inactivation studies were designed to assess how effective the Haemate P/Humate-P man-ufacturing process is in reducing the load of infectious virus in an intermediate plasma fraction. This intermediate consisted of partially fractionated material that still needed further manufacturing steps to become the final Haemate P/Humate-P product. Haemate P/Humate-P intermediate was prepared on a laboratory scale according to the manufacturing process that incorporates the pasteurization step. Chimpanzees were inoculated with either HBV-spiked starting material, the intermediate plasma fraction before pasteurization or the final Haemate P/Humate-P product. Each study utilized a different batch of intermediate. The reduction of HCV was also evaluated using similar inactivation studies. In these HCV inactivation studies, the sample before pasteurization was not tested [22]. Detailed information relating to the three studies and demonstration of the effectiveness of the Haemate P/Humate-P manufacturing process to eliminate HBV/HCV is provided in Table 3.

A range of human pathogenic viruses, including poliovirus, vaccinia virus and three herpes viruses (cytomegalovirus, Epstein-Barr virus, herpes simplex virus), which can all be propagated in cell cultures, were employed in the first virus validation studies [23]. When HIV, initially known as HTLV III (human T-cell leukaemia virus III), was discovered, it was also studied in cell cultures and its considerable heat sensitivity was demonstrated [23,24]. Studies to date have employed a wide range of human and animal pathogenic viruses to assess the virus inactivation capacity of the pasteurization step. Table 4 contains examples of viruses that have been tested and demonstrates how effectively they are inactivated by the pasteurization step.

Validation of virus reduction during the Haemate P/Humate-P production process

Virus inactivation by pasteurization is verified in virus validation studies. Other steps in the production of Haemate P/Humate-P, such as adsorption and precipitation, are validated for their ability to remove viruses during the production process. In 1991, the European authorities released the first guideline on virus validation studies for biological products derived from either cell banks of human or animal origin, blood or other biological fluids or human or animal tissues [25]. The guideline covers the design, implications and the limitations of validation studies, and includes recommendations for the selection of appropriate viruses to be used in virus validation studies. This guideline has since been updated to the current third revision [26].

	HBV		HCV*
	Study 1	Study 2	
Spiked starting material			
Theoretical infectivity in starting material – assuming no virus reduction (CID_{50})	5.6 $\log_{10}$	5.6 $\log_{10}$	5.5 $\log_{10}$
Number of chimpanzees	2	2	2
Volume of inoculum (mL)	1	1	1
Seropositive chimpanzees following infection [HBsAg, α-HBs or α-HBc]	2	2	nd
First signs of infection (weeks)	19, 26	24, 27	10, 15
Intermediate before pasteurization			
Number of chimpanzees	4	nd	nd
Volume of inoculum (mL)	1	nd	nd
Seropositive chimpanzees following infection [HBsAg, α-HBs or α-HBc]	4	nd	nd
First signs of infection (weeks)	9, 12, 13, 25	nd	nd
Final product			
Number of chimpanzees	4	3	4
Volume of inoculum (mL)	1	1	1 or 24 (2 chimpanzees each)
Seropositive chimpanzees following infection [HBsAg, α-HBs or α-HBc]	0	0	nd
Signs of infection at close of study (weeks)	None (28–41)	None (38)	None (22)
Overall virus reduction factor (CID_{50})	>5.6 $\log_{10}$	>5.6 $\log_{10}$	>5.5 $\log_{10}$

Table 3. Reduction of HBV and HCV by the manufacturing process of Haemate P/Humate-P [76].

HBV, hepatitis B virus; HCV, hepatitis C virus; nd, not determined.
*HCV: at the time of experiments, this virus was known as non-A, non-B hepatitis virus.
CID_{50} is defined as the dose that is necessary to initiate infection in 50% of chimpanzees.
α-HBs are antibodies directed against the hepatitis B virus surface antigen.
α-HBc is an antibody directed against the hepatitis B virus core antigen.

Table 4. Pasteurization inactivates a wide range of viruses [CSL Behring on file].

	Enveloped viruses					Non-enveloped viruses		
	HIV-1	BVDV	PRV	HSV-1	WNV	HAV	Poliovirus	B19V
Mean virus inactivation factor ($\log_{10}$)	≥6.4	≥8.9	4.6	≥7.0	≥7.8	4.2	≥7.4	≥3.9

B19V, parvovirus B19; BVDV, bovine viral diarrhoea virus; HAV, hepatitis A virus; HIV-1, human immunodeficiency virus 1; HSV-1, herpes simplex virus 1; PRV, pseudorabies virus; WNV, West Nile virus.

Virus validation studies improve confidence in the safety of a biological product with regard to potential virus transmission. They identify the production steps that are effective in reducing the level of infectious virus, or which contribute to the reduction of infectious virus. Consequently, an estimate of the overall ability of a manufacturing procedure to inactivate and/or remove any contaminating infectious viruses can be obtained.

Selection of viruses for validation studies

Viruses chosen for validation studies should, where possible, closely resemble the viruses that may potentially contaminate the plasma-derived product.

To test the ability of the production process to remove viruses in general, they should also represent a wide range of physico-chemical properties. For these studies, strains of viruses that replicate to high titres in cell culture and can be assayed in an effective, sensitive and reliable *in vitro* infectivity assay were chosen by CSL Behring. Routine infectivity assays are currently not available for HBV, HCV or B19V (although a research infectivity assay for B19V has been established at CSL Behring). Consequently, these viruses cannot be used for virus validation studies, and where appropriate, model viruses are used instead (Table 5).

With respect to the Haemate P/Humate-P manufacturing process, virus validation studies were

Table 5. Viruses used in validation studies at CSL Behring for Haemate P/Humate-P (in accordance with CPMP/BWP/268/95) [1].

Transfusion relevant virus	Test virus (model virus)	Genome	Enveloped	Size (nm)	Resistance to treatment
HIV	HIV-1	RNA	Yes	80–100	Low
HCV	BVDV	RNA	Yes	50–70	Low
HBV	None available*	DNA	Yes	45	Medium
HAV	HAV	RNA	No	25–30	High
B19V	CPV	DNA	No	18–24	Very high
–	Herpesvirus (non-specific model)	DNA	Yes	120–200	Medium

B19V, parvovirus B19; BVDV, bovine viral diarrhoea virus; CPV, canine parvovirus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus 1.
*According to CPMP/BWP/269/95 [26].

performed based on guidelines of the EMEA (formerly known as the European Agency for the Evaluation of Medicinal Products); these documents provide both general guidance on virus validation studies [1] and specific guidance on plasma-derived medicinal products [26]. Virus validation reports were submitted to the authorities for licensing of Haemate P/Humate-P, taking into consideration the overall risk of transmitting blood-borne viruses by the application of the product to recipients [27]. The formal virus validation studies for Haemate P/Humate-P employed a fixed panel of viruses, including those listed below.

Human immunodeficiency virus 1 was employed in virus validation studies as a relevant blood-borne virus that can be propagated in cell culture systems. In addition, limited studies employing HIV-2 were performed, demonstrating that HIV-1 and HIV-2 were equally sensitive. HIV-2 was not studied on a regular basis because it responds to inactivation procedures in a similar manner to that of HIV-1 [26].

Bovine viral diarrhoea virus (pestivirus genus of the family Flaviviridae) was initially introduced to the panel of viruses employed in virus validation studies because it is more closely related to HCV than other model viruses from either the flavivirus genus (e.g. tick-borne encephalitis virus) or the alphavirus genus of the family Togaviridae (e.g. Sindbis virus, Semliki Forest virus). In addition, West Nile virus (WNV – flavivirus genus) was used in pasteurization studies.

Hepatitis A virus was used in validation studies because it is thought to be significantly different from other picornaviruses [26] and is recognized as a blood-borne virus [28].

Canine parvovirus (CPV) was selected for virus validation studies as it does not cross-react with human parvovirus antibodies that may be present in plasma pools, in contrast to bovine parvovirus, which cross-reacts with B19V antibodies [29]. CPV is used as a model virus for B19V and represents small non-

enveloped viruses that are resistant to physico-chemical treatment. B19V was selected as a relevant blood-borne virus to demonstrate the effective inactivation by the pasteurization process. Pseudorabies virus, a porcine herpesvirus, was selected as a non-specific model virus with a complex structure (an enveloped virus with a double-stranded DNA genome).

The validation process

The manufacturing process of Haemate P/Humate-P consists of several steps to purify and concentrate FVIII and VWF: cryoprecipitation, Al(OH)$_3$ adsorption, glycine precipitation, NaCl precipitations, dialysis, ultracentrifugation and sterile filtration. In virus validation studies, selected steps (stages) of the manufacturing process were employed to assess how effectively they could remove a range of viruses. These experiments were based on a scaled-down version of the purification process, which was validated to closely mimic the production scale manufacturing process. Furthermore, the virus inactivation capacity of pasteurization was validated for a wide range of viruses.

The studies were performed by deliberately adding cell culture derived stocks of either blood-borne viruses or appropriately selected model viruses to product intermediates derived from actual Haemate P/Humate-P production lots. The amount of virus infectivity that was removed or inactivated by a subsequent stage or stages of the manufacturing procedure was demonstrated in virus validation studies. The extent of virus removal/inactivation at each individual stage or combination of stages of the production process was tested at least twice, independently. The results of the virus validation studies (in down-scaled procedures) can be used to predict the effectiveness of the manufacturing process in inactivating or removing viruses. The use of a wide range of viruses in these validation studies also

provided indirect evidence that the production process could effectively inactivate or remove novel or unpredictable viral contamination.

'Robustness' studies

In addition to providing an accurate reflection of the full-scale manufacturing procedure, down-scaled versions of the production process were also used in so-called 'robustness' studies. These robustness studies evaluated the production parameters that may have an influence on virus inactivation and/or removal. These include parameters such as concentrations of protein and other components such as precipitating agents, temperature, pH, reaction time, the amount of stabilizer and the temperature of the pasteurization step. These robustness studies were performed in the laboratory using parameters at and beyond the range specified for routine production, and the impact of these extreme production parameters on the effectiveness of virus reduction steps was assessed.

New emerging pathogens

Blood-borne viruses are known to be present in the general population and therefore potentially in blood/plasma donors. The processes outlined above, including selection of donors, screening of donations by serological methods and NAT/PCR assays, detect these currently known relevant viruses, resulting in the exclusion of potentially infectious donations. However, because emerging infectious diseases are a continual threat to the integrity of the starting material, CSL Behring diligently examines all available information on emerging pathogens. Emerging microbial pathogens or parasites are not considered to be a threat to plasma-derived products because of the process of sterile filtration, which takes place prior to the preparation of the finished product and would remove these pathogens. However, the so-called 'new emerging viruses' have to be assessed very carefully with regard to their potential impact on the safety of the plasma pool for fractionation – the starting material for Haemate P/Humate-P and other plasma-derived products.

New emerging viruses

New emerging viruses may be novel zoonotic viruses that are encountered as humans enter new geographical areas and come into contact with (previously undiscovered) animal viruses crossing a species barrier to enter the human disease chain, such as HIV, Yellow Fever virus, severe acute respiratory syndrome coronavirus (SARS-CoV), menangle virus, hendra virus or nipah virus as well as hantaviruses or monkeypox virus. Furthermore, a 'new' virus can emerge/re-emerge in new geographical regions, such as WNV, which was known in Africa and the Middle East, but emerged in North America in 1999 [30]. 'New' viruses may also occur *de novo* by mutation (e.g. influenza viruses). Improved diagnostic methods have resulted in the detection of previously unknown viruses (which should not be considered as emerging viruses but rather as established agents that have been detected or described for the first time), e.g. B19V, which was first detected in 1975 [31], HCV, which was detected in 1989 [4], human herpesvirus type 8 (HHV8), which was discovered in 1996 [32], or transfusion transmission virus (TTV), which was detected in 1997 [33]. It should be noted that viruses detected for the first time because of improved diagnostic methods may be viruses without current attributable symptoms of diseases, such as TTV, the human herpes virus types 6 and 7 (HHV6 and HHV7) [34,35] or GB virus A, GB virus B and GB virus C/hepatitis G virus [36,37], even if some of these viruses were originally detected in the context of clinical symptoms in patients.

CSL Behring diligently assesses the potential threat of emerging diseases with regard to the safety of Haemate P/Humate-P and other plasma-derived products. As outlined by Ludlam *et al.* 2006 [38], newly described agents are of concern regarding plasma-derived products. To meet the challenge for Haemate P/Humate-P, the potential epidemiology of new emerging pathogens in the donor population and the potential load of infectious viruses in a donation during the symptomless incubation period have to be addressed. Emerging viruses cannot be excluded from the donor population, but diligent surveillance of any available information on new emerging viruses may result in (temporal) deferral of donors based on geographical risk, in compliance with regulatory guidance (e.g. WNV, SARS-CoV).

Limited data are available on the virus load of potentially emerging viruses in the donor population. For WNV, the virus load in an asymptomatic donor is generally less than 100 infectious units per mL of blood [39]. For SARS-CoV, a relevant titre in plasma can be excluded, because even in a clinical case the maximum number of genome copies that can be detected is 10^4 mL^{-1} [40], and in a preclinical situation, the detectable virus titre is in the order of 200 genomic copies per mL [41]. Donors may donate during the incubation period provided they have not been deferred based on their geographical and/or travel history. However, the potential virus load in

Table 6. Inactivation of emerging pathogens by pasteurization (60°C for 10 h in aqueous stabilized solution) [CSL Behring on file].

Virus	Inactivation factor ($\log_{10}$)	Time to complete ablation of viral infectivity (h)
Enveloped viruses		
SARS-CoV; TGEV*	≥ 4.0; ≥ 4.1 $(\geq 6.1)^{\dagger}$	1; 1 (4)[†]
Influenza A viruses		
FPV/Rostock/34 (H7N1)	≥ 5.4	1
Chick/Pennsylvania/1/83 (H5N2)	≥ 4.9	2
PR/8/34 (H1N1)	≥ 4.9	1
Non-enveloped virus		
HEV/FCV[‡]	≥ 5.0	1

*Emerging virus studied/model virus studied: BVDV, bovine viral diarrhoea virus; SARS-CoV, SARS coronavirus; TGEV, transmissible gastroenteritis virus; WNV, west nile virus.
[†]Different detection limits at 1 and 4 h.
[‡]Human virus (model virus studied): HEV, hepatitis E virus; FCV, feline calici virus.

such a donation, and in particular, the plasma pool for fractionation would be low; thus, the virus reducing capacity of the manufacturing process would effectively reduce the quantity of these emerging viruses.

In studies assessing the ability of pasteurization to inactivate a wide range of viruses, a selection of emerging viruses or their relevant model viruses was employed. Table 6 shows the very effective virus inactivation capacity of pasteurization for a range of new emerging viruses, demonstrating an effective reduction of infectivity within the 10-hour pasteurization process.

Prions

Transmissible spongiform encephalopathies (TSEs) encompass a group of fatal neurodegenerative diseases of animals and man and include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle and CJD in man. TSEs are characterized by the accumulation of the disease-associated prion protein (PrP^{Sc}) – an abnormal isoform of the cellular prion protein (PrP^{C}) – in the central nervous system. PrP^{Sc} may also accumulate in other tissues, depending on the host and type of TSE involved. In 1996, vCJD was identified [42]. Epidemiological and scientific data indicated a strong association between vCJD and consumption of meat products derived from BSE-infected cattle. In patients with vCJD, abnormal prions are present in both the central nervous system and the lymphoreticular system, which has given rise

to the possibility of blood-borne transmissions. Experimental studies in sheep and rodents have confirmed transmission of TSE through blood from animals in either the preclinical or clinical stage of disease [43,44]. The potential transmission of vCJD by blood transfusion is also reported by Hewitt et al. 2006 [45] (see below).

Prevention of transmission of vCJD from plasma-derived products

Up till now, abnormal prion proteins have never been detected in the blood of an infected human, even if they can be detected in the lymphoid tissue of the same individual [46,47]. The transmission of prions has never been reported from plasma or plasma-derived medicinal products, in contrast to the probable transmission of vCJD in four cases by transfusion of non-leuco-depleted red blood cell concentrates in the United Kingdom [45,48–50]. As these patients were UK residents, they may have had dietary exposure to BSE; however, the likelihood of infection from BSE can be considered far lower than the transmission of prions by the blood transfusion.

Laboratory studies using animals have shown that the cellular components of blood are more likely to contain the pathogenic agents for vCJD (PrP^{Sc}) [51]. Splenic lymphocytes can acquire prions possibly from follicular dendritic cells and have been implicated in the pathogenesis of vCJD, although infectivity does not appear to be associated with circulating lymphocytes [52]. This link between leucocytes and infectivity remains controversial; consequently, the effect of leuco-depletion on vCJD transmission from an infected donor remains uncertain. Even so, since October 1999, leucocytes have been removed from all blood used for transfusion in the United Kingdom [53].

Plasma that is used for fractionation in the production of Haemate P/Humate-P is always leuco-depleted because of the collection method (plasmapheresis plasma) and processing (leuco-depleted, recovered plasma from a whole blood collection). In addition to leuco-depletion, other measures have been implemented to minimize the theoretical risk of contaminating plasma pools with human TSEs. As a precaution, any donors who may potentially present a risk are permanently excluded from donating plasma. This includes donors who have

1 a clinical diagnosis of CJD or vCJD, or any donors suspected of having CJD or vCJD;
2 an increased risk of developing these diseases as recipients of dura mater grafts or substances

derived from human pituitary glands (e.g. growth hormone or gonadotropin);

3 one or more blood relatives diagnosed with CJD and

4 geographical deferral recommendations, including travel and residence in the United Kingdom for a defined period of time from 1980 to 1996 according to regulatory guidance.

To minimize the theoretical risk of human TSE contamination of plasma pools, donors who may present a risk are excluded permanently from donating plasma, as specified in the current FDA Guidance Document [54], which provides guidance for donations collected in the USA. Similar measures are implemented for European donors and plasma-derived protein concentrates marketed in Europe [55], whilst CSL Behring's quality and delivery requirements (a standard contract between plasma supplier and CSL Behring) include these or even more stringent measures. Unpooled units of plasma from donors at risk of developing CJD or those previously unrecognized as being at increased risk of acquiring CJD (according to the above mentioned criteria) are interdicted and excluded from the manufacturing process as a precautionary measure to further minimize the theoretical risk of CJD contamination of plasma pools. In the unlikely event that a donor is diagnosed with vCJD or clinically suspected of having vCJD subsequent to making a donation and after the plasma is processed into a finished product, the batches of affected products are quarantined and withdrawn from the market in close cooperation with the responsible regulatory authority.

As a precautionary measure, CSL Behring does not use plasma from UK donors because the UK has significantly more vCJD cases than any other country [163 deaths have been attributed to either definite or probable cases of vCJD and three people are currently living with definite/probable vCJD (up to January 2008)]. There have been 23 cases of vCJD in France (up to January 2007), and for that reason, CSL Behring currently does not use French plasma, and has not done so since the first vCJD case was reported in France (however, plasma from French donors is generally accepted for fractionation because the risk-benefit ratio is considered favourable [55] by the European authorities, but not by the FDA).

All guidelines and regulations of the concerned authorities are followed wherever plasma is collected or products are marketed. Because of the implementation of these precautionary measures, there is a very low risk of collecting plasma from a donor who subsequently develops vCJD. In addition, based on animal studies, the amount of prion protein present in the plasma of a donor who gives blood whilst in the incubation period for vCJD is considered to be very low [56].

Evaluation of prion protein removal during the Haemate P/Humate-P manufacturing process

Despite the very low risk of vCJD transmission associated with plasma, the ability of manufacturing process steps to eliminate prions is considered. Prion removal studies have been used to evaluate how effective selected steps of the Haemate P/Humate-P manufacturing process are in removing prion proteins [57].

Because PrP^{Sc} has never been isolated from human blood or plasma, prion proteins for use in investigational studies are obtained from deliberately infected laboratory animals. These primarily include the hamster-adapted scrapie agent 263K, which is isolated from hamster brains and used to model human prions. As there is no knowledge of the physico-chemical nature of the potential prion contaminants in plasma, two different prion spike preparations are employed in these evaluation studies, namely, microsomes (membrane-associated prions) and purified PrP^{Sc} (a detergent-extracted, non-membrane associated form of infectious prion protein) [58].

Laboratory studies are performed by spiking experimental plasma pools and an intermediate in the manufacturing process with prion preparations. The conformation-dependent immunoassay (CDI), according to Safar *et al.* [59] described below, is used to quantify the amount of prion material present. Prions are quantified in the spiked starting material, the final sample and inside fractions obtained during the down-scaled manufacturing process to determine the extent of prion reduction.

Conformation-dependent immunoassay

The CDI utilizes the anti-PrP monoclonal antibody, 3F4 (mAb 3F4), which recognizes an epitope present on both forms of the prion protein, PrP^C and PrP^{Sc}. In the native conformation of PrP^{Sc} – the infectious isoform – the epitope recognized by mAb 3F4 is not accessible, whilst in the denatured form of PrP^{Sc} as well as in the native and denatured forms of PrP^C (the normal, non-infectious, cellular isoform), the epitope is exposed and is fully accessible [59].

Conformation-dependent immunoassay records the amount of prion protein by using time-resolved fluorescence (TRF) values to obtain a 'signal'. A sample is tested in parallel in the native form as well as in the denatured form. If PrP^{Sc} is not present, the signals will be comparable because mAb 3F4 detects both the native and denatured forms of PrP^C. If PrP^{Sc} is present, the signal will be higher in the denatured sample because mAb 3F4 detects only the denatured form of PrP^{Sc}. Therefore, a sample contains PrP^{Sc} if the signal of a denatured sample divided by the signal of the sample before denaturation results in a ratio greater than 1.

Calculation of prion removal factors

To calculate the prion removal factor of a given manufacturing step, dilution series are constructed from samples of the manufacturing intermediate that have been collected from both before and after the manufacturing step was performed using prion-spiked starting material. The ratio for each sample dilution (TRF of denatured sample: TRF of native sample) is used to plot a curve in a double logarithmic diagram (Fig. 4) resulting in a trend line. To measure the level of prion removal during the manufacturing step, the distance between the trend lines of the two samples – starting material and final sample of the manufacturing step – is calculated in a parallel line model.

An improved version of the CDI, which is known as the sandwich CDI, has since been developed [60]. In the sandwich CDI, microtitre plates are coated with prion-sp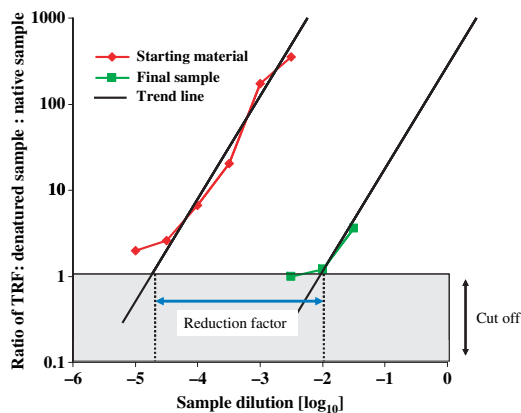ecific capture antibodies. This technique is far more sensitive because the capture antibody specifically enriches prion proteins bound to the plate, whilst in the direct CDI, binding of PrP^{Sc} is not very efficient [60]. Minor *et al.* [61] reported the results of an *in vitro* collaborative study that demonstrated very sensitive detection of prions by the CDI, especially by the sandwich CDI. When comparing the published data, the standard CDI has approximately the same sensitivity as the most sensitive western blot assay and the sandwich CDI is at least 30-fold more sensitive.

Overall prion reduction capability

Prion evaluation studies have shown that various steps in the Haemate P/Humate-P manufacturing process, when studied in combination, are highly effective in removing prion proteins. The overall reduction in prion protein during all the manufacturing steps studied is 6.4 log$_{10}$ for membrane-associated prions (microsomes) and 7.9 log$_{10}$ for purified detergent-extracted PrP^{Sc} (Table 7).

Further evaluations of manufacturing process steps

Additional data supporting the low risk of prion transmission by Haemate P/Humate-P resulted from spiking studies involving single manufacturing steps. The glycine precipitation step was studied employing human prion preparations (sporadic CJD and vCJD) as spiking material, in addition to the hamster-derived 263K scrapie agent. Furthermore, the prion reduction capacity of this precipitation step was studied using the scrapie strain 263K and a hamster bioassay (performed at the Federal Research Institute for Infectious Diseases of Animals, Tübingen, Germany).

The reduction in prion protein (microsomes or purified PrP^{Sc}) achieved during the glycine precipitation step of the manufacturing process was comparable for

Fig. 4. Evaluation of the prion reduction factor by employing a parallel line assay. Ratio of signal of denatured/native samples (dilution series) for prion-spiked starting material and final fraction (sample). The cut-off was defined as the mean ratio for the diluent (phosphate buffered saline/sarcosyl/bovine serum albumin) plus 3 standard deviations. TRF, time-resolved fluorescence.

Table 7. Prion reduction by the manufacturing process of Haemate P/Humate-P [77].

Manufacturing stage (studied as combined steps)	Reduction factor (log$_{10}$)	
	Microsomes	PrP^{Sc}
Cryoprecipitation, adsorption to Al(OH)$_3$, glycine precipitation and NaCl precipitation	3.5	3.9
Pasteurization, second NaCl precipitation, dialysis, ultracentrifugation and sterile filtration	2.9	4.0
Overall prion reduction factor	6.4	7.9

PrP^{Sc}, disease-associated prion protein.

Table 8. Prion reduction by glycine precipitation (part of the manufacturing process of Haemate P/Humate-P) [78].

Prion spike material	Reduction factor ($\log_{10}$)	
	CDI*	Bioassay[†]
Microsomes	2.2 ± 0.1	2.5 ± 0.4
Purified PrP^{Sc}	3.5 ± 0.3	2.9 ± 0.4

PrP^{Sc}, disease-associated prion protein; CDI, conformation-dependent immunoassay.
Prion quantification method: *biochemical methods (CDI) and [†]bioassay (endpoint dilution assay in hamsters).

both forms of prion preparations, regardless of their differing properties, measured either by CDI or hamster bioassay (Table 8). Consequently, the detection and quantification of the prion protein by biochemical methods is an appropriate method for evaluating the prion reduction factor during the manufacturing process of plasma-derived proteins.

Methods for prion quantification

When detecting human prions, Safar *et al.* [62] demonstrated that within the linear range, there was a good correlation between the PrP^{Sc} concentration measured by sandwich CDI and the prion titre measured by titration bioassays in mice, indicating that both assays have comparable sensitivity levels. In studies by Lee *et al.* [63], several plasma protein purification steps were tested by spiking the plasma intermediates with infected hamster brain homogenate and then evaluating the prion reduction capacity using either a western blot assay or a hamster bioassay. The results confirmed the suitability of using a biochemical method to detect and quantify prion protein – a western blot assay – for prion evaluation studies.

In another set of experiments by Gregori *et al.* [64], the removal of prion protein by cold ethanol precipitation steps was studied using either a hamster bioassay to detect prion proteins from the brain homogenate of 263K-infected hamsters or a western blot assay to detect PrP^{Sc} fibrils. Comparable prion reduction factors were demonstrated for both prion quantification methods. As the prion protein is considered to be the disease-causing agent [65], a biochemical/serological method of prion quantification should be sufficient in assessing the prion reduction factor.

Assessment of risk of vCJD transmission from Haemate P/Humate-P

To assess the potential risk of prion transmission by Haemate P/Humate-P, the potential quantity of both prion protein present in the starting material and the removal of prions during the manufacturing process must be considered. The epidemiology of vCJD indicates that currently only donors in the UK and France may pose a vCJD risk, in contrast to plasma donations from all other European countries and from the USA. Based on the donor deferral criteria implemented at CSL Behring, it can be assumed that donations from no more than one potentially infected donor would enter a plasma pool of sufficient size to produce one batch of Haemate P/Humate-P. In addition, the prion load in the donation would be very low. The level of infectivity in blood is estimated to be approximately two ID_{50} per mL of blood – where one ID_{50} has a 50% probability of causing an infection – when administered via intravenous therapy [53]. Therefore, even in the worst case scenario, the prion load in a plasma pool for fractionation is considered to be very low and any prions potentially present in the plasma pool would be removed effectively by the manufacturing process. Overall, the final product would present only a very remote risk of prion transmission.

In principle, the requirements for a safe product are met when the overall prion reduction factor clearly exceeds the potential prion load entering a manufacturing pool, leading to an adequate safety margin of the finished product (as stated for viruses, by the European Regulatory Authority [27]). The overall prion reduction factor (at least 6.4 $\log_{10}$) clearly exceeds the potential prion load and therefore the risk of vCJD transmission by the application of Haemate P/Humate-P is extremely remote.

Another way to assess the risk of prion transmission is to estimate the potential infectivity by prions per vial of product. This is calculated by imagining a scenario with the highest possible prion load in the starting material, then multiplying this prion concentration by the volume of plasma required to produce one vial. The resultant figure is then divided by the prion reduction factor obtained from evaluation studies [27,66]. Based on this worst case scenario, a safety factor of more than 6 $\log_{10}$ can be calculated which meets the sterility assurance level for micro-organisms according to the Pharmacopoeias worldwide and demonstrates very high margin of safety for Haemate P/Humate-P with respect to vCJD transmission.

There are several variables or even unknowns associated with the calculation of a safety margin, e.g. epidemiology of vCJD in the country where CSL Behring is collecting plasma for Haemate P/Humate-P, the potential prion load in the plasma of a donor

who develops vCJD after making the donation, the incubation period for vCJD assuming that infectious prions are present in the blood over the whole incubation period, the volume of the donations, and the yield of VWF/FVIII. As a result, a probability model is more appropriate for calculating the safety margin. This approach was used by the FDA to determine that a prion reduction factor of at least 4 $\log_{10}$ would result in FVIII products with a very remote risk of vCJD transmission [67]. Considering that the prion reduction factor for Haemate P/Humate-P is at least 6.4 $\log_{10}$, calculating the risk of vCJD transmission using this approach results in a negligible risk of transmission following the administration of Haemate P/Humate-P.

Batch-to-batch segregation, cleaning and sanitization of production equipment

Batch-to-batch segregation

Manufacturing areas and procedures utilized in Haemate P/Humate-P production are segregated to prevent cross-contamination of the product by adventitious agents. Appropriate materials have been used for construction of the production facilities and the movements of personnel and of product intermediates are regulated. The manufacturing process uses a dedicated workforce, materials, airlocks and the appropriate required classes of clean rooms. For example, air exchange rates and adequate pressure differentials between rooms of different classification are provided by appropriate heating, ventilation and air conditioning systems. In addition, the construction of the production facilities segregates pre-virus inactivation from post-virus inactivation areas.

Cleaning and sanitization of production equipment

Several studies have provided the assurance that any adventitious agent, potentially retained in the production system, would be adequately destroyed during the equipment sanitization procedure carried out prior to the next production cycle. Studies have demonstrated that treatment with sodium hydroxide (NaOH) or commercial alkaline cleaning solutions efficiently removes and/or inactivates all viruses tested to date [68]. These data agree with unpublished data from CSL Behring, demonstrating that all viruses studied were inactivated effectively even at room temperature (Table 9).

Inactivation or removal of TSEs during the sanitization and cleaning of the fractionation equipment have also been evaluated. TSEs are known to be

Table 9. Inactivation of pathogens by 0.1 M NaOH at room temperature.

Pathogen	Reduction factor ($\log_{10}$)	Time (h)	Reference
Virus			
HIV	≥7.3	≤1	*
BVDV	≥6.8	≤1	*
HHV-1 (HSV-1)	≥7.1	≤0.1	*
PRV	≥5.2	≤0.1	*
Poliovirus	7.2	2	*
HAV	4.0	2	*
BPV	≥6.4	≤0.1	*
CPV	≥6.7	≤0.1	*
Prion (brain homogenate)			
In suspension	≥3.5	≤0.1	[69]
Dried on steel plates	≥4.0	≤0.5	[79]
In suspension	4.0	0.25	[70]
Dried on steel plates (commercial alkaline cleaning solution; 2%)	≥3.3	≤0.1	*

BPV, bovine parvovirus; BVDV, bovine viral diarrhoea virus; CPV, canine parvovirus; HAV, hepatitis A virus; HHV-1, human herpes virus 1; HIV, human immunodeficiency virus; HSV-1, herpes simplex virus 1; PRV, pseudorabies virus.
*Unpublished data (CSL Behring on file).

resistant to a variety of physical and chemical methods; therefore, inactivation of these infectious prion proteins is particularly difficult to achieve. Certain treatments have proved to be reasonably effective, including autoclaving and soaking in ≥ 2% bleach or in 1–2 M NaOH for 1 h [57]. NaOH solutions are commonly used for cleaning of production equipment. Such a treatment also converts PrP^{Sc} into a protease-sensitive form, resulting in the inactivation of prions (Table 9) [69,70, CSL Behring on file]. This conversion of the prion protein by NaOH treatment results in loss of infectivity [71,72]. In summary, production equipment can be re-used following a sanitization period using appropriate concentrations of NaOH or commercial alkaline cleaning solutions.

Conclusion

During the 1970s and 1980s, because of HIV and HCV infections in patients with haemophilia, changes were made in the approach of production of plasma-derived products. When Haemate P/Humate-P was first produced, the manufacturing process relied heavily on pasteurization to inactivate infectious viruses. The current safety of this plasma-derived product can be attributed to pasteurization, further manufacturing steps and several additional safety measures; all designed to minimize the load of infectious viruses in either the plasma pool for

fractionation or the starting material for Haemate P/Humate-P production.

The current process (see Fig. 1) begins with the collection of plasma in approved collection centres from suitable healthy donors. The donations are screened for HBsAg and antibodies against HIV-1/-2 and HCV. The sensitivity of these screening assays has markedly increased over the last 20 years, to a point where, for example, detection rates of HBV assays have been reduced to at least 0.15 ng HBsAg per mL [9]. Because of the delay in antibody production after viral infection, it is possible for a serology assay to generate negative results despite the presence of a high load of infectious virus. The virus load of donations is reduced to a low level by NAT/PCR testing. Furthermore, a positive result from screening plasma donations will result in the removal of any previous donations that are being held in the 60-day inventory hold. This further reduces the potential virus load in a plasma pool for fractionation. These NAT/PCR-based approaches are highly sensitive with a low analytical detection limit of less than 10 IU mL^{-1} for the different virus genomes when using the mini pool assay.

Donations that are non-reactive in serological and NAT/PCR testing are pooled and enter the manufacturing process. During this process, the desired proteins are purified and concentrated; these manufacturing steps also contribute towards the removal of viruses, and their effectiveness has been scrutinized in virus validation studies. Product intermediate is pasteurized to inactivate viruses that may potentially be present; the effectiveness of the pasteurization process in inactivating a wide range of enveloped and non-enveloped viruses has been thoroughly documented. Experimental evidence suggests that all of these procedures are effective in removing or inactivating a wide range of viruses with diverse physico-chemical characteristics, including enveloped and non-enveloped viruses. Although non-enveloped viruses are considered to be more resistant to heat, the pasteurization process alone accounts for a virus inactivation factor of about 4 log$_{10}$ for both HAV and B19V.

The concept of a virus safe Haemate P/Humate-P is based on careful selection of donor centres, screening of each donation for transfusion-relevant viruses and subsequently, the release of the plasma pool for fractionation based on non-reactivity results for HAV, HBV, HCV, HIV-1 and high titres of B19V from sensitive NAT/PCR assays and the virus reduction capacity of the manufacturing process of Haemate P/Humate-P. The virus reduction capacity of the Haemate P/Humate-P manufacturing process includes, especially, the dedicated virus inactivation step of pasteurization and further manufacturing steps that reliably contribute to the overall virus reduction. This approach is in agreement with the European guideline [26] where a so-called 'second step' is required to close the gap of the 'first step' for certain types of virus (for example, an additional manufacturing step is required to reduce non-enveloped viruses from products that are treated only with solvent/detergent).

New emerging viruses that may pose a risk to the safety of plasma-derived products are also subjected to pasteurization evaluation studies. These studies use either the (potential) emerging virus itself or relevant model viruses to confirm that pasteurization will be effective in inactivating the potential threat. TSEs which are heat-resistant and cannot be detected by NAT/PCR because they lack nucleic acid are removed by the manufacturing process of Haemate P/Humate-P. Current prion evaluation studies have successfully shown that the prion reduction factor achieved during the manufacturing process clearly surpasses the potential prion load in the plasma pool.

In conclusion, virus detection measures and thorough validation of process parameters for virus reduction capacity have improved the safety margin of plasma-derived products. Continual improvement of pathogen detection techniques in donations and quality assurance systems for pathogen removal/inactivation procedures, together with surveillance for any new transmissible hazards, should ensure that this high standard of safety continues to be maintained in the future.

Conflict of interest

The author is an employee of CSL Behring.

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