



**Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research**

To: STN 125562/0

From: Pei Zhang, LPD/DH/OBRR

Through: Michael Kennedy, Team Leader, LPD/DH/OBRR

CC: Maruna Thomas, RPM, DBA/OBRR

Applicant: Cangene

Products: Anthrax Immune Globulin Intravenous (Human) [AIGIV]
Anthrasil™

Subject: CMC Review – Viral Safety Evaluation

Recommendation

Approval

Executive Summary - CMC -Viral Safety

Cangene submitted this original Biological License Application (BLA), STN 125562/0, for the licensure of Anthrasil™, Anthrax Immune Globulin Intravenous (Human) [AIGIV]. AIGIV is indicated for the treatment of adult and pediatric patients with toxemia associated with inhalational anthrax in combination with appropriate antibacterial drugs.

AIGIV is a sterile gamma globulin (IgG) fraction of human plasma containing antibodies to *Bacillus anthracis*, the bacterium that causes anthrax. It is prepared from Source Plasma obtained from selected healthy donors who have been immunized with anthrax vaccine adsorbed (AVA) vaccine (BioThrax®).

AIGIV is formulated in 10 % maltose and 0.03 % polysorbate 80. It contains no preservatives, and is frozen and stored at ≤ 15 °C after packaging. It is intended for single use by intravenous injection after thawing. AIGIV is supplied in 50 mL (b) (4) glass vials with 20 mm (b) (4) bromobutyl rubber stoppers, aluminum seals and plastic flip-off caps (3.2.P.7 Container Closure). Each vial contains ≥ 60 Units of activity as determined by the Toxin Neutralization Assay (TNA). The target fill volume is calculated with sufficient excess to allow for variation in the potency assay and for any loss of potency during storage. Currently, the fill volume is calculated based on a target TNA potency of (b) (4) .

The manufacturing process for AIGIV consists of purification of Immune globulin G (IgG) from donor plasma pools containing high titres of antibodies to *Bacillus anthracis* (3.2.S.2.3 Control of Materials). Purification consists of an anion exchange chromatography step followed by viral reduction steps (b) (4)

The purified in-process IgG is concentrated, filtered and formulated, yielding the bulk drug substance. The manufacturing process is physically (b) (4)

The platform process for AIGIV has been evaluated for viral clearance. The virus clearance steps are identical for all Cangene’s hyperimmune products, which include anion exchange chromatography, 20 nm virus filtration and solvent detergent inactivation.

CMC Review- Viral Safety Evaluation

1. Validation reports submitted

Table 1: Viral validation study reports (reviewed)

Validation Study No.	Validation Report Title
854380	Plasma Validation of Virus Inactivation/Removal during the Manufacturing Process of WinRho SD
858762	Validation of Poliovirus Type 1 Reduction During the Manufacturing Process of Immunoglobulin
L.100.00.001	Validation of the Clearance of Small, Non Lipid Enveloped Viruses by Anion Exchange Chromatography Process
PV.HYP.04.002	Evaluation of Porcine Parvovirus Clearance by Planova (b) (4) and 20N Nanofiltration
PV.HYP.04.006	Validation of the Robustness for the Planova 20N Nanofiltration Step for Clearance of PPV
PV.HYP.04.008	Validation of the Viral Clearance Capability of the 20N Nanofilter for EMC, HIV, PRV and BVDV
L.100.00.004	Validation of the Pseudorabies Virus Clearance by Solvent Detergent Treatment in Cangene’s Manufacturing Process
PV.HYP.03.001	Validation of the Virus Inactivation Kinetics for Lipid Enveloped Viruses by Solvent and Detergent Treatment, using Pseudorabies Virus and Bovine Diarrhea Virus
858893	Immunoglobulin Validation of Virus Inactivation During the Manufacturing Process of WinRho SD
856021	Validation of Virus Inactivation/Removal During the Manufacturing Process of WinRho SD

2. Materials of Biological Origin

The raw materials include those used for the (b) (4) which was implemented in the hyperimmune platform process in 2013 to (b) (4)

product (3.2.S.2.6 Manufacturing Process Development). The AIG batches manufactured to date (2005 to 2011) did not include this manufacturing step; however, future lots of AIG manufactured will incorporate this additional step.

(b) (4)



3. Starting Materials of Human Origin – Plasma

All Source Plasma screening, collection, storage and processing procedures used in the manufacture of AIGIV comply with applicable U.S. regulations. Applicant donor procedures and policies are adopted from the Plasma Protein Therapeutic Association (PPTA). Units shipped to the manufacturing facility must have originated from IQPP Qualified Donors. All Source Plasma, including Normal Source Plasma, is screened for the following viral markers as described in 21 CFR 610.40 at FDA licensed laboratories using FDA licensed test kits (except where indicated; see Table below) (3.2.S.2.3 Control of Materials).

(b) (4)

The plasma used in manufacturing AIGIV is tested to minimize potential viral input to finished product. Testing includes:

1. Testing at the donor unit level (3.2.S.2.3 Control of Materials)
2. Mini-pool testing (pre-manufacturing plasma pooling; 3.2.S.2.3 Control of Materials)
3. Manufacturing plasma pool testing.

As outlined in 3.2.S.2.2 Description of Manufacturing Process and Process Controls, the manufacturing plasma pool is tested for viral markers by (b) (4) (3.2.S.2.1 Manufacturers) using Nucleic Acid Testing (NAT).

The steps outlined in the figure below have been validated by spiking studies with viruses belonging to different families and classes. Two steps that were not essential for the purification/concentration of the product were added to the hyperimmune platform process to increase the viral safety of the drug product.

- 20N Filtration (20 nm): Non-selective method validated for removal of both lipid enveloped and non-lipid enveloped viruses, which have sizes at or greater than the nominal filter pore size of 20 nanometers.
- Solvent Detergent Treatment: Selective for the effective inactivation of lipid-enveloped viruses, as it irreversibly destroys the lipid coat. The manufacturing process for all human hyperimmune products, including AIGIV, is operated within the validated parameters for the viral clearance steps.

(b) (4)

(b) (4)

4. Viral Clearance Studies

The platform process for hyperimmune manufacture, which includes AIGIV, has been evaluated for viral clearance. The virus clearance steps are identical for all hyperimmune products; therefore, the studies and reported virus clearance factors are applicable to all of Cangene's hyperimmune products manufactured using the platform process.

Two steps that were not essential for the purification/concentration of the product were added to the hyperimmune platform process to increase the viral safety of the drug product.

- 20N Filtration (20 nm): Non-selective method validated for removal of both lipid enveloped and non-lipid enveloped viruses, which have sizes at or greater than the nominal filter pore size of 20 nanometers.
- Solvent Detergent Treatment: Selective for the effective inactivation of lipid-enveloped viruses, as it irreversibly destroys the lipid coat. The manufacturing process for all human hyperimmune products, including AIGIV, is operated within the validated parameters for the viral clearance steps.

In addition, Cangene provided data of validation of steps contributing to viral safety (log reduction ^{(b) (4)}

The anion exchange chromatographic step is employed for purification of the product and contributes to the viral reduction of both lipid and non-lipid enveloped viruses. Evaluation of this manufacturing step demonstrated some capacity for removal of certain small non-lipid enveloped viruses.

Design of Viral Clearance Studies

Choice of Viruses: The viruses employed for the spiking studies were selected to represent: 1. Those viruses that are potential contaminants of the product, and 2. A wide range of physico-chemical properties in order to challenge the manufacturing process's ability for viral clearance in general. The viruses employed for the pivotal spiking studies and the rationale for their use are summarized.

The selected viruses are considered suitable for validation of the virus clearance capability of the manufacturing process. The virus panel selected for the validation of the process covers a wide range of viral characteristics:

- DNA and RNA genomes
- Lipid and non-lipid enveloped

- Large, intermediate and small size
- Easily inactivated to very resistant
- Relevant, specific model, and non-specific model viruses

Table 3. Viruses Selected for Validation Studies:

Virus	Genome	Size (nm)	Enveloped	Resistance	Rationale
HIV-1	ss-RNA	80–100	Yes	Low	Mandatory use for plasma-derived products. Used as a relevant contaminant and model for other human retroviruses, such as HIV-2 and HTLV II
BVDV	ss-RNA	50–70	Yes	Medium	Model for HCV and WNV, which are potential plasma contaminants
PRV	ds-DNA	120–200	Yes	Medium	Model for herpes viruses, which are a potential human contaminant. PRV is also employed as a model for other enveloped DNA viruses that cannot be readily assayed, i.e.; HBV.
HAV	ss-RNA	25–30	No	High	Potential plasma contaminant. Potential for neutralization by antibodies.
PPV	ss-DNA	18–24	No	Very High	Model viruses for parvovirus B19, a potential plasma contaminant. Models for non-enveloped viruses for which antibodies are unlikely to be present; models for possible unknown non-enveloped viruses.
MMV	ss-DNA	20–25	No	Very high	
EMC	ss-RNA	approx. 30	No	High	

^a Virus characteristics as given by the CPMP/BWP/268/95 Note for Guidance on Virus Validation Studies, where applicable

BVDV = Bovine viral diarrhoea virus

HTLV = Human T-cell lymphotropic virus

DNA = Deoxyribonucleic acid

MMV = Murine minute virus

ds = Double strand

PPV= Porcine parvovirus

EMC = Encephalomyocarditis virus

PRV= Pseudorabies virus

HAV = Hepatitis A virus

RNA = Ribonucleic acid

HBV = Hepatitis B virus

ss = Single strand

HCV = Hepatitis C virus

WNV = West Nile virus

HIV = Human immunodeficiency virus (HIV-1; relevant virus for HIV-1 and model for HIV-2)

Table 4. Summary of Individual Viral Clearance Studies

Manufacturing Step	Study No.	Virus Used	Model for	Enveloped ^a	Genome ^a	Size ^a (nm)	Average Log Reduction	Possible Mechanism of Clearance
Anion Exchange Chromatography	L.100.00.001 Normal conditions	MMV	B19V	No	DNA	20–25	3.4	Removal by viral affinity to resin
		HAV	HAV	No	RNA	25–30	2.3	
20N Virus Filter	PV.HYP.04.002 Normal conditions	PPV	B19V	No	DNA	18–24	4.6 (one run)	Removal by size exclusion
		PPV	B19V	No	DNA	18–24	3.6 (b) (4) (b) (4)	
		BVDV	HCV	Yes	RNA	50–70	≥3.5	
		PRV	herpes	Yes	DNA	120–200	≥5.6 ^b	
		EMC	HAV	No	RNA	20–24	4.8	
Solvent Detergent	L.100.00.004 Robustness	PRV	herpes	Yes	DNA	120–200	≥5.6 (b) (4) (b) (4)	Inactivation
		BVDV	HCV	Yes	RNA	50–70	≥6.6	
		BVDV	HCV	Yes	RNA	50–70	≥7.5	
		HIV	HIV	Yes	retro RNA	80–100	≥4.7	
		PRV	herpes	Yes	DNA	120–200	≥5.3	
		PRV	herpes	Yes	DNA	120–200	≥4.7 ^c	
		BVDV	HCV	Yes	RNA	50–70	≥2.5 ^c	

^a Virus characteristics as given by CPMP/BWP/268/95 Guideline: Note for Virus Validation Studies, where applicable

(b) (4)

^c Study PV.HYP.03.001 was not performed using duplicate runs for each virus. The reductions factors are therefore not employed for calculation of the overall viral reduction capacity of the process. The study is considered a pivotal study since it demonstrates the kinetics of Solvent Detergent (SD) inactivation using SD concentrations that were (b) (4) of the concentration used in full-scale production.

Scale-down Production System: The virus clearance studies were performed at external laboratories (b) (4) The scaled-down process was executed by Cangene and the studies were performed with appropriate intermediates from production batches, ensuring batches were representative of the manufacturing scale. The contract facilities (b) (4) performed the cytotoxicity and interference studies, provided the spiking virus, collected and neutralized the samples, performed the infectivity assays and calculation of the reduction factors, and executed the scale-down studies, if required, using instructions prepared by Cangene. Data showing comparability of the scaled down manufacturing process to the commercial scale process and an integrated overview of the viral clearance studies are provided (Sections 2.3.3.2 and 2.3.4). Summaries of individual viral clearance studies are provided in section 2.3.5.

(b) (4)

Reviewer's comments: Comparison of the data demonstrates that the scaled-down study was executed using conditions consistent with the manufacturing hyperimmune scale, with the exception of the ratio of plasma to filter surface area. The study supports that the pre-treatment step may contribute to viral clearance during the manufacturing process.

Anion Exchange Chromatography:

(b) (4)



Reviewer's comments: The virus spiking studies for the anion exchange (AEX) chromatography step were performed at an approximate scale factor of (b) (4) of the manufacturing scale. The spiking experiments were designed to closely represent the production procedure, by either maintaining the parameters listed below or employing the worst-case conditions, so as not to overestimate the capacity for virus reduction.

(b) (4)

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Comparison of the data demonstrates that the scaled-down study was executed using conditions consistent with the hyperimmune manufacturing scale. The study supports that the anion exchange chromatography step may contribute to viral clearance during the manufacturing process. The study is used as a claim of viral clearance.

(b) (4)

Reviewer's comments: The virus spiking studies for the viral filtration step were performed at an approximate scale factor of (b) (4) of the manufacturing scale used for AIGIV; the scale-down study employed a filter with a surface area of (b) (4) whereas the AIGIV (b) (4) manufacturing scale employed a (b) (4) filter and the (b) (4) scale used two (b) (4)

(b) (4)

(b) (4)

(b) (4)

Reviewer's Comments: The virus spiking studies for the Solvent Detergent (S/D) step were performed at approximately (b) (4) of the manufacturing scale. The S/D step is controlled with respect to:

- (b) (4)

The S/D studies were performed using normal operating conditions and using conditions that challenged the robustness of the step.

Normal S/D Treatment Operating Conditions: These studies employed the following conditions:

- S/D concentrations the same as the target production specification of (b) (4) TnBP and (b) (4) X-100.

- Product temperature maintained at (b) (4) to provide a robust temperature range. The (b) (4)

- (b) (4)

S/D Treatment Kinetics and Robustness Studies: These studies employed the following conditions:

- S/D concentrations at either the minimum specifications of (b) (4) Triton X-100, or at (b) (4)

- (b) (4)

The comparison of the data demonstrates that the scaled-down study was executed using conditions consistent with or worst-case to the AIGIV manufacturing scale. The studies support that the S/D virus inactivation step can be claimed for viral clearance.

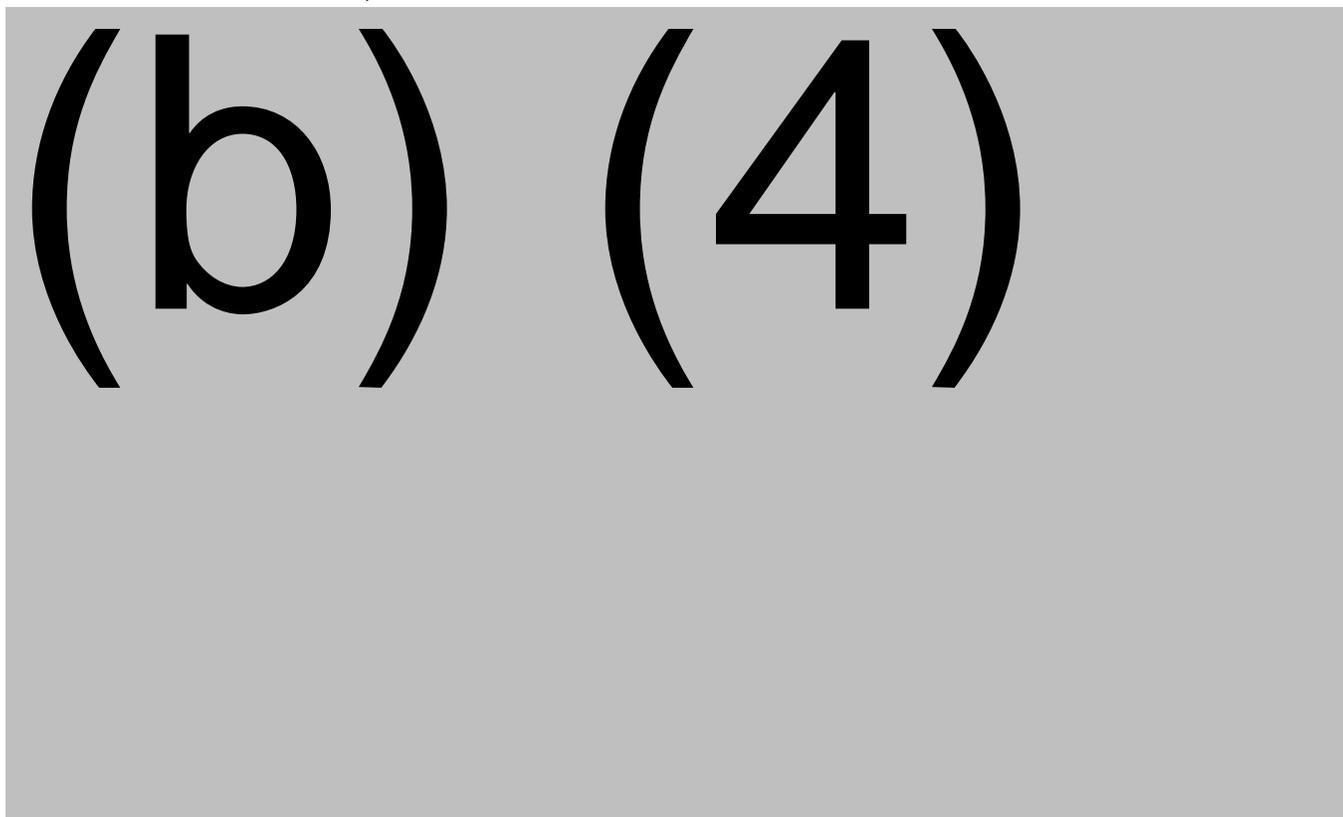
Reduction Capacity for HIV: HIV-1 is used as a relevant contaminant and as a specific model for other human retroviruses, such as HIV-2 and HTLV I and II.

Table 10. Reduction Capacity for HIV (Averages for the Log₁₀ Reduction Factors are Reported as the Geometric Mean)

(b) (4)

Reduction Capacity for BVDV: The model viruses for parvovirus were murine Bovine Viral Diarrhea Virus (BVDV) is a specific model for human Hepatitis C virus (HCV). HCV is a potential contaminant of human blood and plasma-derived products. BVDV is also a model for West Nile Virus (WNV) and other moderately sized, lipid-enveloped viruses.

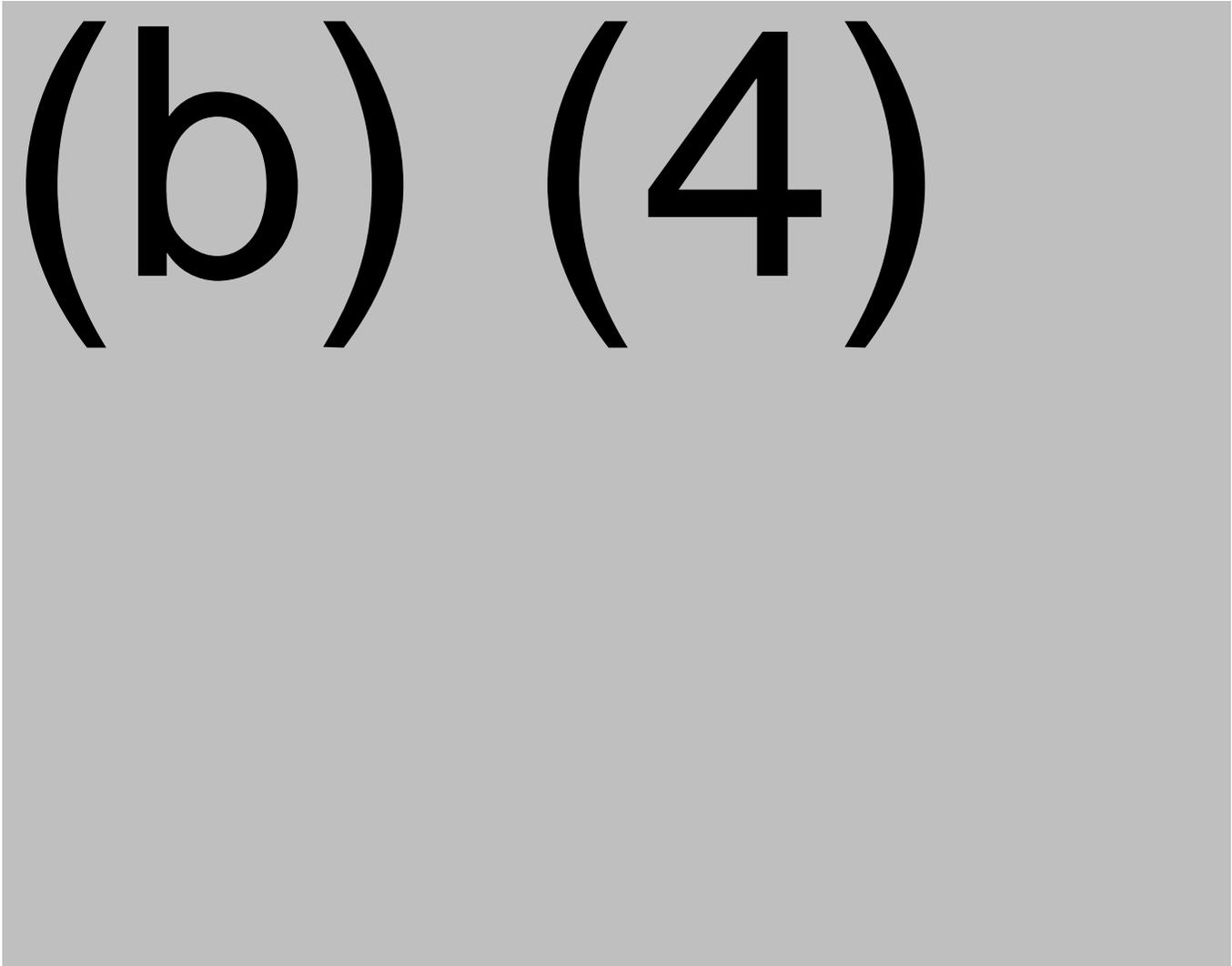
Table 11. Reduction Capacity for BVDV (Averages for the Log₁₀ Reduction Factors are Reported as the Geometric Mean)



(b) (4)

Reduction Capacity for PRV: Pseudorabies virus (PRV) is used as a model for human herpes viruses, which are potential contaminants in human blood and plasma-derived products. PRV is also considered a model for other enveloped DNA viruses, such as Hepatitis B.

Table 12. Reduction Capacity for PRV (Averages for the Log10 Reduction Factors are Reported as the Geometric Mean)



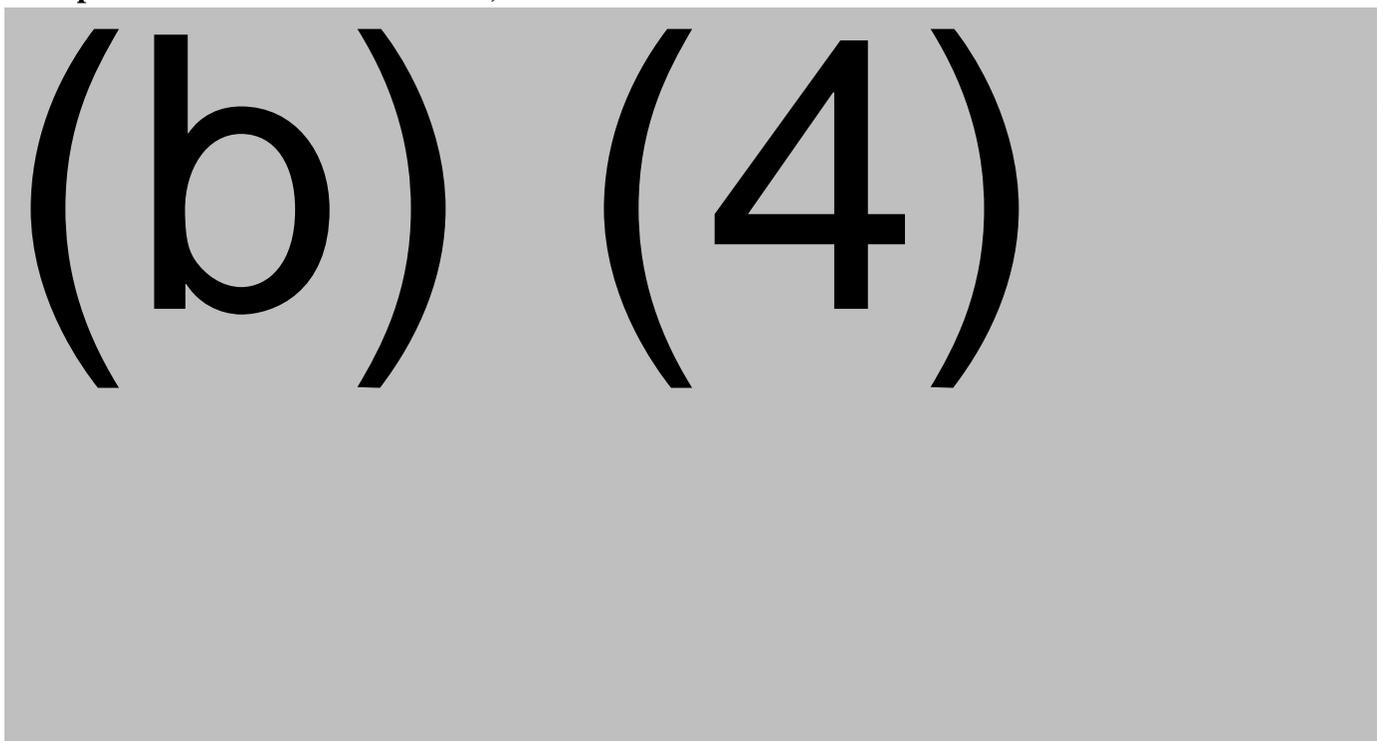
Reduction Capacity for Small Non-Enveloped Viruses: The manufacturing process was investigated for the potential removal/inactivation of small non-enveloped viruses. The virus panel included the relevant virus HAV and the HAV model virus, encephalomyocarditis virus (EMC). The model viruses for parvovirus were murine minute virus (MMV) and porcine parvovirus (PPV). EMC, MMV and PPV are also models for non-enveloped viruses for which antibodies are unlikely to be present and are therefore models for possible unknown or newly emerging small non-enveloped viruses.

Table 13. Reduction Capacity for HAV (Averages for the Log₁₀ Reduction Factors are Reported as the Geometric Mean)



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Table 14. Reduction Capacity for Parvovirus B19 (Averages for the Log₁₀ Reduction Factors are Reported as the Geometric Mean)



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Overall Viral Clearance Capacity: The viral clearance capacity of the manufacturing process is summarized in table below. The AIGIV manufacturing process is effective in virus reduction.

Table 15. Overall Virus Log₁₀ Reduction Factors of the Manufacturing Process

Envelope	Enveloped Virus			Non-Enveloped Virus			
	RNA		DNA	RNA		DNA	
Virus	HIV-1	BVDV	PRV	HAV	EMC	MMV	PPV
Family	Retrovirus	Flavivirus	Herpes virus	Picornavirus		Parvovirus	
Size (nm)	80–100	50–70	120–200	25–30	30	20–25	18–24
Anion Exchange Chromatography (partitioning)	Not evaluated			2.3	n.e.	3.4	n.e.
20N Filtration (size exclusion)	≥4.7	≥3.5	≥5.6 ^a	n.e.	4.8	n.e.	4.1
Solvent Detergent (inactivation)	≥4.7	≥7.3	≥5.5	Not evaluated			
Total Reduction (log₁₀)	≥9.4	≥10.8	≥11.1	2.3	4.8	3.4	4.1

^a The PRV was retained by the 0.1 µm pre-filter during the virus validation. Since manufacturing employs a 0.1 µm pre-filter before the 20N filter, the claim of ≥5.6 reductions is considered applicable.

BVDV = Bovine viral diarrhea virus; model virus for hepatitis C virus (HCV) and West Nile virus (WNV)

DNA = Deoxyribonucleic Acid

EMC = Encephalomyocarditis virus; model for HAV and for small non-enveloped viruses in general

HIV-1 = Human immunodeficiency virus-1; relevant virus for HIV-1 and model for HIV-2

HAV = Human hepatitis A virus; relevant virus for HAV and model for small non-enveloped viruses in general

MMV = Murine minute virus; model for human B19 parvovirus and for small non-enveloped viruses in general

n.e = Not evaluated

PPV = Porcine parvovirus; model for human B19 parvovirus and for small non-enveloped viruses in general

PRV = Pseudorabies virus; model for large enveloped DNA viruses, including herpes

RNA = Ribonucleic Acid

Reviewer's comments: The data provided in this BLA concerning viral validation studies are acceptable. I recommend an approval.