Pathogen safety of a pasteurized four-factor human prothrombin complex concentrate preparation using serial 20N virus filtration

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BACKGROUND: Beriplex P/N/Kcentra/Confidex is a four-factor human prothrombin complex concentrate (PCC). Here, we describe the pathogen safety profile and biochemical characteristics of an improved manufacturing process that further enhances the virus safety of Beriplex P/N.

STUDY DESIGN AND METHODS: Samples of product intermediates were spiked with test viruses, and prions were evaluated under routine production and robustness conditions of the scale-down version of the commercial manufacturing process for their capacity to inactivate or remove pathogens. The PCC was characterized by determining the activity of Factor (F)II, FVII, FIX, FX, protein C, and protein S and the concentration of heparin and antithrombin III in nine product lots.

RESULTS: The manufacturing process had a very high virus reduction capacity for a broad variety of virus challenges (overall reduction factors $\geq 15.5$ to $\geq 18.4$ log for enveloped viruses and $11.5$ to $11.9$ log for nonenveloped viruses). The high virus clearance capacity was provided by two dedicated virus reduction steps (pasteurization and serial 20N virus filtration) that provided effective inactivation and removal of viruses and a purification step (ammonium sulfate precipitation and adsorption to calcium phosphate) that contributed to the overall virus removal capacity. The diethylaminoethyl (DEAE) chromatography and ammonium sulfate precipitation steps removed prions to below the limit of detection. The levels of different clotting factors in the final product were well balanced.

CONCLUSION: The improved manufacturing process of Beriplex P/N further enhances the margin of pathogen safety based on its capacity to remove and inactivate a wide range of virus challenges.

rothrombin complex concentrate (PCC) with concomitant vitamin K is an effective treatment for rapid reversal of oral anticoagulation in bleeding patients and those in need of urgent surgery.1,3 PCCs are prepared from pooled plasma and contain coagulation factors with prohemostatic activity.4,5 Beriplex P/N/Kcentra/Confidex/Coaplex (hereafter referred to as Beriplex P/N) is a four-factor human PCC approved in 35 countries including the United States and Europe. Beriplex P/N is indicated for the treatment and perioperative prophylaxis of bleeding in 1) acquired deficiency of vitamin K–dependent coagulation factors6 and 2) in the European Union for congenital deficiency of vitamin K–dependent factors when specific coagulation factor products are not available.7 Beriplex P/N is derived from plasma donations and contains

ABBREVIATIONS: B19V = human parvovirus B19; BVDV = bovine viral diarrhea virus; CPV = canine parvovirus; DEAE = diethylaminoethyl; PCC(s) = prothrombin complex concentrate(s); PrPSc = proteinase-resistant pathogenic prion protein; PRV = pseudorabies virus; WNV = West Nile virus.

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Factor (F)II, FVII, FIX, FX, protein C, protein S, antithrombin III, and small amounts of heparin.\(^7\)

All biologic products derived from animal or human materials have a potential risk for the transmission of blood-borne viruses and, theoretically, prion proteins.\(^8\) To minimize this risk, multiple complementary safety measures are implemented in their manufacture such as sourcing plasma donations from healthy, low-risk donors; testing donations and plasma pools for certain viruses; and employing production steps that effectively reduce potential virus loads.\(^8,9\)

The effectiveness of these safety measures for Beriplex P/N has been established and confirmed in clinical studies and through pharmacovigilance. While the risk of virus transmission cannot be eliminated, no confirmed case of virus transmission in clinical trials or postmarketing experience has been reported with the use of Beriplex P/N since 1996 when large-pore (approx. 35 nm) virus filtration was added to the manufacturing process.\(^6\) In 2014, filtration over two small-pore (approx. 19 nm) 20N Planova filters connected in series (Fig. S1 [all supplementary material available as supporting information in the online version of this paper]) was introduced in place of the 75N/35N filter series, which served to further improve the safety margin of the manufacturing process. In this article, we focus on those manufacturing steps of Beriplex P/N that effectively inactivate or remove prions and viruses and the small-pore virus filtration step that further enhances the pathogen safety of the product.

**MATERIALS AND METHODS**

**Manufacturing process**

The key steps in the manufacturing process of Beriplex P/N that effectively inactivate or remove prions and viruses include: 1) ion-exchange chromatography and adsorption to diethylaminoethyl (DEAE) Sephadex A-50 resin (DEAE chromatography; prion reduction step); 2) pasteurization (heat treatment) at 60°C for 10 hours (dedicated virus inactivation step); 3) ammonium sulfate precipitation and adsorption to calcium phosphate (purification and prion reduction step); and 4) filtration using two Planova 20N virus filters in series, performed as a single dedicated virus removal step (Fig. S2).

**Bioanalytical characteristics**

The activity of the FII, FVII, FIX, FX, protein C, and protein S and the concentrations of excipients heparin and antithrombin III in Beriplex P/N were determined in nine product lots using automated analyzers (for coagulation factors, protein C, and excipients; Siemens) and enzyme-linked immunosorbent assay kits (for protein S, Stago).\(^10\)

**Scale-down models used to determine pathogen reduction capacity**

Two dedicated virus reduction steps (pasteurization and virus filtration) and a purification step (ammonium sulfate precipitation and adsorption to calcium phosphate) were examined for their capacity to inactivate or remove test viruses. DEAE chromatography and ammonium sulfate precipitation were tested for their capacity to remove prions. These experiments were performed in validated scale-down models of the commercial manufacturing process using process intermediates intentionally spiked with test viruses and prions, with scale-down factors ranging from 1300 to 4000 across the different steps. A scale-down model was not required for pasteurization because the protein content and purity remain constant for this step between the scale-down and commercial manufacturing process.

The critical process variables controlled during the different steps are presented in Table S1. Performance parameters evaluated for the acceptance of the scale-down design are shown in Table S2.

**Virus validation studies**

High levels of virus were spiked into aliquots of production intermediates, and the scale-down process steps were performed to assess their efficacy to remove or inactivate viruses.\(^8,9\) The virus reduction factor of the final sample was calculated as the logarithmic difference of virus load in the starting material to the virus load in the final sample (supplemental text file). Viruses were selected that 1) potentially contaminate blood plasma or 2) serve as a model for blood-borne viruses\(^8,9\) and 3) represent a wide range of physicochemical properties of viruses (Table 1).

In addition to studies at production variables, robustness studies were performed in scale-down experiments with parameters at or beyond the production specifications. Worst-case conditions were investigated to test a potential negative impact of variables on virus reduction.

**Dedicated virus reduction steps: pasteurization and serial 20N virus filtration**

For pasteurization, robustness was tested by examining critical process variables such as temperature (59°C) and stabilizer concentration (90%-110%) beyond the production process specification. For serial 20N filtration, all virus stocks were prefiltred before spiking the product intermediates to remove virus aggregates and small cell debris not removed by centrifugation of the virus harvest. Subsequently, aliquots of spiked intermediates were filtered under production conditions (with filter volume overload up to 125%) through two Planova 20N filters connected in series. Filtration was followed by a pressure release (to zero bar) for up to 10 minutes, and a subsequent postwash buffer chase (approx. 18% of final
### TABLE 1. Virus characteristics, virus reduction factors, and prion reduction factors

| Relevant virus | HIV-1 and-2 | HCV and other flaviviruses (e.g., Zika virus) | Large enveloped viruses, herpesviruses | WNV and other flaviviruses (e.g., Dengue virus, Zika Virus) | HAV | CPV | B19V |
|----------------|-------------|---------------------------------------------|----------------------------------------|-------------------------------------------------|-----|-----|------|
| Strain or isolate used | HTLV III B | Ug59 (DK) | Phylaxia | NY99-35262-11 | HM 175 | Witte | Donations with high titer† |
| Size (nm)<sup>10-13</sup> | 80-100 | 50-70 | 120-200 | 50 | 25-30 | 18-24 | 18-24 |
| Mean virus reduction factor (±SD)*, log | ≥5.9 | ≥8.5 | 3.8 (±0.5) | ≥7.4 | 4.0 (±0.2) | 0.5 (±0.1)§ | 3.5 (±0.7) |
| Pasteurization (n = 2-8) | ≥5.9 | 2.2 (±0.3) | 7.2 (±0.8) | NA | 1.8 (±0.5) | 1.5 (±0.1) | NA |
| Ammonium sulfate precipitation and adsorption to calcium phosphate (n = 2-5) | ≥6.6 | ≥6.0 | ≥6.6 | ≥8.1 | ≥6.1 | 6.5 (±0.1) | NA |
| Serial 20N virus filtration (n = 2) | ≥18.4 | ≥16.7 | ≥17.6 | ≥15.5 | ≥11.9 | 8.0 (±0.1) | 11.5jj |
| Overall mean virus reduction factor | ≥18.4 | ≥16.7 | ≥17.6 | ≥15.5 | ≥11.9 | 8.0 (±0.1) | 11.5jj |
| Mean prion reduction factor (±SD)*, log | Purified PrP<sup>Sc</sup> (n = 2) | Microsomes (n = 2) |
| Ion-exchange chromatography (DEAE) | 1.1 (±0.1) | 1.2 (±0.1) |
| Ammonium sulfate precipitation | ≥3.1 (±0.1) | ≥3.2 (±0.2) |
| Overall mean prion reduction factor | ≥4.2 | ≥4.4 |

*SD not calculated for mean values below the limit of detection.
†Obtained from single donations with high titre of B19V having been discarded from manufacturing of plasma-derived products at CSL Behring.
‡The mean overall virus reduction factor is calculated based on all individual reduction factors except in the case when the stage tested resulted in all experiments in complete virus reduction—then the highest reduction factor is used. Here, reduction factor depends solely on the virus load in the spiked starting material and the detection limit of the assay in the final sample.
§Virus reduction factors less than 1 were not used to calculate the overall virus reduction factor.
jjConsidering CPV removal capacity of virus filtration and ammonium sulfate precipitation and adsorption to calcium, the overall virus reduction factor for B19V can be calculated to be 11.5.

n = number of measurements; NA = not assessed.
volume) under the same filtration conditions. Robustness studies for filtration were performed using canine parvovirus (CPV)—the smallest virus tested—by setting potentially critical process variables beyond the production specifications (Table 2).

**Purification step: ammonium sulfate precipitation and adsorption to calcium phosphate**

In the scale-down studies, this step utilized centrifugation for removal of the ammonium sulfate precipitate rather than depth filtration as used in production scale (Fig. S2).

**Prion studies**

The removal of prions by DEAE chromatography and ammonium sulfate precipitation was investigated in a validated scale-down model. The studies used two prion spike preparations—microsomes and purified prion protein (PrPSc)—prepared from hamster brains infected with hamster-adapted scrapie strain 263K. Conformation-dependent immunoassay and a labeled prion-specific antibody were used for detecting PrPSc.14,15 The prion reduction factor was calculated in a manner consistent with that for the virus reduction factor.

**Statistical analysis**

Results are presented as arithmetic mean (±standard deviation [SD]).

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**Table 2. Robustness studies for the dedicated virus reduction steps (pasteurization and virus filtration)**

| Pasteurization variables | HIV | PRV* | HAV* | BVDV* |
|--------------------------|-----|------|------|-------|
| Pasteurization under production conditions † | ≥5.9 | 3.8 (±0.5) | 4.0 (±0.2) | ≥6.5 |
| Temperature 59°C | ND | 2.6 | 3.9† | ≥6.4 |
| Stabilizer 90% | ND | 4.5 | ND | ≥6.1 |
| Stabilizer 109%/110% | ND | 2.8 | 3.4 | ≥6.3 |
| 0% stabilizer, 0% protein in buffer | ND | ≥4.9‡ | ND | ND |
| Temperature 58°C and stabilizer 109% | ≥4.5 | ND | ND | ND |

Filtration variables

| Mean virus reduction factor (±SD), log | CPV§ |
|---------------------------------------|-----|
| Virus filtration under production conditions (n = 2) | 6.5 (±0.1) |
| High protein concentration at maximum temperature | 5.5 |
| Low protein concentration at maximum temperature | ≥6.9 |
| Minimum inlet pressure at maximum temperature | 6.5 |
| Low protein concentration at maximum temperature | ≥6.9 |
| Minimum inlet pressure at maximum temperature | 6.5 |
| Low pH at minimum temperature | 6.2 |
| High pH at minimum temperature | ≥6.7 |
| Overload volume per filter | >6.7 |
| Three pressure drops to 0 bar | 6.5 |
| Placebo buffer without protein | ≥6.5 |
| 1× 20N only | 4.4 |

*PRV, n = 1 for all tested robustness conditions; HAV, n = 3 for all tested robustness conditions; BVDV, n = 2 for all tested robustness conditions.
†Pasteurization conditions: 60°C, 99%/100% stabilizer; n = 2 for HIV, n = 1 for PRV, n = 9 for HAV, and n = 7 for BVDV.
‡Complete virus inactivation after 5 minutes of heat treatment.
§The smallest of the tested viruses is presented to demonstrate the robustness of the virus filtration step. n = 1 for all robustness conditions. n = number of studies; ND = not determined.

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**RESULTS**

The manufacturing process of Beriplex P/N results in a lyophilized plasma protein preparation of human PCC suitable for intravenous use after reconstitution.

**Bioanalytical characterization**

The different clotting factors and the coagulation inhibitors protein C and protein S, heparin, and Antithrombin III were well balanced and within the acceptance criteria for the nine lots of Beriplex P/N that were tested. The mean (±SD) ratio between FII and FIX was 1.0 (±0.1; Table S3).

**Scale-down models used to determine pathogen clearance capacity**

Percent protein yields of the scale-down models and commercial manufacturing process were comparable during the steps of ion-exchange chromatography, ammonium sulfate precipitation and adsorption to calcium phosphate and virus filtration, and within the 3 × SD acceptance range of production scale (Table S4). The mean (±SD) protein yield for the ammonium sulfate precipitation and adsorption step was slightly higher in the scale-down experiments (9.9% [±1.0%]) than in the production scale (7.1% [±1.6%]); however, it remained within the 3 × SD acceptance range. Thus, the scale-down process was...
considered to reflect manufacturing conditions based on a worst-case approach concerning virus removal. The analytical characteristics were shown to be comparable between the scale-down and the commercial-scale process for the key steps (Table S3).

Virus validation studies
The manufacturing process of Beriplex P/N has a very high capacity for virus removal or inactivation, with the overall virus reduction factors ranging from 11.5 to at least 18.4 log for both enveloped and nonenveloped viruses (Table 1). Pasteurization was effective in inactivating the West Nile virus (WNV) to below the limit of quantification within 1 hour, human immunodeficiency virus (HIV) within 2 hours, and bovine viral diarrhea virus (BVDV) within 3 hours (Fig. S3). Pseudorabies virus (PRV; a model for herpes viruses) and hepatitis A virus (HAV) were effectively heat inactivated (<1 log reduction), but the human parvovirus B19 (B19V) was substantially inactivated (3.5 log reduction; Table 1). Test viruses that were effectively inactivated (HIV, HAV, PRV, and BVDV) were also studied under robustness conditions; the capacity to inactivate HAV, HIV, and BVDV was not significantly affected (Table 2). For PRV, the mean virus reduction factor was slightly decreased at higher stabilizer concentration (109%; never occurring under standard production conditions) and at a lower temperature of 59°C (Table 2). No significant impact on the reduction factors was observed for the other viruses tested (WNV, CPV, and B19V; data not shown).

The virus filtration step using two Planova 20N filters in series decreased the virus load of enveloped viruses and nonenveloped virus HAV to below the limit of detection (Table 1). Notably, virus reduction factors of medium to smaller sized viruses (BVDV, HAV, and CPV) were considerably enhanced versus 75N/35N filtration (Table 3). Even CPV, the smallest virus within the virus panel, was effectively removed (6.5 log); robustness of the virus filtration step for this virus is presented (Table 2). Effective removal of CPV (4.4 log) was even achieved when only one 20N filter was used. The ammonium sulfate precipitation and adsorption to calcium phosphate step was effective in removing HIV to below the limit of detection (≥5.9 log); PRV was also highly effectively removed (7.2 log). BVDV, HAV, and CPV were moderately reduced (Table 1).

Prion evaluation studies
Percent protein yields of the scale-down models and the commercial manufacturing process were comparable during the steps of DEAE chromatography and ammonium sulfate precipitation and within the 3 × SD acceptance range of the production scale (Table S5). The DEAE chromatography step reduced both prion spikes equivalently (purified PrPSc, 1.1 log; and microsomes, 1.2 log). The ammonium sulfate precipitation step also reduced both prions spikes to below the limit of detection (≥3.1 log for purified PrPSc and ≥3.2 log for microsomes). The overall prion reduction factors were at least 4.2 log for purified PrPSc and at least 4.4 log for microsomes (Table 1).

| Virus/virus filter* | 20N/20N† | 75N/35N† | 1 x 35N |
|---------------------|----------|----------|---------|
| BVDV                | 2        | 11       | ND      |
| Number              |          |          |         |
| Mean VRF (±SD), log| ≥6.0     | 4.2 (±0.5)| ND      |
| HAV                 | 2        | 6        | ND      |
| Mean VRF (±SD), log| ≥6.1     | ND       | 2.5 (±0.3)|
| CPV                 | 2        | 8        | <1      |
| Number              |          |          |         |
| Mean VRF (±SD), log| 6.5 (±0.1)| <1       | <1      |

*Filtration conditions between the 20N/20N process, the previous 75N/35N process, and research studies for 35N filtration differed slightly with regard to filter load (lower volume and lower or no protein load for the 75N/35N studies) and type of filtration (tangential flow or dead end filtration). Input pressure was always set around 0.9 bar.
†Performed with Beriplex intermediate.

DISCUSSION
The manufacturing process of Beriplex P/N results in a stable (shelf life of 3 years at ≤25°C), lyophilized preparation of PCC with a high margin of safety regarding blood borne viruses. Analytical tests demonstrate that the levels of different clotting factors in Beriplex P/N are well-balanced. The thrombogenic potential of a PCC is largely determined by its concentration of FII. Older PCCs containing greater quantities of FII than FIX had a higher risk of thrombogenicity. In Beriplex P/N, the mean (SD) ratio of FII to FIX is 1.0 (±0.1); that is, the product contains an equal quantity of FII and FIX and, therefore, administration of a sufficient dose of FIX is not accompanied by a high, unbalanced dose of FII. 

Biologic materials such as human antithrombin III (CSL Behring), human albumin (CSL Behring), and porcine heparin are added before the virus filtration step. These are manufactured using validated virus reduction steps that provide effective virus clearance (data not shown) in addition to the Beriplex P/N virus filtration. Addition of antithrombin III and heparin to Beriplex P/N ensures that the coagulation factors in the finished product are inactive. Normally, the coagulation factors get activated after release of the tissue factor after any injury to the vascular endothelium. PCCs containing activated factors are associated with an increased risk of
thrombosis.\textsuperscript{18-21} It was demonstrated that the mere presence of heparin was not sufficient to reduce the thrombogenic potential of a PCC, and addition of antithrombin III reduced the thrombogenic potential of a PCC known to cause thrombosis.\textsuperscript{17} The manufacturing process of Beriplex P/N not only adds these components, but also controls for activated coagulation factors during batch release.

Pasteurization was effective under production and robustness conditions for inactivating all enveloped viruses tested except the herpes virus, PRV, which is known to be moderately heat resistant in the presence of sucrose.\textsuperscript{22} Substantial inactivation of PRV was observed under all conditions studied. Under routine manufacturing conditions, the virus was inactivated by a mean reduction factor of 3.8 log over 10 hours and the overall process demonstrated a very high virus reduction capacity for PRV. Based on the rapid inactivation of BVDV and WNV, the pasteurization step would be expected to provide similarly high reduction capacity for emerging arboviruses such as dengue virus, Zika virus, and chikungunya virus. The method remained effective for inactivating HIV, BVDV, and WNV despite varying critical process variables such as temperature and stabilizer concentration. Among the nonenveloped viruses, HAV was effectively inactivated. The animal parvovirus (CPV), which is known to be highly resistant to physicochemical treatment, was inactivated only to a limited degree. However, B19V was inactivated with a mean reduction factor of 3.5 log consistent with previous reports that have shown that human B19V is more susceptible to heat inactivation than animal paroviruses.\textsuperscript{23,24}

Percent protein yields of scale-down models were comparable with those in commercial manufacturing process for all the steps tested. The slightly higher protein yield in the supernatant for the ammonium sulfate precipitation and adsorption step may be attributed to the use of centrifugation for separation in scale-down experiments (vs. filtration in production scale), which is a less effective method for precipitate removal.

The effectiveness of virus filtration using 20N filtration in series was tested for all viruses. HIV, BVDV, PRV, and HAV were effectively removed to below the limit of detection. The serial 20N virus filtration step provided highly effective reduction of the small parvovirus model CPV (6.5 log) and the robustness of this removal step was clearly demonstrated for diverse, potential worst-case conditions. The ammonium sulfate precipitation and adsorption to calcium phosphate purification step was highly effective for HIV and PRV, and moderately for HAV, CPV and BVDV, thus contributing to the overall safety of the Beriplex P/N manufacturing process.

The overall mean reduction factors for the enveloped viruses ranged from at least 15.5 log (for WNV; ammonium sulfate precipitation and adsorption to calcium phosphate purification step not studied) to at least 18.4 log (for HIV). For nonenveloped viruses, the virus reduction factor was 11.5 log for paroviruses and at least 11.9 log for HAV. The introduction of the Planova 20N serial virus filtration step, in place of the Planova 75N/35N virus filtration step, further enhances the virus reduction capacity of the production process, especially for small (approx. 20 nm) and medium sized viruses (approx. 50 nm; Table 3). Furthermore, the testing, identification, and exclusion of positive plasma units during the screening of donors and plasma also contribute to the overall virus reduction capacity of the manufacturing process. The overall virus reduction factors significantly exceed the worst-case virus concentrations that could be present in the starting material, that is, the plasma required to produce one vial of Beriplex P/N. Thus, the manufacturing process of Beriplex P/N provides a substantial margin of safety with a capacity to reduce a wide range of viruses with diverse physicochemical characteristics, and it can be concluded that the overall manufacturing process is effective for the enveloped blood-borne viruses HIV, HCV, and HBV; arthropod transmitted viruses; and the nonenveloped viruses HAV and B19V.

Prions are the causative agents of Creutzfeldt-Jakob disease (CJD), variant CJD, and other related neurodegenerative disorders in animals and humans. Prion spike experiments showed that the two steps studied—DEAE chromatography and ammonium sulfate precipitation—were effective in reducing prions. In addition, cryoprecipitation, adsorption to calcium phosphate, and filtration using two 20N filters in series are likely to contribute to overall prion reduction, although these steps were not studied in prion spike studies. It was shown that virus filtration has a prion reduction capacity of approximately 2 to 5.5 log.\textsuperscript{25,26} Thus, it is assumed that 20N serial filtration would also enhance the overall prion reduction capacity of the manufacturing process of Beriplex P/N.

In conclusion, the improved manufacturing process of Beriplex P/N using 20N Planova filters in series is effective in further enhancing the safety margin of the product, especially for small nonenveloped viruses. The pathogen safety has been clinically demonstrated through pharmacovigilance reporting. The manufacturing process also results in well-balanced levels of different clotting factors.

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\textbf{CONFLICT OF INTEREST}

All authors are current or previous employees of CSL Behring.
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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s website.

Fig. S1. Illustration of serial filtration. Note: Serial filtration is operated as a single manufacturing step with
two 20N Planova filters connected directly one after the other, in dead end configuration mode with no tank in between the filters.

**Fig. S2.** Outline of the manufacturing process of Beriplex P/N showing the product intermediates and the pathogen reduction steps. *Rigorous selection of donor populations and screening of donations and the plasma pools for fractionation by nucleic acid amplification technique ensure that the starting material for Beriplex P/N is non-reactive for certain blood borne viruses; the testing, identification and exclusion of a reactive plasma unit has a virus reduction capacity of more than 5 log_{10}† Ammonium sulphate precipitate is removed by depth filtration (versus centrifugation in scale-down models).

**Fig. S3.** Kinetics of virus inactivation during pasteurization for enveloped (A) and non-enveloped (B) viruses. B19V: human parvovirus B19; BVDV: bovine viral diarrhoea virus; HAV: hepatitis A virus; HIV: human immunodeficiency virus; N: number of measurements; PRV: pseudorabies virus; SD: standard deviations; WNV: West Nile virus. Given is the mean (SD) (N = 1–9). The arrows mark the time points at which the virus titre was below the limit of detection. For WNV and HIV, results are presented for the time point when virus titre was below the limit of detection, and 1 timepoint thereafter. N = 1 for B19V. *A different volume was used for testing at this time point.

**Table S1.** Main critical process parameters controlled during different manufacturing steps

**Table S2.** Performance parameters evaluated for the acceptance of the scale-down design

**Table S3.** Characteristics of the lyophilized PCC product

**Table S4.** Comparison of the mean yield for different parameters between the production scale process and the scale-down model for the steps IEC, ammonium sulphate precipitation/adsorption to calcium phosphate, and virus filtration

**Table S5.** Comparison of the mean yield for different parameters between the production scale process and the scale-down model for prion evaluation studies