



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

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**Date:** February 24, 2010  
**To:** STN: 125350.0  
**From:** Lilin Zhong, MS, LPD, DH, OBRR, HFM-345  
**Through:** Mei-ying W Yu, Ph.D. LPD, DH, HFM-345  
Dorothy Scott, M.D., LPD Chief, DH, HFM-345.3  
**CC:** Pratibha Rana, RPM, HFM-370  
**Applicant:** CSL Behring AG, Bern, Switzerland  
**Product:** Immune Globulin Subcutaneous (Human), 20% Liquid, IgPro20  
Trade name: Hizentra™  
**Subject:** **Final CMC Review:** Viral Validation

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**RECOMMENDATION**

Approval

**EXECUTIVE SUMMARY**

The Biologics License Application (BLA) from CSL Behring AG, (Bern, Switzerland) was received by CBER on April 30, 2009 requesting U.S.-licensure of a 20% Liquid Immune Globulin Subcutaneous (Human) product, trade name Hizentra™, indicated for the treatment of patients with primary immunodeficiency (PID), and received a standard 10 month BLA review schedule. The firm provided viral safety data from its validation studies including 1) Plasma screening; 2) Analytical assay validation (serological testing for antibodies and antigen and Nucleic Acid testing); and 3) Manufacturing procedures, to support this BLA. Upon revisions, three robust and validated manufacturing steps are claimed by the firm which contribute to the viral inactivation or the removal - 1) pH 4 incubation; 2) -----(b)(4)----- depth filtration -----(b)(4)-----; and 3) Nanofiltration with -(b)(4)- ----- . The reviewer find that sufficient data and information have been provided on the viral validation to support the licensure of CSL Behring's IgPro20.

**BACKGROUND SUMMARY**

IgPro20 is a 20% solution of human normal immunoglobulin G (IgG) for subcutaneous administration. It is manufactured from large pools of human Source Plasma or recovered plasma by a combination of cold alcohol fractionation, octanoic acid precipitation, anion-exchange chromatography, and nanofiltration. The manufacturing process of IgPro20 is based on the manufacturing process of its parent product, Immune Globulin Intravenous (Human), 10% Liquid, IgPro10 or Privigen® (BLA STN 125201) which was licensed on July 27, 2007. The manufacturing procedures for IgPro20 are identical down to the active substance solution step of IgPro10 --- (b)(4) --- -----.

After production of -----(b)(4)----- and pre-formulation, the protein solution is concentrated to the final protein concentration of 20%, resulting in IgPro20. IgPro20 is formulated with 250 mmol/L of L-proline (used as a stabilizer) and 10-30 mg/L polysorbate 80. The final pH of the product is between 4.6 and 5.2. IgPro20 is filled at

5 mL (1 g), 10 mL (2 g), ----(b)(4)---- and 20 mL (4 g) sizes in -(b)(4)- glass infusion vials with -----(b)(4)----- stoppers. The product is stable at 2-25 °C, protected from light for up to 18 months.

In this BLA submission, CSLB provided data from its viral safety studies to support the licensure of IgPro20. Viral validation studies are performed based on the procedures for Privigen (IgPro10), which was previously approved by the FDA. These studies include 1) Plasma screening; 2) Analytical assay validation (serological testing for antibodies and antigen and NAT testing); and 3) Manufacturing procedures that are intended for virus clearance. This review is limited to the viral validation. Maria Luisa Virata and Douglas Frazier of LPD/DH/OBRR, HFM-345 reviewed both plasma screening and analytical assay validation.

**CMC REVIEW - Viral Validation**

**I. Plasma and Intermediates**

All plasma collection centers are licensed by the FDA. All Plasma donations including Source Plasma and recovered plasma used for manufacturing IgPro20 are collected in the United States. Plasma collection centers chosen are inspected and audited by CSL Behring. All donations are tested by using FDA-licensed serological assays for hepatitis B surface antigen (HBsAg) and antibodies against human immunodeficiency virus (type 1 and 2) (HIV-1/2) and hepatitis C virus (HCV). -----(b)(4)-----  
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FDA licensed Nucleic Acid Testing (NAT) procedures for HIV-1 and HCV are used to screen all plasma donations. For HBV, an investigational NAT procedure is used and the plasma units found to be negative. In addition, plasma has been tested for parvovirus B19 by several in-process NAT procedures and the limit for B19 DNA in the manufacturing pools is set as less than or equal to 10<sup>4</sup> IU/mL.

IgPro20 is manufactured at the licensed Bern, Switzerland facility, meanwhile the licensed -----(b)(4)----- facility will manufacture the -----(b)(4)-----, which can be used as -----(b)(4)-----  
----- made in Bern. Both -----(b)(4)----- have been accepted as comparable (see IgPro10 original BLA).

**II. Viral Reduction Steps in Manufacturing Process for IgPro20**

The viral log reduction by manufacturing steps obtained from validation studies are based on those in the Original BLA and BLA supplement of Privigen (STN 125201/0 and STN 125201/113 - Attachment, previously approved by the FDA, June 2009). In this submission for IgPro20, CSLB proposed to include the following steps for viral reduction: -----(b)(4)----- 2) pH 4 incubation, 3) -----(b)(4)----- depth filtration -----(b)(4)-----; and 4) Nanofiltration with -----(b)(4)----- Upon revisions, CSLB agreed to include only the following three steps for the virus reduction (Fig 1, Table 1):

**Fig 1. Manufacturing steps that are designed for viral reduction**

--(b)(4)--

### Table 1. Viral Reduction Steps

Designation	Process conditions
pH 4 Incubation	--(b)(4)----- ----- ----- -----
----- (b)(4)----- depth filtration ----- (b)(4)----- (step CZ0800 to CZ1050)	--(b)(4)-- ----- ----- -----
Virus filtration (Nanofiltration) (step CZ1300)	--(b)(4)----- ----- -----

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**2. Validation studies on the viral clearance steps in manufacturing process**

There are three steps that contribute overall virus clearance; 1) pH 4 incubation, 2) ----- (b)(4) ----- depth filtration ----- (b)(4) -----, and 3) Nanofiltration with ----- (b)(4) ----- . The same steps were also claimed for Privigen previously.

Model viruses are appropriately chosen including HIV, PRV, BVDV, WNV, EMCV, MVM and B19V to cover enveloped, non-enveloped, DNA and RNA virus types.

Down-scale viral validation studies are conducted appropriately which are the same as Privigen except:

- a). ----- (b)(4) -----
- b). Parvovirus B19 (B19) testing is included.

**2.1. Virus stocks and infectivity assay**

One of critical parameters for validating virus reduction is the quality of virus stocks, such as spiking virus stock preparation with proper filtration to eliminate virus aggregation; virus stock stability during the validation process, etc. Information Request regarding these issues was sent to CSLB on Nov 5, 2009.

**FDA IR:** You have provided summaries of study reports for evaluating LRF including PRV, BVDV, EMCV, and MVM. Please submit the raw data that support these summaries.

**CSLB response:** We have submitted in BL 12350/0 34 complete GLP conforming study reports evaluating LRFs for PRV, BVDV, EMCV, and MVM and not summaries of study reports.  
All virus validation studies have been performed in compliance with the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97) 186/Final] and with US FDA Good Laboratory Practice regulations (21 CFR 58). Study plans were written and predefined for all experiments. All results can be traced back to the original raw data, which have been archived in accordance with the applicable SOP's. Raw data collection and the calculation of results are in accordance with GLP rules. As documented in the study reports, each study plan, all study procedures and the final reports were inspected by the responsible GLP Quality Assurance Unit.  
Please find the raw data appended as pdf files to each individual study report in 3.2.A.2 Adventitious Agents Safety Evaluation.

**Reviewer's comments:** Acceptable.

**FDA IR:** Please provide data from the “untreated bench sample” to demonstrate that the loss of virus infectivity was taken into account during both your robustness studies and viral validation studies for PRV, BVDV, EMCV, and MVM.

**CSLB response:** Stability of virus was investigated in all studies and addressed in the study reports; raw data can be found in the attached pdf files (see answer to Question 11). -----(b)(4)-----  
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**Reviewer’s comments:** Acceptable.

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## 2.3. Parvovirus B19 (B19)

In this submission, CSLB included additional validation studies on B19 for low pH incubation step. However, the B19 ---(b)(4)--- assay is considered as experimental and not well established. Therefore, the validation results should not be included in the LRF table of the Package Insert. Instead, it is recommended to be included as a footnote to the LRF table as “In addition, virus clearance of human parvovirus B19 was investigated experimentally at the pH 4 incubation step. The estimated Log Reduction Factor obtained was  $\geq 5.3$ .” An Information Request was sent to CSLB on Nov 5, 2009.

**FDA IR:** Please be advised that the agency regards the B19 --(b)(4)--- assay as experimental and not well established. As such, the B19 validation results should not be claimed in the viral clearance table, but may be included as a footnote to the table. We suggest the following language for the footnote: “In addition, virus clearance of human parvovirus B19 was investigated experimentally at the pH 4 incubation step. The estimated Log Reduction Factor obtained was  $\geq 5.3$ .”

**CSLB response:** CSL Behring agrees to the solution as suggested above and will include the following footnote to the viral clearance table: “In addition, virus clearance of human parvovirus B19 was investigated experimentally at the pH 4 incubation step. The estimated Log Reduction Factor obtained was  $\geq 5.3$ .” This is the same wording that appears in the Privigen PI as approved by the FDA in June 2009.

**Reviewer’s comments:** Acceptable.

## 2.4. Virus Inactivation/Removal in Hizentra\*

	HIV-1	PRV	BVDV	WNV	EMCV	MVM
<b>Virus Property</b>						
Genome	RNA	DNA	RNA	RNA	RNA	DNA
Envelope	Yes	Yes	Yes	Yes	No	No

Size (nm)	80-100	120-200	50-70	50-70	25-30	18-24
<b>Manufacturing Step</b>	<b>2.1.1.1.1.1 Mean LRF</b>					
pH 4 incubation	≥5.4	≥5.9	4.6	≥7.8	nt	nt
Depth filtration	≥5.3	≥6.3	2.1	3.0	4.2	2.3
Virus filtration	≥5.3	≥5.5	≥5.1	≥5.9	≥5.4	≥5.5
<b>Overall Reduction (Log<sub>10</sub> Units)</b>	<b>≥16.0</b>	<b>≥17.7</b>	<b>≥11.8</b>	<b>≥16.7</b>	<b>≥9.6</b>	<b>≥7.8</b>

HIV-1, human immunodeficiency virus type 1, a model for HIV-1 and HIV-2; PRV, pseudorabies virus, a nonspecific model for large enveloped DNA viruses (e.g., herpes virus); BVDV, bovine viral diarrhea virus, a model for hepatitis C virus; WNV, West Nile virus; EMCV, encephalomyocarditis virus, a model for hepatitis A virus; MVM, minute virus of mice, a model for a small highly resistant non-enveloped DNA virus (e.g., parvovirus); LRF, log<sub>10</sub> reduction factor; nt, not tested; na, not applicable.

\*Virus clearance of human parvovirus B19 was investigated experimentally at the pH 4 incubation step. The estimated Log Reduction Factor obtained was ≥ 5.3.

## REVIEWERS' SUMMARY COMMENTS

Steps including low pH, -(b)(4)-, and nanofiltration have been validated for virus reduction. These steps are identical to those for Privigen, which have been approved by the FDA (Refer to STN: 125201/0 and see attachment STN: 125201/113).

------(b)(4)-----

------(b)(4)-----

## APPENDIX

### Supporting documents submitted that were reviewed: Viral clearance validation studies

- 3.2.S.2.2 Description of the Manufacturing Process and Process Controls
- 3.2.S.2.3 Control of Materials
- 3.2.S.2.4 Control of Critical Steps and Intermeidates
- 3.2.P.1 Description and Composition of the Drug Product
- 3.2.P.3.3 Description of the Manufacturing Process and Controls
- 3.2.A.2 Adventitious Agents Safety Evaluation (IgPro20)
- 3.2.A.2 Attachment 01 Comparability of the scale down process sequence OA-fractionation
- 3.2.A.2 Attachment 02 Comparability of the down-scale process sequence of OA-fractionation for -----(b)(4)-----  
----- as starting material
- 3.2.A.2 Attachment 03 Comparability of the scale down process sequence combined -(b)(4)- filtration
- 3.2.A.2 Attachment 04 Comparability of the scale down process sequence Nanofiltration
- 3.2.A.2 Attachment 05 Scale – Up Report
- 3.2.A.2 Attachment 06 Validation of the clearance of Human Immunodeficiency Virus ------(b)(4)-----  
----- in the IVIG-CZ manufacturing process
- 3.2.A.2 Attachment 07 Validation of the PRV clearance during ------(b)(4)----- in the IVIG-CZ manufacturing process
- 3.2.A.2 Attachment 08 Robustness of the PRV clearance during ------(b)(4)----- in the IVIG-CZ manufacturing process – Effect of minimal amount of -(b)(4)--
- 3.2.A.2 Attachment 09 Validation of the BVDV clearance during ------(b)(4)----- in the IVIG-CZ manufacturing process
- 3.2.A.2 Attachment 10 Robustness of the BVDV clearance during ------(b)(4)----- in the IVIG-CZ manufacturing process – Effect of minimal amount of --(b)(4)--
- 3.2.A.2 Attachment 11 Validation of the WNV reduction by ------(b)(4)----- in the IgPro10 manufacturing process
- 3.2.A.2 Attachment 12 Validation of the EMCV clearance during ------(b)(4)----- in the IVIG-CZ manufacturing process
- 3.2.A.2 Attachment 13 Validation of the MVM clearance during ------(b)(4)----- in the IVIG-CZ manufacturing process
- 3.2.A.2 Attachment 14 Validation of the inactivation of Human Immunodeficiency Virus Type 1 during pH 4 incubation in the IVIG-CZ manufacturing process

3.2.A.2 Attachment 15	Validation of PRV inactivation during pH 4 incubation in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 16	PRV inactivation during pH 4 incubation in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 17	Robustness of the PRV inactivation during pH 4 incubation in the IVIG-CZ manufacturing process – Effect of pH
3.2.A.2 Attachment 18	Robustness of the PRV inactivation during pH 4 incubation in the IVIG-CZ manufacturing process – -----(b)(4)-----
3.2.A.2 Attachment 19	BVDV inactivation during pH 4 incubation in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 20	Robustness of the BVDV inactivation during pH 4 incubation in the IVIG-CZ manufacturing process – Effect of pH
3.2.A.2 Attachment 21	Robustness of the BVDV inactivation during pH 4 incubation in the IVIG-CZ manufacturing process – -----(b)(4)-----
3.2.A.2 Attachment 22	Robustness of the BVDV inactivation during pH 4 incubation in the IVIG-CZ manufacturing process – -----(b)(4)-----
3.2.A.2 Attachment 23	Validation of the inactivation of WNV during the pH 4 incubation in the IgPro10 manufacturing process
3.2.A.2 Attachment 24	Validation of the clearance of Human Immunodeficiency Virus during the combined -(b)(4)- filtration in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 25	Validation of PRV clearance during the combined -(b)(4)- filtration in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 26	Robustness of the PRV clearance during the combined -(b)(4)- filtration in the IVIG-CZ manufacturing process – -----(b)(4)-----
3.2.A.2 Attachment 27	Validation of the BVDV clearance during the combined -(b)(4)- filtration in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 28	Robustness of the BVDV clearance during the combined -(b)(4)- filtration in the IVIG-CZ manufacturing process – -----(b)(4)-----
3.2.A.2 Attachment 29	Validation of the WNV reduction by ----(b)(4)----- filtration in the IgPro10 manufacturing process
3.2.A.2 Attachment 30	Validation of the EMCV clearance during the combined -(b)(4)- filtration in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 31	Robustness of the EMCV clearance during -(b)(4)- filtration in the IVIG-CZ manufacturing process – -----(b)(4)-----
3.2.A.2 Attachment 32	Validation of the MVM clearance during the combined -(b)(4)- filtration in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 33	Robustness of the MVM clearance during -(b)(4)- filtration in the IVIG-CZ manufacturing process – -----(b)(4)-----
3.2.A.2 Attachment 34	Validation of the clearance of Human Immunodeficiency Virus type 1 during -(b)(4)- nanofiltration in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 35	Validation of the PRV clearance by nanofiltration in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 36	Validation of the PRV clearance by nanofiltration in the IVIG-CZ manufacturing process by the use of -----(b)(4)-----
3.2.A.2 Attachment 37	Validation of the BVDV clearance by nanofiltration in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 38	Validation of the BVDV clearance by nanofiltration in the IVIG-CZ manufacturing process by the use of -----(b)(4)-----
3.2.A.2 Attachment 39	Validation of the WNV clearance by nanofiltration in the IgPro10 manufacturing process
3.2.A.2 Attachment 40	Validation of the EMCV clearance by nanofiltration in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 41	Validation of the MVM clearance by nanofiltration in the IVIG-CZ manufacturing process
3.2.A.2 Attachment 42	Robustness of the MVM clearance during nanofiltration in the IVIG-CZ manufacturing process – Combined worst case
3.2.A.2 Attachment 43	Robustness of the MVM clearance during nanofiltration in the IVIG-CZ manufacturing process – -----(b)(4)-----
3.2.A.2 Attachment 44	Robustness of the MVM clearance during nanofiltration in the IVIG-CZ manufacturing process – -----(b)(4)-----
3.2.A.2 Attachment 45	Validation of WNV reduction by the manufacturing procedure of IgPro10

- 3.2.A.2 Attachment 46 Validation of the virus reduction by -----(b)(4)----- in the IgPro10 manufacturing process using -----(b)(4)-----
- 3.2.A.2 Attachment 47 Validation of the virus reduction by -----(b)(4)-----  
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- 3.2.A.2 Attachment 63 Inactivation of B19V by pH 4.0 treatment in the manufacturing procedure of Privigen
- 3.2.A.2 Attachment 64 Validation of PRV reduction by the manufacturing procedure of Privigen
- 3.2.A.2 Attachment 66 Virus titration - Summary of ---(b)(4)--- experiments
- 3.2.A.2 Attachment 67 EMC virus titration - Summary of EMCV ---(b)(4)--- experiments
- 3.2.A.2 Attachment 68 Evaluation of the virus reduction capacity of -----(b)(4)----- in the manufacturing process of Privigen