



Department of Health and Human Services
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To: STN 125683

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Applicant: Grifols Therapeutics LLC / 1871

Product: Immune Globulin Subcutaneous (Human), 20% (IGSC 20%)

Proprietary Name: XEMBIFY

CMC Review: Viral Validation

Recommendation

Approval

Summary

Immune Globulin Subcutaneous (Human), 20% (IGSC 20%) is a solution of purified human immunoglobulin (IgG) made from large pools of human plasma based on modifications to the Immune Globulin Injection (Human), 10% Caprylate/Chromatography Purified (IGIV-C) manufacturing process. The IGIV-C process is approved under BLA 125046 in the US (trade name Gamunex®-C) for both intravenous and subcutaneous use. The liquid formulation, containing human immune globulin proteins, glycine, polysorbate 80 and water for injection, is filled into (b) (4) glass vials. This product is indicated for the treatment of Primary Humoral Immunodeficiency (PI) in patients 2 years of age and older (1). This includes, but is not limited to, congenital agammaglobulinemia, common variable immunodeficiency, X-linked agammaglobulinemia, Wiskott-Aldrich syndrome, and severe combined immunodeficiencies.

To ensure the viral safety of this product, the manufacturing procedures are implemented to include: selection of donors, testing of individual donations and plasma pools for markers of infection with known viruses, and validation of the manufacturing process for its capacity to inactivate or remove viruses.

The capacity of the IGSC 20% manufacturing process to inactivate and/or remove viruses was evaluated in viral validation studies. Virus inactivation or removal was determined using infectivity-based assay systems. These studies were conducted by staff with expertise in methods for the work separate from the manufacturing facility.

The IGSC 20% manufacturing steps evaluated in this summary are:

- 1.(b) (4) Caprylate Precipitation/Depth Filtration
2. Caprylate Incubation
3. Column Chromatography
4. Nanofiltration
5. Low pH Final Container Incubation

All studies were performed using a scaled-down model of each process step with (b) (4) that were deliberately spiked with virus. The products from each of the scaled-down experiments were evaluated to determine their equivalence to the products from manufacturing scale, based on comparison of characterization data at the two scales.

These studies were performed using relevant viruses or models of human viruses that represent a wide range of physico-chemical properties with respect to the virus clearance process. Human immunodeficiency virus type 1 (HIV-1) was used as a relevant bloodborne pathogen, bovine viral diarrhea virus (BVDV) was used to model hepatitis C virus, and pseudorabies virus (PRV) was used as a surrogate for large, enveloped DNA viruses and can be used to model hepatitis B virus, for which there is no practicable assay system. Reovirus type 3 (Reo3) was used to model non-enveloped viruses, hepatitis A virus (HAV) was used as a relevant non-enveloped virus and porcine parvovirus (PPV) was selected as a surrogate for human parvovirus B19. Inactivation of the relevant bloodborne pathogen West Nile virus (WNV) was evaluated for the caprylate incubation and low pH final container incubation steps, and the model enveloped viruses duck hepatitis B virus (DHBV) and Sindbis virus (SINV) were evaluated for the caprylate incubation step only. This section of my review memo summarizes the studies concerning (1) the characterization of scaled-down models for the virus clearance capacity studies, and (2) the evaluation of virus clearance capacity across the IGSC 20% process. For the process steps including (b) (4) caprylate precipitation/depth filtration, column chromatography and nanofiltration, a full validation is performed using the scaled-down models. The caprylate and at low pH of final container incubation steps are performed in simple scaled-down models; (b) (4)

These scaled-down studies were performed originally to evaluate Grifols's Immune Globulin (Human), 10% Caprylate/Chromatography Purified (IGIV-C) process. These steps are the same for IGSC 20% process, and therefore, the data are applicable to the IGSC 20% process. Grifols submitted the data from scaled-down studies of the (b) (4) caprylate precipitation/depth filtration, the column chromatography and the nanofiltration steps:

1. PS-RSD-000002. (b) (4) Caprylate Precipitation/Depth Filtration Process Scaledown.
2. PS-RSD-000003. Column Chromatography Process Scaledown.
3. PS-RSD-000004. Nanofiltration Process Scaledown.

Documents Reviewed:

1. PS-RSD-000002. (b) (4) Caprylate Precipitation/Depth Filtration Process Scaledown.
2. PS-RSD-000003. Column Chromatography Process Scaledown.

3. PS-RSD-000004. Nanofiltration Process Scaledown.
4. PS-RSD-000005. (b) (4) Caprylate Precipitation/Depth Filtration Virus Clearance Capacity Studies.
5. PS-RSD-000006. Caprylate Incubation Virus Inactivation Capacity Studies.
6. PS-RSD-000009. Caprylate Incubation West Nile Virus Inactivation Capacity Studies.
7. PS-RSD-000007. Column Chromatography Virus Clearance Capacity Studies.
8. PS-RSD-000008. Column Chromatography (b) (4) Virus Clearance Capacity Studies.
9. PS-RSD-000010. Nanofiltration Virus Clearance Capacity Studies.
10. PS-RGLP-VV-000003. Validation of the Enveloped-Virus Clearance Capacity of the Terminal Low pH Incubation of IGSC 20% at a Target pH 4.8.

CMC Review- Viral Validation

1. (b) (4) **caprylate precipitation and depth filtration:** The validation process of the (b) (4) caprylate precipitation and depth filtration step is reviewed. (b) (4)

[REDACTED]

[REDACTED]

[REDACTED]

The (b) (4) caprylate precipitation/depth filtration step was demonstrated to provide effective clearance capacity for non-enveloped viruses. The Log₁₀ reduction determined for this step for Reo3, HAV and PPV were ≥3.5, ≥3.6 and 4.0, respectively (Table 10-1, [PS-RSD-000005. (b) (4) Caprylate Precipitation/Depth Filtration Virus Clearance Capacity Studies.]). (b) (4)

[REDACTED]

[REDACTED] Caprylate Precipitation/Depth Filtration Virus Clearance Capacity Studies.]). BVDV was the only enveloped virus studied because of its relative resistance to caprylate inactivation under the conditions of the (b) (4) precipitation. The log₁₀ for BVDV was 2.7^{(b) (4)}.

2. **CAPRYLATE INCUBATION:** The caprylate incubation step was demonstrated to provide effective inactivation capacity for enveloped viruses; inactivation to the limit of

detection was achieved for all viruses evaluated. The Log₁₀ reduction was ≥4.5 for HIV-1, ≥4.5 for BVDV, ≥4.6 for PRV (5), ≥5.1 for WNV (6), ≥3.6 for DHBV and ≥6.0 for SINV (Table 10-1).

The caprylate incubation step also demonstrated robust and effective enveloped virus inactivation with respect to changes in (b) (4) (Table 10-2, [PS-RSD-000006. Caprylate Incubation Virus Inactivation Capacity Studies.]).

- 3. COLUMN CHROMATOGRAPHY:** The column chromatography step was demonstrated to have clearance capacity for enveloped and non-enveloped viruses. The log₁₀ reductions were ≥3.0 for HIV-1, 4.0 for BVDV, ≥3.3 for PRV, ≥4.0 for Reo3, ≥1.4 for HAV and 4.2 for PPV (Table 10-1, [PS-RSD-000007. Column Chromatography Virus Clearance Capacity Studies.]). The low clearance capacity determined for HAV was due to (b) (4)

The column chromatography step was demonstrated to provide robust clearance of BVDV and PPV when evaluated at the (b) (4) (Table 10-2, [PS-RSD-000007. Column Chromatography Virus Clearance Capacity Studies.]).

Note: (b) (4) studies evaluated virus clearance capacity with (b) (4). The log₁₀ reductions, (b) (4)

- 4. NANOFILTRATION:** Studies were performed to evaluate the virus clearance capacity of the nanofiltration step of the IGIV-C manufacturing process. Nanofiltration for the IGSC 20% process is performed using the (b) (4) nanofilter as used for IGIV-C; therefore, the virus clearance capacities determined for the IGIV-C nanofiltration process are also applicable to the IGSC 20% process. HIV-1, BVDV and Reo3 were cleared to the limit of detection; the log₁₀ reductions were ≥3.7, ≥4.1 and ≥1.8, respectively (Table 10-1, [PS-RSD-000010. Nanofiltration Virus Clearance Capacity Studies.]). The relatively low reduction for Reo3 is attributable to (b) (4). Clearance of PRV and HAV by nanofiltration was also evaluated; however, due to interfering effects of the process intermediate matrix, the virus clearance capacity could not be determined for these two viruses. (b) (4).

Evaluation of BVDV clearance capacity under robustness conditions showed that the nanofiltration step including a (b) (4) is robust. The nanofiltration step provided clearance to the limit of detection across the range evaluated for (b) (4) (Table 10-2, [PS-RSD-000010. Nanofiltration Virus Clearance Capacity Studies.]).

- 5. LOW PH FINAL CONTAINER INCUBATION:** The low pH final container incubation was demonstrated to provide effective inactivation capacity for enveloped viruses. With incubation conditions of (b) (4) WNV, PRV and HIV-1 were inactivated to the limit of detection by (b) (4), respectively. At (b) (4), log₁₀ reductions for HIV-1, BVDV, PRV and

WNV were ≥ 5.3 , 4.9, ≥ 5.1 and ≥ 5.3 , respectively (Table 10-1, Table 10-2 [PS-RGLP-VV-000003. Validation of the Enveloped-Virus Clearance Capacity of the Terminal Low pH Incubation of IGSC 20% at a Target pH 4.8.]).

Table 10-1 Summary of Virus Clearance Capacity (Log10) - IGSC 20% Process

Process Step	Enveloped Virus				Non-Enveloped Virus		
	HIV-1	BVDV	PRV	WNV	Reo3	HAV	PPV
(b) (4) Caprylate Precipitation/Depth Filtration	ND ^a	2.7	ND ^a	ND ^a	≥ 3.5	≥ 3.6	4.0
Caprylate Incubation ^b	≥ 4.5	≥ 4.5	≥ 4.6	≥ 5.1	NA ^c	NA ^c	NA ^c
Column Chromatography	≥ 3.0	4.0	≥ 3.3	ND	≥ 4.0	≥ 1.4	4.2
Nanofiltration	≥ 3.7	≥ 4.1	ND ^d	ND	≥ 1.8	ND ^d	0.5
Low pH Final Container Incubation	≥ 5.3	4.9	≥ 5.1	≥ 5.3	NA ^c	NA ^c	NA ^c
Overall Clearance Capacity	≥ 16.5	≥ 20.2	≥ 13.0	≥ 10.4	≥ 9.3	≥ 5.0	8.2

- ND = Not determined: Interference by caprylate precluded determination of virus clearance capacity for this step.
- DHBV and SINV were also evaluated for the caprylate incubation step only. The log10 clearance capacities were ≥ 3.6 and ≥ 6.0 , respectively.
- NA = Not applicable: This step is not applicable to non-enveloped viruses.
- Due to interfering effects of the process intermediate matrix the virus clearance capacity could not be determined.

Table 10-2 Summary of Process Robustness for Virus Clearance Capacity - IGSC 20% Process (Setpoint Values in Bold)



2 pages determined to be not releasable: (b)(4)

(b) (4)

6. Caprylate Incubation West Nile Virus Inactivation Capacity Studies: In addition, Grifols also included recent data of Caprylate Incubation West Nile Virus Inactivation Capacity Studies to support the clearance of WNV (PS-RSD-000009. Caprylate Incubation West Nile Virus Inactivation Capacity Studies.). Viral validation studies were performed to evaluate the capacity of the caprylate incubation step of the Immune Globulin manufacturing process to inactivate the relevant enveloped virus West Nile virus (WNV). Inactivation of deliberately spiked WNV was evaluated at (b) (4)

The WNV (b) (4) used to spike the samples was provided by (b) (4)

Experimental Parameters for WNV Inaction

(b) (4)

(b) (4)

2 pages determined to be not releasable: (b)(4)

(b) (4)

The results of this study demonstrated that the caprylate incubation step is a step with effective inactivation capacity for WNV. Even under worst case conditions of (b) (4) the caprylate incubation step provided rapid and complete WNV inactivation.

Based on these studies, WNV inactivation by caprylate is included in the package insert See Tables below **Viral Clearance- GAMUNEX®-C vs. Xembity**

GAMUNEX®-C - Log Virus Reduction

Process Step	Log Virus Reduction					
	Enveloped Viruses			Non-enveloped Viruses		
	HIV	PRV	BVDV	Reo	HAV	PPV
Caprylate Precipitation/Depth Filtration	C/I	C/I	2.7	≥ 3.5	≥ 3.6	4.0
Caprylate Incubation	≥ 4.5	≥ 4.6	≥ 4.5	NA	NA	NA
Depth Filtration	CAP	CAP	CAP	≥ 4.3	≥ 2.0	3.3
Column Chromatography	≥ 3.0	≥ 3.3	4.0	≥ 4.0	≥ 1.4	4.2
Nanofiltration	≥ 3.7	M/I	≥ 4.1	≥ 1.8	M/I	< 1.0
Low pH Incubation	≥ 6.5	≥ 4.3	≥ 5.1	NA	NA	NA
Global Reduction	≥ 17.7	≥ 12.2	≥ 20.4	≥ 9.3	≥ 5.0	8.2

C/I - Interference by caprylate precluded determination of virus reduction for this step. Although removal of viruses is likely to occur at the caprylate precipitation/depth filtration step, BVDV is the only enveloped virus for which reduction is claimed. The presence of caprylate prevents detection of other, less resistant enveloped viruses and therefore their removal cannot be assessed.

NA - Not Applicable: This step has no effect on non-enveloped viruses. Some mechanistic overlap occurs between depth filtration and other steps. Therefore, Grifols Therapeutics LLC has chosen to exclude this step from the global virus reduction calculations.

CAP - The presence of caprylate in the process at this step prevents detection of enveloped viruses, and their removal cannot be assessed.

M/I - Interference by the process intermediate matrix precluded determination of virus removal capacity for this step.
Sum of reduction factors greater than or equal to 1 log₁₀.

XEMBIFY: Virus Clearance Capacity (Log₁₀)

Process Step	Enveloped Virus				Non-Enveloped		
	HIV-1	BVDV	PRV	WNV	Reo3	HAV	PPV
Caprylate Precipitation/Depth	C/I [*]	2.7	C/I [*]	C/I [*]	≥3.5	≥3.6	4.0
Caprylate Incubation [†]	≥4.5	≥4.5	≥4.6	≥5.1	NA [‡]	NA [‡]	NA [‡]
Column Chromatography	≥3.0	4.0	≥3.3	ND [§]	≥4.0	≥1.4	4.2
Nanofiltration	≥3.7	≥4.1	ND [§]	ND [§]	≥1.8	ND [§]	0.5
Low pH Final Container Incubation	≥5.3	4.9	≥5.1	≥5.3	NA [‡]	NA [‡]	NA [‡]
Overall Clearance Capacity	≥16.5	≥20.2	≥13.0	≥10.4	≥9.3	≥5.0	8.2

Note: Besides the addition of WNV inactivation, there is a minor difference in PRV reduction of between these two tables (less than 1 log₁₀), which is considered insignificant.

Reviewer's comments: These data demonstrate that the IGSC 20% manufacturing process could effectively remove and/or inactivate both enveloped and non-enveloped model viruses.