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ORIGINAL RESEARCH ARTICLE

Pathogen Safety of a New Intravenous Immune Globulin 10% Liquid

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Abstract

Background The manufacturing process of a new intravenous immune globulin (IVIG) 10% liquid product incorporates two dedicated pathogen safety steps: solvent/detergent (S/D) treatment and nanofiltration (20 nm). Ion-exchange chromatography (IEC) during protein purification also contributes to pathogen safety. The ability of these three process steps to inactivate/remove viruses and prions was evaluated.

Objectives The objective of this study was to evaluate the virus and prion safety of the new IVIG 10% liquid.

Methods Bovine viral diarrhea virus (BVDV), human immunodeficiency virus type 1 (HIV-1), mouse encephalomyelitis virus (MEV), porcine parvovirus (PPV), and pseudorabies virus (PRV) were used as models for common human viruses. The hamster-adapted scrapie strain 263K (HAS 263K) was used for transmissible spongiform encephalopathies. Virus clearance capacity and robustness of virus reduction were determined for the three steps. Abnormal prion protein (PrP^{Sc}) removal and infectivity of the samples was determined.

Results S/D treatment and nanofiltration inactivated/removed enveloped viruses to below detection limits. IEC supplements viral safety and nanofiltration was highly effective in removing non-enveloped viruses and HAS 263K. Overall virus reduction factors were: $\geq$9.4 $\log_{10}$ (HIV-1), $\geq$13.2 $\log_{10}$ (PRV), $\geq$8.2 $\log_{10}$ (BVDV), $\geq$11.7 $\log_{10}$ (MEV), $\geq$11.6 $\log_{10}$ (PPV), and $\geq$10.4 $\log_{10}$ (HAS 263K).

Conclusion Two dedicated and one supplementing steps in the manufacturing process of the new IVIG 10% liquid provide a high margin of pathogen safety.

Key Points

The manufacturing process for products derived from human plasma is required to include steps to remove any potential infectious agents.

Three process steps of a new liquid intravenous immune globulin product were investigated regarding their pathogen safety capacity.

It was demonstrated that the manufacturing process is capable of inactivating/removing viruses and prions and provides a high margin of safety.

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1 Introduction

Intravenous immune globulin (IVIG) products derived from human plasma play an important role as replacement therapy in the treatment of primary and secondary immune deficiencies with recurrent infections, and as immunomodulatory therapy in autoimmune diseases [1–5]. The development of effective IVIG products marks an important advance in the treatment of severe antibody deficiencies, and current IVIGs are considered to be very safe in clinical practice with respect to the transmission of viruses [6–9].

The manufacturing process for any medicinal product derived from human plasma is required to include steps to remove any potential infectious agents [10, 11]. A number of processes are mandatory before relevant regulatory authorities grant marketing authorization for these products. These processes include donor selection, screening of individual donations and plasma pools, a look-back procedure for retrospective identification of any infectious donation entering the production process by the retraceability of each single donation, and a defined communication procedure between manufacturer and plasma supplier, validated production processes that include effective measures to inactivate and/or remove a wide range of viruses and other infectious agents (such as the prions that cause transmissible spongiform encephalopathies [TSEs], e.g., variant Creutzfeldt-Jakob disease [vCJD]), as well as adherence to good manufacturing practice (GMP) during production [10–16]. Steps for removal and/or inactivation of viruses and prions are always necessary because screening of donors and plasma donations is limited by the number of viruses for which they are screened and the sensitivity of the tests. Furthermore, there is currently no screening test for the detection of prion diseases available [12] as this is still under investigation [17]. Therefore, effective and robust inactivation and/or removal procedures need to be incorporated into the manufacturing processes used in the production of IVIGs.

Steps for the pathogen safety of human plasma-derived IVIG products are important and may include pasteurization, solvent/detergent (S/D) treatment, caprylate treatment, inactivation by low pH with elevated temperature, cold ethanol precipitation, chromatography, and nanofiltration [18–26]. These procedures have been comprehensively examined in several recent literature reviews [9, 27–29].

The new IVIG 10% liquid (Panzyga®) was developed by Octapharma AG (Lachen, Switzerland) and the first marketing authorization was granted by the Paul-Ehrlich-Institut (Langen, Germany) in 2016. In addition to its application in traditional primary immune deficiency, this new IVIG product has been investigated in immune thrombocytopenic purpura. It is a high-purity glycine-formulated human normal immune globulin product using a production process designed to provide a more efficient extraction of gammaglobulin from plasma. To ensure optimal efficacy and safety of the new IVIG product (Panzyga®), three steps—S/D treatment, ion-exchange chromatography (IEC), and nanofiltration—used during the manufacturing process were validated for pathogen safety of the final IVIG product. This article describes these individual steps and the results of the study evaluating their effectiveness in inactivating and/or removing pathogens.

2 Materials and Methods

2.1 Pathogen Safety Steps in the Manufacturing Process

The manufacturing process of the new IVIG product (Panzyga®) starts with the fraction I + II + III obtained by cold ethanol precipitation based on the Kistler-Nitschmann fractionation method. The further process comprises protein precipitation, IEC, S/D treatment, S/D removal, nanofiltration, ultrafiltration/diafiltration for protein adjustment, and final formulation. Further information about the product is given elsewhere [30]. Three pathogen safety steps are included in this manufacturing process. The S/D treatment was implemented to inactivate enveloped viruses, including human immunodeficiency virus (HIV) type 1 (HIV-1), HIV type 2 (HIV-2), hepatitis B virus (HBV), and hepatitis C virus (HCV). IEC is a part of the protein purification process during the manufacturing of IVIG, which also removes non-enveloped viruses and protease-resistant prion protein (PrP^{Sc}). As a further dedicated pathogen safety step, nanofiltration (mean pore size 19 ± 2 nm) was introduced into the manufacturing process. Nanofiltration utilized a filter mechanism designed to exclude infectious agents by size while allowing recovery of the functional protein component. It has been shown to be a robust process for the removal of both enveloped and non-enveloped viruses [31], and has the capability to remove prions. Special process conditions seem to disfavor the removal capacity of small viruses, e.g., low pH and high conductivity in combination with low pressure/pressure release. However, even under extremely unfavorable conditions, the reduction factor for small viruses can be maintained at >4 log, mainly as long as the nanofilter is not overloaded with small viruses [32].

All three process steps were validated for virus removal/inactivation, whereas IEC and nanofiltration were also validated for prion removal. Pathogen safety studies were

performed in duplicate for each virus safety step to demonstrate the reproducibility of the results. The lowest of both results observed in each study are shown.

2.2 Scale-Down Model and Robustness Conditions

The process steps contributing to pathogen safety—S/D treatment, IEC, and nanofiltration—were transferred to laboratory scale. Scale-down models were established by comparing relevant process control parameters (Table 1). The comparability of the process performance between the scale-down models and the production process was proven. For every virus inactivation or virus removal step, critical parameters were determined and their influence on virus reduction was investigated. For S/D treatment, the critical factors examined were a lower concentration of S/D reagents and a lower temperature than standard process conditions. Critical factors investigated for IEC were varying conditions of the product matrix, the amount of resin, and contact time, while for nanofiltration the impact of a higher load compared to standard loading was investigated.

2.3 Viruses

The viruses used in the pathogen safety studies and the corresponding susceptible cells used for virus titration are shown in Table 2. These viruses were selected in order to demonstrate inactivation/removal of viruses (or suitable models) that may be present in the source material from which the product is manufactured, and to demonstrate the inactivation/removal of viruses with a wide range of biophysical and structural properties that may reflect those of unknown or unidentified contaminants in the source material [11]. Viruses were pre-filtered at 0.22 µm (bovine viral diarrhea virus [BVDV], mouse encephalomyelitis virus [MEV], porcine parvovirus [PPV]) or 0.45 µm (pseudorabies virus [PRV], HIV-1) before spiking for removal of any potential aggregates.

2.4 Methods for Virus Titer Calculations

Virus titers were determined using the 50% tissue culture infectious dose ($TCID_{50}$) assay based on endpoint dilution. Prior to the assay (in a separate pre-study), the sample dilution that was not cytotoxic and did not interfere with the virus/cell system was derermined. The $TCID_{50}$ assay was performed in 96-well plates with susceptible cells for the respective virus seeded 1 day before titration. Three-fold serial dilutions were prepared and eight replicates per dilution were tested. The tested sample volume per replicate was 100 µL. The titer was estimated using the Spearman–Kaerber method [33]. In cases where only a few positive cultures were found and the Spearman–Kaerber method was not applicable, the most probable number (MPN) method, a Poisson-based maximum likelihood method, was used [34, 35]. The MPN method estimated the virus concentration that could be transferred to $TCID_{50}$ [36, 37].

The large volume plating (LVP) assay was used to improve the limit of detection (LOD) by increasing the tested sample volume up to 48 mL. The LVP assay was performed for process samples, which were expected to contain no or only a few infectious viruses (e.g., 30 and 60 min kinetic point S/D treatment, IEC flow through, and nanofiltrate samples). The virus titers for samples where no positive cultures were found were determined according the Poisson distribution at 95% confidence limits [13]. If positive cultures were found in the LVP assay, the MPN method was used to estimate the virus titer. The $\log_{10}$ reduction factor (LRF), which quantitatively determines the capacity of a process step to inactivate and/or remove viruses, was calculated from the ratio of the virus load detectable in the starting material at the beginning of the test and the virus load actually or potentially present after the virus safety step. The calculations of the virus reduction factor and 95% confidence intervals were undertaken using the methodology outlined in European regulatory guidelines [14].

Table 1 Relevant process control parameters, which are used similarly for manufacturing scale as well as for scale-down models

Control parameters for each process		
S/D treatment (scale-down factor: 2696)	IEC (scale-down factor: 9802)	Nanofiltration (scale-down factor: 6875)
pH	pH	pH
Temperature	Contact time	Pressure
Incubation time	Applied amount of target protein	Filter area
Concentration of S/D reagents		Temperature

IEC ion-exchange chromatography, *S/D* solvent/detergent

Table 2 Viruses used in the pathogen safety studies

Virus	Family	Genome	Size (nm)	Envelope	Model for	Cell line
BVDV	*Flaviviridae*	ssRNA	45–50	Yes	HCV	MDBK
HIV-1	*Retroviridae*	ssRNA	90–120	Yes	HIV	C8166
PRV	*Herpesviridae*	dsDNA	120–200	Yes	Enveloped DNA viruses, e.g., HBV	Vero
MEV	*Picornaviridae*	ssRNA	22–30	No	HAV	BHK-21
PPV	*Parvoviridae*	ssDNA	18–26	No	Parvovirus B19	PK 13

BHK-21 baby hamster kidney-21, *BVDV* bovine viral diarrhea virus, *dsDNA* double-stranded DNA, *HAV* hepatitis A virus, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *HIV* human immunodeficiency virus, *MDBK* Madin–Darby bovine kidney, *MEV* mouse encephalomyelitis virus, *PPV* porcine parvovirus, *PRV* porcine pseudorabies virus, *ssRNA* single-stranded RNA

2.5 Virus Safety Studies

2.5.1 Solvent/Detergent Treatment

The process intermediate was cooled to +6 °C and spiked 1:10 with virus. The pH value was adjusted to pH 4.2, if necessary. The final concentrations of tri(*n*-butyl)phosphate (TNBP) and octoxynol-9 were 0.3% (w/w) and 1.0% (w/w), respectively. This starting material was incubated for 60 min at 6 ± 2 °C.

To determine the kinetics of virus inactivation, test samples were collected at pre-defined intervals during the process (5, 15, 30, and 60 min), and investigated by end-point dilution assay (LOD 1). In addition, the samples were analyzed by LVP assay (LOD 2) after 30 and 60 min. The S/D treatment was terminated by C-18 resin processing in batch mode (0.3 g resin/mL sample) to remove the S/D reagents and simultaneously lower the cytotoxic side effects of the S/D reagents. The sample was incubated for 1 min, centrifuged, and the supernatant passed through a 0.45 μm filter. Control samples were implemented to verify the termination of the S/D treatment and to ensure that no significant loss of virus occurred during C-18 processing. Differences of <1 log between control samples were accepted and stated as being not significant. Experiments were performed with the enveloped viruses HIV-1, PRV, and BVDV.

To demonstrate the robustness of the process step, studies were also performed at a lower S/D concentration (0.75% [w/w] octoxynol-9 and 0.23% [w/w] TNBP) and a lower process temperature (3 ± 1 °C).

2.5.2 Ion-Exchange Chromatography

The process intermediate was passed through a filter (0.2 μm). This starting material was spiked with pre-filtrated virus at a ratio of 1:21 and 220 mg of IgG per mL of resin (approximately 46 g applied during viral safety standard conditions) was loaded with a flow rate of 1.5 mL/min onto the equilibrated chromatography column (XK16, GE Healthcare Europe GmbH, Freiburg, Germany) packed with 10 mL of a strong ion exchanger. The column was then washed with 3.7 column volume (CV) equilibration buffer (0.01 M of sodium dihydrogen phosphate). While loading the column, the flow-through fraction (containing the IgG) was sampled when the UV signal of the loading peak was raised to 50 mAU until the signal dropped to <400 mAU. The remaining equilibration buffer was sampled separately if technically feasible. The column was further washed with 2 CV elution buffer (0.01 M of sodium dihydrogen phosphate, 1 M of sodium chloride). The elution buffer fraction was sampled as a third fraction. After elution, the column was regenerated.

Test samples were taken out of the flow-through, equilibration buffer, and elution buffer fractions to investigate the virus removal and partitioning. Experiments were performed with the non-enveloped viruses PPV and MEV.

To demonstrate the robustness of this process step, studies were performed with an increased product load (25%) and a lower contact time (5.9 min instead of 6.7 min) between the intermediate and chromatography resin. The influence of altered product matrix conditions [pH value below production range (A) and above production range (B)] in combination with an increased product load (≥15%) and re-used resin material (>200 cycles) was also tested.

2.5.3 Nanofiltration

The process intermediate was passed through a Pegasus™ LV6 filter (Pall Life Science, Port Washington, NY, USA) and spiked with pre-filtered virus at a ratio of 1:100. Up to 80.0 g of this starting material was then passed through the Planova™ 20N filter (mean pore size 19 ± 2 nm, effective surface area 0.001 m^2) (Asahi Kasei Pharma Corp., Osaka, Japan), followed by a post-wash step with 3.0 ± 0.2 g of water.

Nanofiltration was performed at 37 ± 1 °C, pH 4.0 ± 0.1, and at a pressure of 0.7 ± 0.2 bar. A flow

rate of 0.5 g/10 min was defined as the stop criterion. Therefore, the weight of the filtrate was measured every 10 min. The filtration was stopped as soon as the specified product amount was filtered or in cases where the stop criterion was reached. After a pressure release due to the technical design, a post-wash was performed. Test samples were taken from the nanofiltrate without and with post-wash and from the post-wash itself. Experiments were performed with BVDV, MEV, HIV-1, PRV, and PPV viruses.

To investigate the robustness of this process step, the filtration load was increased by at least 15%, depending on the occurrence of the stop criterion.

2.6 Prion Safety Studies

Preparations derived from the hamster-adapted scrapie strain 263K (HAS 263K) (microsomal/cytosolic fraction) were used as the model for TSE. This fraction was prepared from crude brain homogenate by differential centrifugation to remove larger aggregates, leaving only the smaller microsomal membrane fragments in the supernatant. This spike preparation was used for the chromatography studies.

For the nanofiltration studies, the spike was additionally sonicated at 39% power for 2 min in pulses (10 s on, 30 s off) whilst being maintained on melted ice using a Sonics VCX750 sonicator (Sonics, Newtown, CT, USA) fitted with a stepped micro tip. Following sonication, the spike material was filtered through a 0.1 µm syringe filter.

Prion safety studies were performed for the IEC and nanofiltration steps. Process intermediate was spiked with HAS 263K and the samples collected following chromatography or nanofiltration were assessed for PrP^{Sc} levels using Western blot analysis [38]. Studies were performed in duplicate to show reproducibility.

Animal bioassays were performed to determine the infectivity of samples collected during the IEC and nanofiltration steps. Hamsters were inoculated with either spiked starting material or post-chromatography or post-nanofiltration product samples. The proportion of animals with signs of infection and the proportion of animals without signs of scrapie but with positive PrP^{Sc} Western blots were determined for each sample.

2.7 Process Intermediates

The studies were performed using in-process materials collected from the commercial scale batches during the production of IVIG 10%. For the studies performed in duplicate, different batches were used.

3 Results

3.1 Virus Safety Studies

3.1.1 Solvent/Detergent Treatment

The S/D treatment was effective in completely inactivating the enveloped viruses—HIV-1, PRV, and BVDV—under both standard and robustness conditions. All enveloped virus loads were completely inactivated to below detectable levels (LOD 1) within 5 min of exposure to S/D, which was confirmed after 15, 30, and 60 min (Fig. 1a, b). Further, no infectivity was detected on improving the LOD

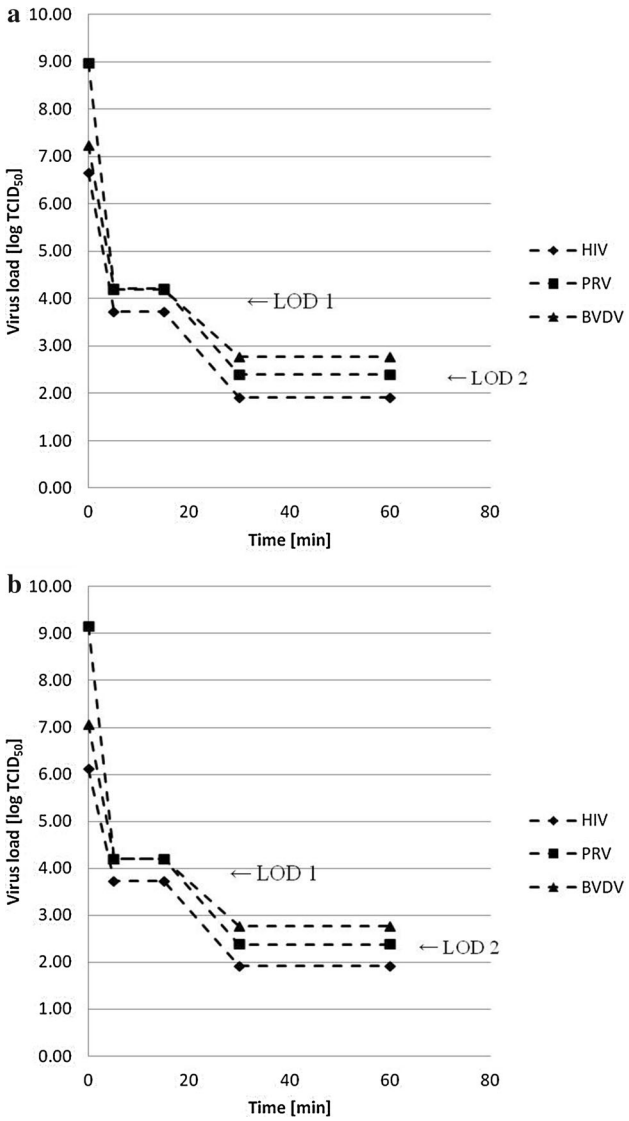

Fig. 1 Change in viral load after the solvent/detergent treatment step, under (**a**) standard conditions and (**b**) robustness conditions (see Sect. 2.5.1). *BVDV* bovine viral diarrhea virus, *HIV* human immunodeficiency virus, *LOD* limit of detection, *PRV* porcine pseudorabies virus, *$TCID_{50}$* 50% tissue culture infectious dose

(LOD 2) after 30 and 60 min of S/D treatment by increasing the sample volume tested (LVP).

The LRFs for HIV-1, PRV, and BVDV after 30 min under standard process conditions are shown in Table 3. Even under robustness conditions, the virus reduction factors remained below the LOD (30 min), i.e., $\geq$4.20 $\log_{10}$ for HIV-1, $\geq$6.77 $\log_{10}$ for PRV, and $\geq$4.29 $\log_{10}$ for BVDV.

3.1.2 Ion-Exchange Chromatography

It was demonstrated that the IEC step removes the non-enveloped viruses MEV and PPV under standard and robustness conditions, including modified product matrix conditions, reduced contact time, and high protein loads.

Levels of virus present in the flow-through fraction (which contained the target IgG protein) and in the equilibration buffer were very low or below the detection limit. The main load of virus was found in the elution buffer fraction.

The log reduction factors for MEV and PPV under standard conditions are shown in Table 3. The viral loads of the different fractions and the reduction factors for MEV and PPV under standard and robustness testing and with used resin material (>200 cycles) are shown in Table 4.

3.1.3 Nanofiltration

Nanofiltration effectively removed both enveloped and non-enveloped viruses under standard and robustness conditions. The results of the endpoint titration showed that HIV-1, PRV, BVDV, and MEV infectivity was below detectable levels in the Planova^{TM} 20N nanofiltrate. The effectiveness of the nanofiltration step was investigated more extensively using the LVP assay. The tested volume of the nanofiltrate, including post-wash, was increased by 60-fold compared with the standard method. Despite the significant increase in volume tested, the infectivity remained below the LOD. The investigation with PPV showed residual infectivity in the endpoint titration of the nanofiltrate sample. This finding was confirmed by the LVP assay. The LRFs for HIV-1, PRV, BVDV, MEV, and PPV under standard process and robustness conditions and the viral loads in the starting material, controls, and filtrate sample are shown in Table 5.

The influence of post-wash on the removal capacity of the nanofiltration was investigated by the PPV samples. The LRF of the filtrate sample was 5.80 $\log_{10}$ and the filtrate including post-wash was 5.78 $\log_{10}$. An influence of the post-wash was therefore not observed; this finding was confirmed by the robustness study. Control samples also indicated inactivation capacity of the process step for HIV-1, PRV, and BVDV due to the low pH and temperature conditions during nanofiltration to different degrees. In contrast, the loss of PPV and MEV infectivity in the nanofiltrate was entirely due to removal of the virus and not by inactivation. However, the nanofiltration step resulted in effective and robust virus removal/inactivation.

3.2 Transmissible Spongiform Encephalopathy Safety Studies

3.2.1 Ion-Exchange Chromatography

IEC was effective in removing prions as determined by the infectivity assay. Prion load in the starting material based on hamster bioassay was 8.62 $\log_{10}$ and the load in the flow-through sample (which contained the target IgG protein) was $\leq$3.66 $\log_{10}$. Animal bioassay showed that the infectious agent was removed below the detection limit, with a reduction factor of $\geq$4.96 $\log_{10}$, confirming the results by Western blot assay (data not shown).

3.2.2 Nanofiltration

The prion load in the starting material based on the hamster bioassay was 9.02 $\log_{10}$ and the load in the nanofiltrate sample was $\leq$3.58 $\log_{10}$. The reduction factor for the

Table 3 Pathogen log reduction factors of the investigated manufacturing process steps

Production step	Pathogen reduction factor [$\log_{10}$]					
	Enveloped viruses			Non-enveloped viruses		Prion
	HIV-1	PRV	BVDV	MEV	PPV	HAS 263K
S/D treatment	≥4.67	≥6.59	≥4.47	Not applicable		
Ion-exchange chromatography	Not done			5.88	5.83	≥4.96
Nanofiltration (20 nm)	≥4.70	≥6.57	≥3.69	≥5.78	5.78	≥5.44
Global reduction factor	≥9.37	≥13.16	≥8.16	≥11.66	11.61	≥10.40

BVDV bovine viral diarrhea virus, *HAS 263K* hamster-adapted scrapie strain 263K, *HIV-1* human immunodeficiency virus type 1, *MEV* mouse encephalomyelitis virus, *PPV* porcine parvovirus, *PRV* porcine pseudorabies virus, *S/D* solvent/detergent, ≥ indicates below the limit of detection,

Table 4 Mouse encephalomyelitis virus and porcine parvovirus removal by ion-exchange chromatography

Condition	Cycle 0	Cycle 200	High load, reduced contact time	High load, matrix condition A, reduced contact time	High load, matrix condition B, reduced contact time
Mouse encephalomyelitis virus					
Starting material	8.67	8.68	8.49	8.53	8.84
Flow-through fraction	2.79	3.39	3.19	4.83	2.42
Remaining equilibration buffer	≤1.74	≤1.74	≤1.77	2.68	≤1.79
Elution buffer fraction	8.40	8.22	8.09	8.06	8.55
LRF	5.88	5.29	5.30	3.70	6.42
Porcine parvovirus					
Starting material	9.37	9.37	9.19	9.15	9.29
Flow-through fraction	3.54	3.12	3.14	4.05	2.83
Remaining equilibration buffer	≤0.87	≤1.51	≤0.77	≤1.18	≤0.76
Elution buffer fraction	9.33	9.55	9.28	9.23	9.67
LRF	5.83	6.25	6.05	5.11	6.47

Viral loads of the starting material and the different fractions are expressed as $\log_{10}$ $TCID_{50}$

condition A pH below production range, *condition B* pH above production range, *LRF* log reduction factor of the process step as $\log_{10}$, *$TCID_{50}$* 50% tissue culture infectious dose, ≤ indicates below the limit of detection

Table 5 Virus removal by nanofiltration

Pathogen	HIV-1		PRV		BVDV		MEV		PPV	
Condition	Std	Rob	Std	Rob	Std	Rob	Std	Rob	Std	Rob
Starting material	6.06	5.58	8.38	8.17	6.36	6.76	8.09	8.59	9.03	8.94
Hold control pH 4, 37 °C	≤3.14	≤3.20	≤3.61	≤3.79	5.23	≤4.25	8.39	7.87	8.87	8.92
Hold control pH 7, 2–8 °C	6.06	6.12	8.03	7.93	6.66	6.34	8.15	8.35	8.93	8.62
Filtrate including post-wash	≤1.36	≤1.42	≤1.81	≤1.94	≤2.67	≤2.83	≤2.31	≤2.45	3.25	4.44
LRF	≥4.70	≥4.16[a]	≥6.57	≥6.23[a]	≥3.69	≥3.93[a]	≥5.78	≥6.14[a]	5.78	4.50[a]

Viral loads of the starting material, controls and filtrate sample are expressed as $\log_{10}$ $TCID_{50}$

BVDV bovine viral diarrhea virus, *HIV-1* human immunodeficiency virus type 1, *LRF* log reduction factor express as $\log_{10}$, *MEV* mouse encephalomyelitis virus, *PPV* porcine parvovirus, *PRV* pseudorabies virus, *Rob* robustness, *Std* standard, *$TCID_{50}$* 50% tissue culture infectious dose, ≤ indicates below the limit of detection

[a] Stop criterion reached

nanofiltrate in the hamster bioassay was ≥5.44 ± 0.48 $\log_{10}$. None of the animals inoculated with nanofiltered samples showed clinical signs of scrapie, confirming the results by Western blot assay (data not shown).

4 Discussion

The results of the present study demonstrate that incorporating the pathogen safety steps into the immune globulin manufacturing process can provide robust and effective clearance of viruses and TSEs from the new IVIG (10%). Such removal or inactivation is important to prevent the transmission of pathogens when IVIGs are administered. Although the safety profile of currently marketed IVIG products is excellent [6–9], this has not always been the case in the past [39]. Outbreaks of viral infection from IVIG products in the 1990s led to the more stringent regulatory guidelines for virus and prion removal/inactivation that exist today [40]. These historical lessons reinforce the need to use robust processes in the manufacturing of the plasma-derived products in accordance with modern regulatory guidelines [10, 11].

Different procedures may be used to remove or inactivate pathogens from human plasma-derived IVIG, including ethanol or caprylate fractionation, heat treatment (pasteurization), S/D treatment, IEC, precipitation, and incubation at low pH [23, 28]. However, current guidelines recommend the use of at least two complementary process

steps for the removal of enveloped viruses and one for the removal of non-enveloped viruses, in order to maximize the number and type of pathogens removed and/or inactivated [10]. Most of the currently available IVIG preparations use a combination of three or more process steps for virus and prion inactivation and removal [24, 41–43].

Accordingly, two dedicated pathogen safety steps were incorporated into the manufacturing process of the new IVIG, supplemented by IEC. In addition to validating these manufacturing processes under standard conditions, the robustness of each process step was investigated in varying conditions. The results of the robustness studies demonstrated the reliability of the processes with a wide margin of safety. The broad range of model viruses used in the studies and particularly the mode of action of the dedicated pathogen safety steps, S/D treatment and nanofiltration, indicate that the manufacturing process can be expected to also provide protection against emerging or unknown pathogens.

S/D treatment is considered the gold standard for the inactivation of viruses with a lipid envelope [20, 23]. Our validation studies of the S/D process, under both standard and worst-case (robustness) conditions, showed that S/D treatment was an efficient and robust step to inactivate enveloped viruses and was effective in reducing the enveloped viruses below the LODs. These findings are in accordance with previously published data [20]. Reducing the S/D concentration and decreasing the process temperature did not have any negative impact on the efficacy of the process to inactivate BVDV, HIV-1, or PRV, which ensures a substantial margin of viral safety. All investigated viruses were inactivated below the detection limit after 5 min, and even improving the detection limit by 60-fold resulted in no infectivity detected after 30 min, whereas the manufacturing process time is at least 60 min in the production unit. Our data confirm that the S/D process step in the manufacture of the new IVIG provides rapid viral inactivation kinetics and a high safety margin.

Similarly, the studies evaluating IEC demonstrated that the process supplements the removal of non-enveloped viruses such as PPV and MEV, even under worst-case robustness conditions and throughout many cycles. Varying the matrix of the product solution, in combination with high load and reduced contact time, influenced the removal capacity. Even if the matrix conditions were out of product specification, the removal of non-enveloped viruses in the order of 5.1 log (PPV) and 3.7 log (MEV) was verified. IEC was also highly effective in removing the HAS 263K scrapie model of prion contamination. Moreover, the removal of prions was confirmed by an infectivity study. Importantly, during the manufacturing of IVIG, the chromatographic equipment including resin used during IEC is sanitized with 1 M sodium hydroxide solution after each run, minimizing the risk of batch-to-batch contamination.

With regard to nanofiltration, a combination of removal by Planova^{TM} 20N nanofiltration and inactivation due to matrix/pH effects demonstrated that the process provided an effective, reliable, and robust virus removal/inactivation step for both enveloped and non-enveloped viruses, under both standard and robustness conditions. The reduction factors of 4–6 $\log_{10}$ achieved by nanofiltration with different viruses in our studies is comparable to the reductions reported elsewhere [30, 41, 44]. High load in combination with a decreasing flow rate (stop criterion reached) appear to have minimal effect on removal of PPV in the given process setting. The removal capacity for this robustness investigation was in the order of 4.5 log, which is still acknowledged as effective [14]. Prion removal below the detection limit was confirmed through the infectivity bioassay [45]. The effectiveness of the nanofilter (20 nm) in removing prions during IgG manufacturing has also been shown by Diez et al. [45].

The new IVIG (10%) product is manufactured using a process that results in a high yield. Demand for IVIG throughout the world has been growing steadily since the 1990s [46]. Currently, there is a shortfall between production of IVIG and demand, and this situation is expected to worsen over the next decade [47]. Therefore, any process that has the potential to improve the yield of IVIG from plasma represents an important advance in maintaining supply to meet the growing demand and sustainability. It has been suggested that effective pathogen step measures may lower the yield of IgG during manufacture [46]. However, in contrast, data from our study indicate that the optimal combination of pathogen safety processes can effectively remove or inactivate viruses and prions within a process that also yields high levels of IgG.

5 Conclusions

The pathogen inactivation and/or removal processing steps used in the manufacturing of this new IVIG product provides reliable protection from pathogen transmission. When used in combination, these process steps provide highly effective pathogen safety.

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Compliance with Ethical Standards

Conflict of Interest KUR, TS, GL, and JR are employees of Octapharma.

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Ethical Considerations Pathogen safety studies were conducted in accordance with the principles of Good Laboratory Practice (Octapharma Biopharmaceuticals GmbH, Frankfurt am Main, Germany; ViruSure GmbH, Vienna, Austria), as required by the Committee for Proprietary Medicinal Products of the European Medicines Agency [14]. Animal infectivity studies were undertaken at a specialized animal facility (ViruSure). All animal procedures performed at ViruSure were approved by the Austrian Federal Ministry of Science and Research (Authorization No. BMWF-68.205/0056-II/10b/2010) and conducted according to the Austrian animal rights act (BGBL. I Nr. 169/1999) and legislative decree (BGBL II Nr.198/2000).

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