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Pathogen inactivation and removal procedures used in the production of intravenous immunoglobulins

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Abstract

Patients with immunodeficiencies or some types of autoimmune diseases rely on a safe therapy with intravenous immunoglobulins (IVIGs) manufactured from human plasma, the only available source for this therapeutic. Since plasma is predisposed to contamination by a variety of blood-borne pathogens, ascertaining and ensuring the pathogen safety of plasma-derived therapeutics is a priority among manufacturers. State-of-the-art manufacturing processes provide a high safety standard by incorporating virus elimination procedures into the manufacturing process. Based on their mechanism these procedures are grouped into three classes: partitioning, inactivation, and virusfiltration.

Keywords: IVIG; Safety; Biotechnology; Blood; Plasma

1. Introduction

One of the most important clinical applications of intravenous immunoglobulin (IVIG) is to supply a broad spectrum of antibodies to patients who are antibody deficient. Throughout their lives, patients with inherited (primary) antibody deficiencies are treated with relatively high doses of IVIG. Patients who develop secondary antibody deficiencies because of disease or disease therapy may also receive high dose IVIG for long periods of time. Since IVIG was first developed, other indications have been found to benefit from high dose IVIG to correct immunological disorders such as autoimmune diseases. The original immunomodulatory application was to induce increased platelet levels in patients with immune thrombocytopenic purpura (ITP) [1]. After this application was discovered, single or multiple courses of high dose IVIG were successfully used to treat a wide variety of other autoimmune disorders (for review see: refs. [2–5]). Since regular exposure to large quantities of a human plasma protein carries the risk of infection with blood borne pathogens, increasing the pathogen safety of IVIG, without diminishing its clinical efficacy, is a high priority.

Transmission of ‘‘homologous serum hepatitis’’ through whole blood, plasma, and serum was a great concern during development of plasma fractionation procedures to produce human serum albumin during World War II [6]. Yellow fever vaccines stabilized with human serum produced 23,000 cases of hepatitis in American military personnel. Most epidemiological investigations strongly suggested that pooled human plasma presented a higher risk of hepatitis transmission than whole blood. This was attributed to the increased probability that pooled plasma would be contaminated by one or several donors. Since plasma pools from 250 to 2000 blood donations were being used to produce albumin, efforts were initiated to inactivate hepatitis virus in human serum albumin solutions [6].

In 1948 Gellis and his co-workers reported that hepatitis transmission by albumin was eliminated by heating for 10 h

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at 60 °C [6]. This procedure was possible because of the discovery that 40 mM acetyltryptophan and 20 mM sodium caprylate increased the heat resistance of albumin. Unfortunately, other plasma proteins in solution are inactivated by heat and early attempts to inactivate viruses in high risk products were unsuccessful. According to the FDA, high risk plasma products included fibrinogen, Factor VIII concentrate, and Factor IX. Heated albumin solutions and immunoglobulins produced by cold ethanol fractionation were considered low risk products [7].

The perception that immunoglobulins produced by cold ethanol fractionation had a low risk of transmitting virus infections changed in 1983 when Lane reported that an experimental IVIG produced by cold ethanol fractionation transmitted non-A, non-B hepatitis [8]. At this time, the human immunodeficiency virus was isolated and proven as transmissible by blood and blood products [9,10]. The emergence of human immunodeficiency virus (HIV) and reports of non-A, non-B hepatitis transmission by some IVIG products [11,12] but not others caused manufacturers and regulatory agencies to examine existing IVIG manufacturing processes for their capacity to eliminate viruses [13–22]. Development of dedicated virus inactivation procedures for IVIG production was also initiated [23,24].

Studies of IVIG manufacturing procedures suggested that cold ethanol fractionation removes viruses by two mechanisms: 1) inactivation and 2) partitioning. Several laboratories demonstrated that retroviruses, such as HIV, are inactivated by cold ethanol under conditions used in IVIG production [14–17,22]. However, vesicular stomatitis virus (VSV) and Sindbis virus (SIN), both used as models for hepatitis C virus (HCV), were stable under similar conditions [22]. Over the years, several procedures have been developed to prevent pathogen transmission and increase the safety of IVIG and other therapeutic proteins. We will describe these procedures, including the most recent technology termed ‘‘virusfiltration’’. The term ‘‘virusfiltration’’ has become the accepted nomenclature for what was previously called ‘‘nanofiltration’’ [25]. Virusfiltration removes viruses regardless of the presence or absence of a lipid envelope. It also eliminates other pathogens that have the potential to transmit infections. Virusfiltration is the first totally new principle in pathogen removal incorporated into plasma protein manufacturing in the past 20 years.

2. Donor screening to decrease the viral load of pooled plasma

Although people with illnesses are always excluded from donating blood or plasma, some donors do not feel sick or have clinical symptoms even though they are infected by a pathogen. During this ‘‘window period’’ blood or plasma donations may transmit the pathogen. For this reason, donor screening tests were not only developed for new pathogens but also to have more sensitivity and thereby reduce the window period. Gürtler has reviewed blood-borne pathogens with respect to their relevance to transfusion [26]. Human pathogens that cause chronic, progressive wasting or lethal diseases, and some infectious agents that are not prevalent in the transfused population, were considered relevant. Using these criteria, hepatitis B virus (HBV), HCV, and human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2) were characterized as relevant. B19 virus (B19V; formerly named: Parvovirus B19), cytomegalovirus (CMV) and hepatitis A viruses (HAV) were classified as occasionally relevant. Since this review was published, non-enveloped viruses such as B19V and HAV have become more relevant and new viruses (e.g. West Nile Virus, SARS corona virus) have emerged. Thus relevance of pathogens to transfusion is an evolving concept. Identification of hepatitis viruses and the development of sensitive donor screening tests became a high priority because of early concern for hepatitis transmission. A sensitive test for HBV was developed in 1972 [27] and was used to eliminate infected donors. Unfortunately, the HBV test did not eliminate transfusion-related hepatitis and the search for one or more non-A, non-B hepatitis viruses was initiated. The AIDS epidemic led to the rapid development of a screening test for antibodies to HIV-1 in 1984 [28]. In 1989, the genome of a non-A, non-B hepatitis virus was isolated and used to develop a donor screening test for HCV [29]. Today, plasma is screened for antibodies to syphilis, HIV-1, HIV-2, HCV, and HBV. Extremely sensitive tests for HCV, HIV-1, HAV, HBV and B19V nucleic acids have been introduced recently and are being used to reduce the window period further.

3. Virus removal by partitioning

During plasma fractionation, classes of proteins are precipitated and separated from proteins that remain in solution either by centrifugation or by filtration. Viruses are frequently precipitated along with the proteins. When frozen plasma is thawed at 2 °C, some proteins called cryoglobulins remain insoluble. This fraction called cryoprecipitate contains many proteins belonging to the coagulation cascade (e.g. factor VIII). But also viruses tend to partition into the cryoprecipitate. Viruses are also precipitated by cold ethanol into the fibrinogen fraction (Cohn-Oncley Fraction I) and the IgG fraction (Cohn-Oncley Fraction II + III or Kistler-Nitschmann Precipitate A; see Fig. 1) [15–19,24]. The most effective virus removal step during IgG production occurs during fractional precipitation of Fraction II+III. Almost all the virus in Cohn Fraction II + III is removed during precipitation of Fraction III (Kistler-Nitschmann Precipitate B), a waste fraction that contains IgA, IgM, plasminogen and other proteins [15–19,24] (Fig. 1). The distribution of model viruses into plasma fractions produced by cold ethanol precipitation is illustrated in Table 1.

4. Virus inactivation

The virus inactivation capacity is often correlated with log reduction factors (LRF). Table 2 shows LRFs, which have been reported for dedicated virus inactivation procedures. However, inactivation kinetics are also relevant. Rapid inactivation kinetics are regarded as evidence of excess capacity. Lengthy inactivation steps, after which a virus can still be detected or

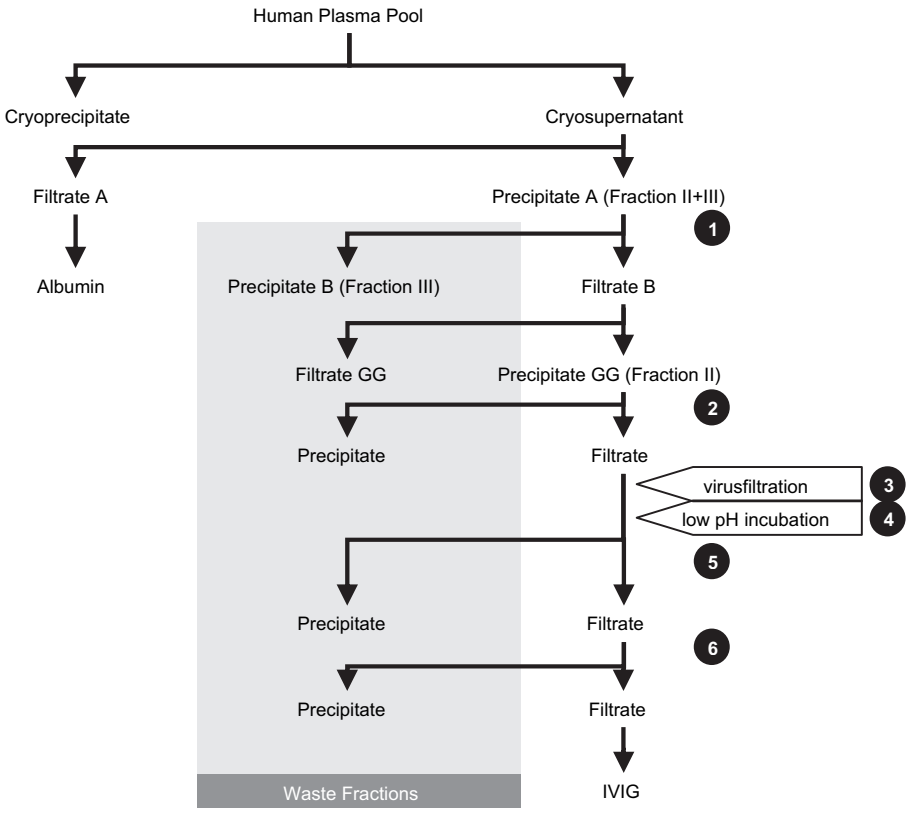

Fig. 1. Kistler-Nitschmann fractionation scheme for IVIG manufacturing. Filter aid is added at each depth filtration clarification step. In black cycles are the numbers of validated process steps.

only becomes non-detectable near the end of the process, is believed to provide less assurance of viral safety [30].

4.1. Heat inactivation

Viruses are composed of nucleic acids complexed with proteins in structural units of varying complexity. The structural units are assembled into a whole infective virus and are held together by non-covalent bonds. Enveloped viruses have lipid-bilayer membranes with membrane associated glycoproteins. Non-enveloped viruses are frequently small, stable icosahedral particles.

The challenge in developing virus inactivation procedures for protein solutions is to inactivate viruses without harming the therapeutic protein. The non-covalent bonds involved in virus assembly are the same as those that maintain proteins in their native, biologically active, three dimensional structure (conformation). Consequently, processes that inactivate viruses may also denature proteins. Some proteins can withstand small changes in conformation without losing their biological activity or may renature spontaneously. Other proteins lose biological activity from minor changes in conformation. Heat can be used to inactivate viruses and proteins. The denaturation temperature of a protein is sharply defined and is different for each protein [31]. Heating for a definite time to a temperature just below the denaturing temperature of a particular protein is used in some protein purification procedures to inactivate viruses. In the presence of substrate, enzymes can be heated to temperatures 10 °C higher than in the absence of substrate [31]. This explains how albumin was successfully stabilized. Human

Table 1
Distribution of viruses during cold ethanol fractionation of human plasma

Virus	Plasma	Fraction I	Fraction II + III (Ppt A)	Fraction III (Ppt B)	Fraction II (Ppt GG)
Mouse retrovirus [16]	8.4	NA	7.6	3.2	ND
HIV [16]	5.6	NA	4.7	3.2	ND
BVDV [19]	6.9	>5.9	6.8	6.4	NT
HBV antigen [62]	5.6	ND	4.7	7.7	ND

Data are expressed as $\log_{10}$ of virus concentrations (per ml). NT = not tested; NA = not applicable; ND = not detectable.

Table 2
Log_{10} reduction factors for virus inactivation procedures used in IVIG production

Inactivation step	Enveloped virus reduction ($\log_{10}$)		
	HIV	BVDV	PRV
SD (TNBP-cholate) [63]	≥5.2	≥4.2	≥4.6
SD (TNBP-Triton X-100, Tween 80) [64]	>3.7	>4.9	>4.1
20 mM caprylate [40]	≥4.5	≥4.5	≥4.6
10 h @ 60 °C [65]	>5.4	>6.4	>3.6
pH 4-pepsin [66]	>6.1	>4.4	>5.3
pH 4.25, 21 d @ 25 °C [40]	>6.5	3.5	≥4.3

SD = solvent and detergent.

albumin has many binding sites for hydrophobic molecules and plays a major role in the transport of fatty acids. Filling these sites with the stabilizers acetyltryptophan and caprylic acid (a fatty acid also known as octanoic acid) allows albumin to withstand heating for 10 h at 60 °C. Since albumin has no measurable biological activity, the full impact of heating albumin is not known. Factor VIII is rapidly inactivated when heated in solution. However, dried Factor VIII is relatively heat-stable under certain conditions. In the 1980s, this observation led to the development of heat treated Factor VIII preparations [32,33]. Fortunately, HIV and non-A non-B hepatitis was inactivated in heated Factor VIII but the products had lower biological activities, were relatively insoluble, and produced a higher incidence of Factor VIII inhibitors. Moreover, HBV was not completely inactivated [32].

Heat treatments for IVIG in the presence of sugar stabilizers have been developed. One IVIG is stabilized with 33% (w/w) sorbitol at pH 5.5 and heated to 60 °C for 10 h [34]. Several enveloped viruses and one non-enveloped virus (ECHO virus type 6) were studied. All viruses were completely inactivated within the detection limits of the assay systems used. The log reduction of ECHO virus was >3.8. No substantial changes in physicochemical and biological properties were reported. A slight increase in IgG1 subclass was observed.

Another IVIG is heated to 60 °C for 10 h in the presence of sucrose and potassium acetate as stabilizers. Enveloped viruses were inactivated to their detection limits within 6 h of heating. The non-enveloped encephalomyocarditis virus (EMCV) and porcine enterovirus (VIR918) showed a more or less steady decrease over time resulting in approximately 4.5 log inactivation after 10 h at 60 °C. However residual infectivity was still observed after completion of the pasteurization process [35,36].

Thus it appears that heating is a relatively efficient process for enveloped viruses but it is not as effective for non-enveloped viruses. The susceptibility of enveloped viruses to elevated temperatures may be explained by the fact, that heat disintegrates lipid bilayers by converting them from solids into liquids [37].

4.2. Low pH virus inactivation

Reid et al. in 1988 [20] reported virus inactivation by treating IVIG with low levels of pepsin at pH 4. Enveloped viruses such as vaccinia, herpes simplex (HSV), mumps and Semliki Forest virus (SFV) were found to be susceptible to pH 4-pepsin treatment but poliovirus type 2, a non-enveloped, acid-stable virus, was resistant. The authors also observed decreased infectivity of vaccinia and HSV in IVIG samples incubated at pH 6.9 without pepsin. These decreases were attributed to antibody mediated neutralization. Similar results were observed by Kempf et al. who studied virus inactivation in IVIG samples incubated at pH 4 with and without pepsin [21]. HIV, HSV type 1, CMV, VSV, and SFV were inactivated by pH 4 incubation with or without trace amounts of pepsin. Inactivation of HSV and CMV was also observed at pH 7. Additional experiments demonstrated that the addition of pepsin at pH 4 accelerated VSV inactivation. Further evidence of the effectiveness of low pH virus inactivation was published by Louie et al. who reported inactivation of bovine viral diarrhea virus (BVDV) and HCV in IVIG incubated at pH 4.25 for 21 days at 21 °C [19]. Examination of BVDV inactivation under the same conditions but at different pHs demonstrated increased inactivation with decreasing pH.

Boschetti et al. recently reported that B19V in an IgG solution is inactivated by pH 4 incubation at 37 °C. Minute virus of mice (MVM), a common model virus for B19V, was stable under the same conditions [38].

As previously observed with other virus inactivation procedures (heat and solvent-detergent), low pH inactivation is most effective for enveloped viruses [19–21]. Recent evidence suggests that non-enveloped viruses may also be inactivated [38]. Therefore, virus inactivation at low pH may result from denaturation, and degradation if pepsin is present, of membrane-associated glycoproteins of enveloped viruses or capsid proteins of non-enveloped viruses.

4.3. Solvent-detergent virus inactivation

The presence of lipid envelopes on blood-borne viruses makes them uniquely susceptible to inactivation by chemicals that dissolve or dissociate lipids such as solvents and detergents. Although proteins can also be denatured by solvents and detergents, they can be exposed to low levels for limited periods of time without significant irreversible effects on structure or function. This observation was exploited by Horowitz and his co-workers who developed a virus inactivation process that involves addition of both a solvent and detergent [39]. Solvent-detergent treatments were first applied to Factor VIII preparations and rapidly replaced heat treatments as the standard virus inactivation procedure [39]. Solvent-detergent virus inactivation was soon applied to a wide variety of other plasma proteins considered at risk of transmitting viruses. After hepatitis C transmission by IVIG was reported [11,12], solvent-detergent virus inactivation was incorporated into several IVIG manufacturing processes [22,23]. The use of this procedure requires incubation periods and reagent removal steps that are reported to reduce IVIG recovery and to lengthen manufacturing times [39]. It is also a considerable environmental burden. Since this reduces the capacity of IVIG production, some IVIG manufacturers are beginning to abandon solvent-detergent virus inactivation in favour of other processes [40].

5. Virusfiltration

Filtration has long been used to remove blood-borne pathogens from plasma products. Sterile or germ filtration through 0.22 μm filters removes bacteria and fungi. This process has been so effective that the development of filters with pore sizes small enough to remove viruses was a logical consequence. Development of virus removal filters for protein solutions was handicapped by the need to process large volumes at reasonable flow rates. Initial problems were resolved in the early 1990s and the viral safety of plasma products was improved by

implementing virusfiltration at the process scale. Virusfiltration is a simple, robust, non-destructive process that adds size exclusion, a new mechanism, to conventional virus inactivation and partitioning. The use of virusfiltration to remove viruses is the first major advancement in virus safety since solvent-detergent treatment was introduced. Since it does not discriminate between enveloped and non-enveloped viruses, virusfiltration has the potential to remove the broadest range of pathogens. In 1994, Burnouf-Radosevich et al. [41] reported excellent virus removal from Factor IX and Factor XI solutions using Planova® (Ashai Kasei) virusfilters with mean pore sizes of 15 and 35 nanometres (nm). Subsequently, these new virusfilters were introduced in the manufacturing processes of other plasma derived therapeutics.

In studies with immunoglobulin (IgG) solutions at protein concentrations up to 12 mg/mL, O'Grady et al. demonstrated that the Planova® 35 nm filter removed 6 to 7 $\log_{10}$ of mouse type C retrovirus, SV 40 and pseudorabies virus (PRV), whereas polio virus was only removed by the 15 nm filter [42].

Complete elimination from IgG-solutions by Planova 35 nm filtration, to below the detection limit was also confirmed by Troccoli et al. [43] for all viruses over 40 nm from a 70 mg/mL IgG solution, and by Dichtelmüller [44] from a 30 mg/ml IgG solution. In addition, Dichtelmüller demonstrated the excellent robustness of Planova® 35 nm dead-end virusfiltration for BVDV in IgG-solution with regard to protein and salt concentrations, pH, pressure, temperature, and volume per filter area. A >5 $\log_{10}$ clearance for viruses >40 nm (HIV, BVDV and PRV) was also found by van Holten [45] using a Viresolve 180 (Millipore) filter and a 5 mg/mL IgG solution. We obtained similar results with an Ultipore VF grade DV50 filter (Pall Corporation) in a 10 mg/mL IgG solution (Table 3). Virusfiltration has become a generally accepted, efficient, and very robust method for the removal of viruses larger than the pore size of the filter. However, differences were observed in removal studies with viruses of about the same size, or smaller than, the pore size of the virusfilter. In addition to pore size, other factors such as the composition of the IgG solution, the model virus used, and, probably the most important factor, the presence or absence of neutralising or cross-reacting antibodies may play a role. The elimination capacity for small viruses has been studied by several authors.

Table 3
Log_{10} reduction factors of model viruses observed during laboratory experiments of IVIG production (Carimune NF®)

Process (step in Fig. 1)	Mechanism	HIV	PRV	BVDV	SIN	BEV
Fractionation/depth filtration (1)	*p*	4.0	3.6	1.5	3.2	3.4
Fractionation/depth filtration (2)	*p*	5.3	4.7	1.6	4.6	4.1
DV50 Virusfiltration (3)	*n*	>4.9	>4.4	>4.5	>7.5	>5.1
pH 4 pepsin inactivation (4)	*i*	>6.1	>5.3	>4.4	>6.7	<1*
Clarification/depth filtration (5)	*p*	4.0	4.7	3.0	2.9	3.8
Clarification/depth filtration (6)	*p*	2.2	3.0	<1*	1.7	2.8
Total reduction		>26.5	>25.7	>15.0	>26.6	>19.2

Mechanism: *p*: partitioning; *i*: inactivation; *n*: virusfiltration. *Not significant and not included in total reduction.

The Planova® 35 nm filter was able to eliminate 4.3 $\log_{10}$ of EMCV and >4.7 log of HAV, whereas porcine parvovirus (PPV) was only eliminated to some extent (2.6 $\log_{10}$) when two Planova® 35 nm filters were used in series [43]. Using Viresolve 180, van Holton demonstrated elimination capacities of 4.1 $\log_{10}$ for EMCV, >5.1 $\log_{10}$ for HAV and 3.3 $\log_{10}$ for PPV [45]. Omar and Kempf [46] specifically studied the effectiveness of virusfiltration in the removal of small non-enveloped viruses. The viruses studied were bovine enterovirus (BEV, 27 to 30 nm), bovine parvovirus (BPV, 18 to 25 nm) and minute virus of mice (MVM, 18 to 25 nm). Virusfiltration was performed with Ultipore VF grade DV50 and DV20 virus removal filters. The DV50 filter removed >5.7 $\log_{10}$ BEV, whereas the DV20 filter removed 4.5 $\log_{10}$ of MVM and $\geq$5.5 $\log_{10}$ of BPV (down to the detection limit) from 10 mg/mL IgG solutions. The authors demonstrated that removal of viruses with a diameter smaller than the virusfilter pore size was due to cross-reacting human antibodies bound to the viruses.

These results clearly demonstrate the significant contribution virusfiltration makes to the overall viral safety of IVIG products.

6. Removal of TSE infectivity during IVIG manufacturing

Transmissible Spongiform Encephalopathies (TSEs) are degenerative brain diseases transmitted by inoculation or ingestion of nervous system tissues from diseased subjects. TSE diseases include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in deer/elk, Kuru, Creutzfeld-Jacob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and variant CJD (vCJD) in humans. Typical disease symptoms of the human forms are dementia, progressive loss of brain function, and death.

Agents responsible for TSE transmission are believed to be composed of a protease resistant protein (PrP) and are called prions. Animal studies have demonstrated infectivity in blood and transmission of blood infectivity by intracerebral injection [47]. Experiments with a mouse-adapted strain of human TSE demonstrated a need for at least five to seven times more infectious agent to transmit disease by the intravenous than the intracerebral route in mice [48]. Recent studies also demonstrated that BSE and scrapie can be transmitted to sheep by intravenous injection of blood from experimentally infected sheep [49]. In another study, primates were infected with BSE by the intravenous or the oral route [50]. However, there is no evidence that plasma products have ever transmitted CJD or vCJD. Nevertheless, the theoretical risk of transmission has prompted laboratory studies to determine the behaviour of prions during plasma fractionation. TSE infectivity is mainly eliminated from IgG solutions by two mechanisms; partitioning by depth filtration and by elimination by virusfiltration. Efficient elimination of TSE infectivity from IgG solutions by

Table 4
Elimination of hamster scrapie infectivity during manufacturing of IVIG (Carimune NF®) [51]

Process (step in Fig. 1)	Mechanism	Log_{10} reduction
Precipitate A to filtrate B (1)	*p*	3.5
IgG precipitate to IgG filtrate 1 (2)	*p*	4.5
IgG filtrate 1 to IgG Virusfiltrate (3)	*n*	4.4
Clarification (depth) filtration (5)	*p*	2.8
Step (2) and (5) combined	*p*	7.2
Overall reduction		15.2

Mechanism: *p*: partitioning; *n*: virusfiltration.

depth filtration was recently reported for three different IVIG products by Gregori et al. [51] (Table 4), Trejo et al. [52] and by van Holten and Autenrieth [53]. The authors reported log_{10} reduction factors of 2.5 to 6.9. Different TSE preparations were reduced to a similar extent, and as shown in Table 4, consecutive steps were additive.

The potential of virusfiltration to reduce TSE agents was investigated by Tateishi et al. in an albumin solution spiked with scrapie brain homogenate [54]. A significantly reduced removal of infectivity was observed in the presence of detergents by using Planova® 35 nm filter (1.61 log_{10} versus 4.93 log_{10}). No such difference in the removal capacity was observed for the Planova® 15 nm filter. The efficiency of TSE removal from IgG solution by virusfiltration was demonstrated by Gregori et al. [51]. Virusfiltration with an Ultipore VF grade DV50 filter reduced TSE infectivity by 4.4 log_{10} (see Table 4).

7. Evaluation of the risk for pathogen transmission

To date IVIG therapeutics have reached a high safety standard due to consequent blood and plasma testing and the introduction of virus elimination steps in the manufacturing process. Table 3 shows LRFs obtained during the manufacturing process of one IVIG (Carimune NF®). The following calculation is an example that estimates the risk for exposure to an assumed pathogen for a single dose. This example assumes the manufacturing process contains three independent virus elimination mechanisms (e.g. partitioning, inactivation, virusfiltration). It also assumes the lower detection limit (LOD) of the pool NAT-test (currently HCV = 48 geq/ml; HBV = 13 geq/ml; HIV = 48 geq/ml). This sets the highest possible contamination of a manufacturing pool.

Other assumptions are:

1.	LOD	LOD in pool testing	50	geq/ml
2.	*s*	Pool size	3000	l
3.	*y*	Yield	4	g/l
4.	*d*	Dose/body mass	0.4	g/kg
5.	*m*	Body mass	75	kg
6.	LRF	Production process with three elimination mechanisms each clearing ≥ 4 log_{10} of virus. Total log_{10} reduction factor:	≥ 12	

The risk for exposure to such a pathogen in a single dose is calculated using the following formula:

$$\text{Risk} = \frac{d \times m \times \text{LOD} \times s \times 1000}{y \times s \times \text{RF}} = \frac{0.4 \times 75 \times 50 \times 3000 \times 1000}{4 \times 3000 \times 10^{12}} \leq 3.75 \times 10^{-7}$$

Since single-donation and mini-pool testing were not taken into consideration, the calculation may be considered as worst-case scenario. The result shows the very low risk associated with the administration of a single dose of IVIG.

A dedicated elimination step is generally supposed to reduce the viral load by at least 4 log_{10} (LRF $\geq$ 4). In practice larger reduction factors may result in virus validation studies (see Table 3). However, validation studies of very effective steps may not fully elucidate their elimination potential due to the limitations by the titer of the virus spiking material and the detection limit of the assay leading to minimal LRFs (expressed as LRF $\geq$ X).

Finally the risk of transmitting a pathogen is smaller than the risk of exposure. Contact with a pathogen does not necessarily lead to infection. However, since exact numbers for infection efficiencies of pathogens vary widely (depending on the pathogen and the host's health), we assume the risk of transmission is equal to the risk of exposure.

8. Discussion

The tragic consequences of the AIDS epidemic emphasize the importance of incorporating specific pathogen inactivation or removal procedures in the production of therapeutic proteins from human plasma. Optimal procedures should not only eliminate known pathogens but should also have the potential to inactivate or remove emerging or unknown pathogens that may infect future blood supplies.

There are two current examples of emerging pathogens causing epidemics that could affect blood and blood products. These are the 2002–2003 epidemics of severe acute respiratory syndrome (SARS) and West Nile virus (WNV) [55,56].

SARS corona virus (SARS-CoV) is a marginal threat to blood product safety, because of the disease progresses rapidly. However, viremia in symptomatic patients was reported [57]. The SARS epidemic has produced 8098 probable cases in 29 countries with a case fatality rate of 9.6% [55]. SARS-CoV represents a new strain of human coronavirus. Coronaviruses are enveloped RNA viruses that range in diameter from 80 to 200 nm and are roughly spherical [58]. Information on the pathogenesis of SARS-CoV is currently being developed [55]. It is difficult to predict the inactivation and removal by partitioning of SARS-CoV during manufacturing of IVIG and data is not available. However, the removal of this virus using virusfiltration can be reasonably assumed.

Of potentially greater concern for producers of human plasma proteins is WNV. As of August 2003, approximately 0.03% of US blood donations were reactive for WNV in nucleic acid tests [56]. WNV is a spherical, 50 nm diameter, enveloped flavivirus. Currently available data strongly suggests, that WNV is unlikely to be transmitted through plasma

derivatives because of the effectiveness of viral clearance procedures, such as filtration, acidification, solvent-detergent, and heat treatments. This has been demonstrated utilizing model viruses (e.g. BVDV) as well as WNV [59–61].

To date several commercial IVIGs are produced using a variety of dedicated virus elimination procedures. These procedures can be categorized into three different elimination mechanisms, each attacking different physico-chemical properties of the pathogens:

- Partitioning
- Inactivation
- Virusfiltration

Partitioning steps are classical procedures incorporated in IVIG manufacturing from the beginning. Low pH treatment is an inactivation step, which was incorporated in the manufacturing of some IVIGs to make them intravenously tolerable. Other inactivation procedures were specifically designed and introduced into manufacturing processes to eliminate viruses. Virusfiltration is a simple, robust, non-destructive process that adds size exclusion, a totally new mechanism, to conventional virus inactivation and partitioning procedures. Virusfiltration has the potential to eliminate emerging viruses that may contaminate future blood supplies.

Research into resistant and emerging pathogens that may become relevant in transmitting diseases through blood products in the future continues.

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